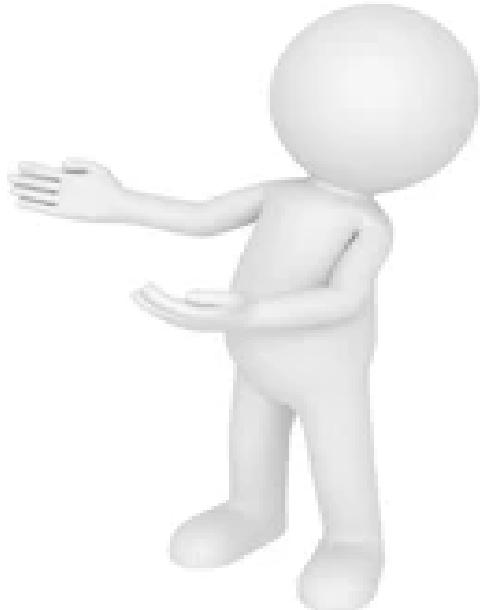


INTRODUCTION



1. INTRODUCTION:

The intranasal drug administration has gained growing interest recently. The nasal tract represents a non-invasive route for administration of active pharmaceutical ingredients for local, systemic and CNS action. Although, the nasal epithelium appears as a tight barrier, the tightness of the intercellular junctional complex of the nasal mucosa is low due to leaky epithelial tissue¹⁻³. In spite of its advantages, nasal delivery has some limitations which includes mucosal irritation due to frequent intranasal administration of drugs, the active mucociliary clearance which rapidly clears the drug from nasal cavity and interference of nasal congestion with absorption of drug⁴⁻⁶. Permeability of intranasally administered drugs is influenced by various biological factors, drug formulation and device related factors. The intranasal absorption and delivery rate of drugs varies with section of the nasal cavity. The nasal cavity is divided into five anatomical regions: nasal vestibule, the olfactory region, respiratory region, atrium and nasopharynx⁷. Most of absorption occurs in the respiratory region because of its high vasculature and large surface area. The olfactory region offers direct access to cerebrospinal fluid so most of the intranasal drugs are transported directly to CNS through this section of nasal cavity. It is important to understand the physiology and anatomy of the nasal cavity to address the obstacles that impact absorption and delivery of intranasal agents^{8,9}.

1.1 BRAIN:

The brain is a complex organ that controls thought, memory, emotions, touch, motor skills, vision, breathing, temperature, appetite and all the processes that control our body. Together, the brain and spinal cord that extends from it make up the central nervous system, or CNS. Weighing about 3 pounds in the average adult, the brain is about 60% fat¹⁰. The remaining 40% is a combination of water, protein, carbohydrates and salts. It contains blood vessels and nerves, including neurons and glial cells. It has a mass of pinkish grey tissues containing networks known as neuron. Interneurons mediate simple reflexes as well as it is responsible for the highest function of the brain and gives a protective cover by blood brain barrier (BBB)¹¹. The glial cells support brain function as well as make an important contribution in the development of CNS and the function of the adult brain. Various diseases can affect the brain such as epilepsy, HIV encephalopathy, cerebrovascular diseases, brain tumors and all neurodegenerative disorders¹².

1.2 BLOOD BRAIN BARRIER (BBB):

BBB is a semi-permeable membrane barrier at the interface between blood and brain tissue, composed of a complex system of endothelial cells, striated cells, pericardium, and perivascular mast cells. BBB strictly controls the transport of various substances from blood to brain¹³. In general, only low molecular weight lipophilic molecules (400-600 Da) can cross the blood-brain barrier¹⁴. The diffusion of drugs from the blood to the brain depends on the ability of the biologically active substances to cross the lipid membrane.

BBB penetration efficiency is hard to reach therapeutic concentration in brain parenchyma for effective treatment¹⁵. The BBB is a selective barrier that permits nutrients to diffuse in a controlled manner. The BBB is missing in the vomiting center, and the hypothalamus is required to check the chemical composition of the blood¹⁶.

1.2.1 FUNCTION OF BLOOD-BRAIN BARREIR:

The blood-brain barrier (BBB) protects the neural tissue from variations in blood composition and toxins. Extracellular concentrations of hormones, amino acids and potassium are constantly changing, especially after meals, exercise or stressful times. Since many of these molecules regulate neuronal excitability, a similar change in the composition of interstitial fluid in the CNS can lead to uncontrolled brain activity¹⁷. Blood borne infections of the brain are rare. BBB becomes permeable during inflammation, allowing macrophages to move across. Antibodies are too large to cross BBB. Also certain Biochemical poisons are too large to pass BBB¹⁸.

1.3 BLOOD CSF BARRIER: The CSF system is the primary route through which a variety of substances enter the brain. It's unclear why some substances enter through the blood-CSF barrier while others enter through the blood-brain barrier. Because the tight junctions of the blood-CSF barrier are quantitatively leakier than those of the blood-brain barrier, it could reflect the difference in passive permeability of the two barriers. It could also reflect the needs of regions adjacent to the ventricular system¹⁹.

1.4 BRAIN TUMOR BARRIER (BTB): The brain tumor barrier is a protective layer formed as a result of anatomical and physiological changes in the BBB by regenerating new blood vessels that loosen the tight junctions of endothelial cells and supply oxygen to growing tumors. These newly formed blood vessels had several small pores that prevented the penetration of molecules, including hydrophilic ones²⁰.

1.5 EFFLUX PUMPS: Efflux pumps present in the lining of endothelial cells, serve as additional hurdles in medication delivery to the brain. The permeability of the endothelium barrier is principally regulated by these efflux pumps, which are made up of protein complexes termed adherens junctions²¹.

1.6 PHYSIOLOGY AND FUNCTION OF THE NOSE:

1.6.1 BREATHING:

In the first several weeks of life, breathing by nose is the most critical and favored method for human babies. Nasal breathing has several advantages in neonates, including preventing aspiration during swallowing during breast or bottle feeding and humidifying and warming the inspired air. During inhalation, nose breathing provides approximately 30 to 50% of total resistance, which is sufficient to preserve lung elasticity and integrity; however, mouth breathing provides less resistance and may lead to the development of lung disease such as emphysema²².

1.6.2 FILTRATION:

The nose is thought to be the first filter that removes particulate matter from inspired air before it reaches the alveoli. Extraneous particle deposition in such areas may have serious consequences in the lung due to slow clearance activity and structural fragility of alveoli. Several factors, including nasal disease, particle diameter, and surgical changes to the internal structure of the nose (turbines and valves), can all have an impact on the nose's filtration function. Particles with a diameter of 10um or greater are trapped on the nasal

mucosa and moved to the oropharynx within 30 minutes to be swallowed. Electrostatic interaction, mucociliary clearance, impingement, and deposition on vibrissae are some of the mechanisms used to filter and clean particles from inspired air²³.

1.6.3 HEATING AND HUMIDIFICATION:

Another important nasal function is the simultaneous heating and humidification of inhaled air. Because of the nasal cavity's specialized vascularization and the high secretory function of the epithelial gland and goblet cells, incoming air can be efficiently and continuously warmed and humidified. The arterial blood flows in the opposite direction to the inspired air in the nasal mucosa, allowing for rapid and efficient heat exchange. At room temperature, 23°C, or very low temperatures, -18°C, the nose can effectively condition the inspired air to 30°C and 98 percent relative humidity, whereas mouth breathing was found to be less effective in cold air conditioning^{24,25}.

1.6.4 OLFACTION AND SENSATION:

Olfaction is the nose of smell, which aids humans and animals in identifying various types of odors. The mechanism of smell detection and discrimination is still unknown²⁶. The odorant binding protein binds and interacts with odorant molecules, increasing their solubility thousands of times greater than in ambient air. The superior turbinate and the nasal septum are covered by the olfactory area, which is located on the roof of the nasal cavity. It is made up of a variety of cells, including olfactory receptor cells, supporting cells, basal cells, and Bowman's gland. The trigeminal system also influences odor perception by enhancing or suppressing the olfactory system^{27,28}.

1.7 MUCOCILIARY CLEARANCE ACTIVITY:

Mucociliary clearance is an innate defense mechanism that protects the pulmonary system from the harmful consequences of inhaled agents, including those of biological, chemical, and physical nature²⁹. Ciliated cells, which line the surface epithelium of the airways, provide the force necessary for mucociliary clearance by the coordinated beating of their cilia³⁰. The nasal mucosal cilia act as a barrier to outside particle penetration. They can be found in the respiratory and olfactory systems. These, however, are not motile in the olfactory zone. Cilia in the respiratory system transport mucus from the nose to the oropharynx. This mechanism flushes the mucus every 10–15 minutes. This process is called mucociliary clearance³¹. Mucociliary clearance is responsible for the generally observed rapid clearance of nasally administered drugs. Therefore, there is an opposing mechanism in the absorption process of drugs following intranasal delivery³². Approaches to cilia suppression improve processing time in the nasal mucosa and can thus be used to increase the amount of medication that can be absorbed into the brain via the nasal mucosa^{33,34}.

1.8 ANATOMY OF THE NOSE

1.8.1 EXTERNAL NOSE:

The outer nose consists of a pair of nasal bones and cartilage on the upper and lower sides. Inside, the nasal septum separates the nose from the right and left. The lateral nasal wall contains lower and middle turbinates and sometimes the turbinate bone is very high or very low. The opening of the sinuses is also found under the turbinates in the middle of the nasal

wall on the side. The lacrimal system drains into the nasal cavity below the anterior inferior aspect of the inferior turbinates³⁵.

1.8.2 NASAL CAVITY:

The average size of the human nasal cavity is 12–14 cm long, its capacity is 12–14 ml, and its surface area is 160 cm sq. Due to its relatively small volume (25 cm³), the nasal cavity can only be used in small quantities (100-200L) at a time. The human nose is divided into two cavities by the nasal septum. Nasal cavities are classified into three regions, i.e. vestibule, respiratory, and olfactory region³⁶.

1.8.3 NASAL VESTIBULE

The nasal vestibule is the entrance to the nasal cavity and the first point of contact with the external environment. The nasal vestibule, unlike the rest of the nasal cavity, is lined with keratinized squamous epithelium, which is similar to the epithelial lining of the skin. The nasal vestibule also contains vibrissae (thick hair) and sebaceous glands, which help the nose's filtration function by filtering out large particles. The vestibular area contains thermoreceptors, which detect nasal airflow and change nasal airway resistance with cold or warm air inhalation. The nasal vestibule has low vascularization, a small surface area of approximately 0.6 cm², and keratinized cells. All of these factors could explain the low permeability and absorption of the drugs through the vestibular area³⁷.

1.8.4 NASAL VALVE:

Valves are structures that control the flow of air or fluid inside a person's body. In the nose, cartilage and nasal passages - especially those of the lower nasal conchae and the nasal septum act as valves, which regulate air flow. The anterior part of the nasal cavity, from the nose to the nasal cavity (NV), is the area where the nasal passages are held narrowest segments of the nasal cavity thus, being very important for nasal physiology and the main nasal symptom – obstruction such as trauma, surgery or congenital alteration in nasal structure may cause increase in total nasal resistance and even block nasal airways flow^{38,39}.

1.8.5 THE RESPIRATORY REGION:

The respiratory area is one of the largest parts of the nasal cavity and is lined with columnar pseudostratified epithelium. This type of epithelium, also known as respiratory epithelium or non-olfactory epithelium, covers 80-90% of the human nasal cavity. It is characterized by the presence of various types of cells and glands, including microcytic cells, basal cells, ciliated cells, secretory glands, and serous glands. Most respiratory cells are surrounded by microvilli that help increase the total absorption surface area of the nasal cavity, so the respiratory area is considered to be the major compartment of drug

absorption throughout the body⁴⁰. Less than 20% of respiratory cells are covered with cilia. Cilia take advantage of their active mobility to support the protective function of the nose by transporting and excreting mucus containing trapped particles to the throat and digestive system. Mucus is continuously secreted by goblet cells and secretory glands and is thought to be the main component of the mucosal layer that covers the epithelium. The respiratory area is highly angiogenic and the internal and external carotid arteries^{41,42}.

1.8.6 NASOPHARYNX

Nasopharynx forms the upper part of pharynx and it is situated above the soft palate and posterior to the nasal cavity. The upper part of nasopharynx is covered with ciliated epithelium while lower part is covered with squamous epithelium. This structural organization acts as channel to transport the filtered, warmed air from the nasal cavity to the lungs. It has many other functions associated with vocalization and pressure equalization in the middle ear and also acts as a drainage route for most of nasal secretions⁴³.

1.9 BRAIN TARGETING

As mentioned earlier, directing drugs to the human brain has always been a challenge for prescribers, as there are strong barriers such as the blood-brain barrier and the blood-cerebrospinal fluid barrier. Extensive research has identified the intranasal route as a potential route for delivering the drug directly to the brain. Drug delivery via the nose is considered to be a non-invasive route. The ability of the nasal mucosal layer to transport small molecules has also been extensively investigated. Various drug delivery technologies have been developed, including nanoparticulate systems. The nanoparticulate system is easy to manufacture, long-term stability, site of action, strong drug encapsulation, and protection from environmental degradation^{44,45}.

1.10 THE MECHANISM FOR INTRANASAL DELIVERY:

The exact mechanism underlying intranasal drug delivery to the CNS is not fully understood, but there is increasing evidence that the neural pathways that connect the nasal passages to the brain and spinal cord are important. In addition, pathways involving the vascular, CSF, and lymphatic systems are used to transport molecules from the nasal passages to the CNS. The combination of these routes may be the cause, but one route may be exceeded, depending on the properties of the neurotherapeutic drug, the properties of the formulation, and the delivery device used. The nasal septum separates the two halves of the nasal cavity. Each half has three areas-Nasal atria, respiratory area, and olfactory area⁴⁶.

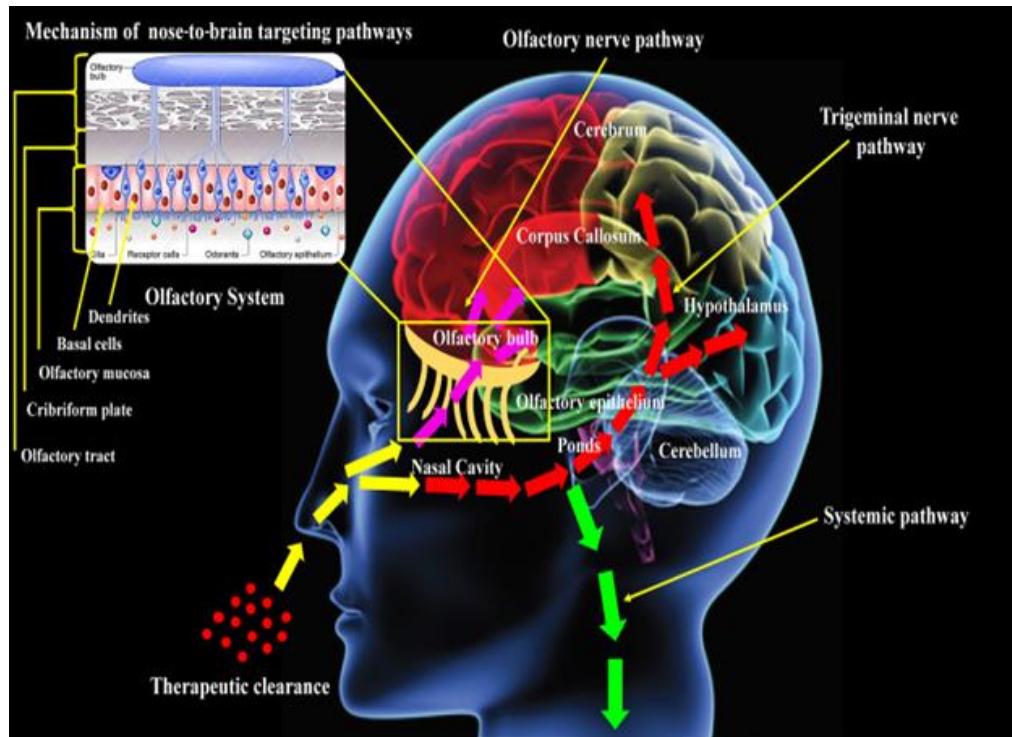


Figure 1: Mechanism of Nose to brain delivery.

1.10.1 OLFACTORY NERVE PATHWAY: In order for the drug to reach the fluid or brain parenchyma from the olfactory region of the nasal cavity, it must pass through the olfactory epithelium of the nose, and in some routes, the arachnoid membrane surrounding the subarachnoid space. In principle, we can consider two different pathways through the olfactory epithelium⁴⁷.

- i) Transcellular pathway- Perhaps by receptor-mediated endocytosis, liquid phase endocytosis, or bypass diffusion, especially via sustentacular cells. Passive diffusion is most likely with more lipophilic drugs. It is delivered quickly. This pathway is involved in the transport of lipophilic active substances and exhibits their lipophilic rate dependence.
 - ii) Paracellular pathways- through tight junctions between sustentacular cells, or so-called crevices between sustentacular cells and sensory neurons. Nasal absorption of hydrophilic drugs is most likely due to diffusion through aqueous channels (pores). This path is slow and passive. This pathway is involved in the transport of the hydrophilic active ingredient and shows its rate dependence on the molecular weight of the active ingredient. Drugs that do not contain absorption enhancers and have a MW up to 1000 Da show good bioavailability. It can be expanded to drugs with a MW up to 6000 Da, by including absorption enhancers in the formulations⁴⁸.

1.10.2 TRIGEMINAL NERVE PATHWAY -The trigeminal nerve pathway involves endocytosis and axonal transport (i.e., intracellular transport) followed by transport via the trigeminal nerve. The trigeminal nerve is the largest cranial nerve that innervates the olfactory and respiratory epithelium and has three different branches (mandibular, ophthalmic and maxillary) that converge to the trigeminal ganglion, enter the CNS, and end in the brainstem⁴⁹. In the olfactory epithelial pathway, substances are absorbed by the lamina propria and utilize the gaps around the olfactory nerve tract to continue to the

central nervous system. The substance travels through the perineural space between the olfactory envelope cells and the olfactory nerve fibroblasts by bulk flow. This space leads to the subarachnoid space of the brain, from which more material may be distributed⁵⁰.

1.10.3 LYMPHATIC PATHWAY-Drugs can be transported in a number of ways outside the cell, such as the perivascular, perineural, and lymphatic channels from the submucosal region of the olfactory region. These external pathways are connected to the olfactory nerves from lamina propria to the olfactory bulb of the brain. Therefore, the lymphatic system also plays an important role in transporting the drug from nose to the brain⁵¹.

1.10.4 THE SYSTEMIC PATHWAY-The systemic route is an indirect route that the drug takes to reach the lungs and bloodstream before it reaches the brain. Therefore, the drug must pass through the BBB and reach the brain by this route. This increases the time it takes to achieve a therapeutic effect and limits the amount of drug that effectively reaches the brain⁵². In addition, the amount of drug in the brain after intranasal administration varies from patient to patient and is excreted via renal and hepatic mechanisms. This allows the drug to reach the brain directly and systemically, primarily depending on the needs of the drug's properties. For example, some lipophilic drugs enter the brain via systemic routes after intranasal administration⁵³. Transport of drugs to CNS through intranasal route involved all of the above pathways. However, olfactory & trigeminal nerve pathway plays most important role in treating neurodegenerative disorders.

2. NEURODEGENERATIVE DISEASES:

It is a type of diseases where an extensive degeneration of neuron or neuro cell death. The nervous systems are made with the help of neurons and there is a difficulty in regeneration of cells when damage or dead. This is due to the inability in generating of new cells by the body. Some of these are Alzheimer's, Sclerosis, Huntingtons and Parkinsons. However, current researcher's focuses on Alzheimer's disease it is growing more fastly in present decades.

2.1 ALZHEIMER's DISEASE:

Alzheimer's disease is a progressive neurologic disorder that causes the brain to shrink (atrophy) because of dead brain cells. Alzheimer's disease is the most common cause of dementia - a progressive decline in thinking, behavioral and social skills that affects a person's ability to function independently. At present, there is no known cure for AD when it comes to the lack of understanding of its cellular mechanisms. However, treatment is available to improve symptoms and further research is aimed at finding a solution. The accumulation of amyloid-beta peptides (A β), neurofibrillary tangle formation of phosphoric tau proteins, and destructive neuroinflammation are signature features of AD progression⁵⁴. Major biochemical markers for diagnostic purposes include A β plaque around the affected brain and the presence of soluble A β and tau protein in cerebrospinal fluid. Inhibition of the formation of A β plaque / tau tangle and the reduction of its binding to neurons is a key focus of advanced therapeutic strategies. Current clinically approved drugs can only alleviate symptoms and delay the progression of AD by promoting interactions between neurons in the AD brain by neurotransmitters. Therefore, the discovery of AD markers and the development of advanced and potent nanomedicine drugs to correct these AD symptoms are essential for effective treatment and treatment⁵⁵.

2.2 SYMPTOMS:

Memory loss is a major symptom of Alzheimer's disease. Early symptoms include difficulty remembering recent events or conversations. As the disease progresses, memory impairment becomes worse and more symptoms appear. At first, a person with Alzheimer's disease may find it difficult to remember and organize thoughts. A family member or friend can see how serious the symptoms are. Alzheimer's disease-related brain changes lead to growing problems:

❖ Memory

Everyone has some memory loss from time to time, but the Alzheimer's disease-related memory loss persists and worsens, impairing one's ability to work at office or at home.

People with Alzheimer's may:

- Repeat statements and questions over and over
- Forget conversations, appointments or events, and not remember them later
- Routinely misplace possessions, often putting them in illogical locations
- Get lost in familiar places
- Eventually forget the names of family members and everyday objects
- Have trouble finding the right words to identify objects, express thoughts or take part in conversations

❖ Thinking and reasoning

Alzheimer's disease creates difficulties in concentrating and concentrating, especially on seemingly insignificant ideas.

Doing many things is very difficult, and it can be a challenge to manage finances, balance check books, and pay bills on time. Finally, a person with Alzheimer's may not be able to see and deal with numbers.

❖ Making judgments and decisions

Alzheimer's disease results in decreased ability to make sound decisions and judgment in everyday situations. For example, a person may make bad or immoral decisions in public relations or wear clothes that are not appropriate for the weather. It can be very difficult to respond effectively to everyday problems, such as burning food on the stove or unexpected driving conditions.

❖ Planning and performing familiar tasks

Tired activities that require a series of steps, such as preparing and cooking food or playing a favorite game, become a struggle as the disease progresses. Finally, people with advanced Alzheimer's often forget to do basic activities like dressing and bathing.

❖ Changes in personality and behavior

Alternative brain changes in Alzheimer's can affect mood and behavior. Problems may include the following:

- Depression
- Apathy
- Social withdrawal
- Mood swings
- Distrust in others
- Irritability and aggressiveness
- Changes in sleeping habits

- Wandering
- Loss of inhibitions
- Delusions, such as believing something has been stolen

❖ Preserved skills

Many important skills are stored for a long time or the symptoms get worse. Preserved skills may include reading or listening to books, storytelling and memory, singing, listening to music, dancing, drawing, or doing crafts.

2.3 STAGES:

There are five categories associated with Alzheimer's disease: Alzheimer's preclinical disease, moderate cognitive impairment due to Alzheimer's disease, mild dementia due to Alzheimer's disease, moderate dementia due to Alzheimer's disease and severe dementia due to Alzheimer's disease.

1) Preclinical Alzheimer's disease

Alzheimer's disease begins long before any symptoms appear. This stage is called preclinical Alzheimer's disease, and usually appears only in research settings. You will not notice signs in this section, and those around you will not notice them. This stage of Alzheimer's disease can last for years, perhaps decades. While you may not notice any changes, new photography technology can now detect deposits in a protein called amyloid-beta that is a symptom of Alzheimer's disease. The ability to identify these early contributions may be very important in clinical trials and in the future as new treatments for Alzheimer's disease are developed.

2) Moderate cognitive impairment (MCI) due to Alzheimer's disease

People with mild mental retardation have minor changes in their memory and thinking ability. These changes are not important enough to affect work or relationships at the moment. People with MCI may have fond memories when it comes to information that is often easily remembered, such as interviews, recent events or appointments. People with MCI may also have difficulty judging the amount of time needed at work, or they may have difficulty correctly judging the number or sequence of steps needed to complete a task. The ability to make sound decisions can be difficult for people with MCI.

3) Minor dementia due to Alzheimer's disease

Alzheimer's disease is most commonly diagnosed in a mild form of dementia, in which it becomes clear to family and doctors that a person has a serious memory and thinking problem that affects daily functioning.

In the intermediate stage of dementia, people may have:

- **Loss of memory of recent events.** People may have a very difficult time remembering newly learned information and asking the same question over and over again.
- **Difficulty in solving problems,** difficult tasks and good judgment. Planning a family event or evaluating a test book can be difficult. Many people face judicial failure, as when making financial decisions.
- **Personality changes.** People may feel humiliated or withdrawn - especially in challenging social situations - or show unusual irritability or anger. Decreased motivation to complete tasks is also common.
- **Difficulty in planning and expressing ideas.** Finding the right words to describe things or expressing ideas clearly becomes increasingly difficult.

- **Lost or misplaced.** Individuals have a growing problem finding their way, even in places they are familiar with. It is also common to lose or misplace things, including important things.

4) Moderate dementia due to Alzheimer's disease

During the intermediate phase of Alzheimer's disease, people grow confused and forget and begin to need more help with daily activities and self-care.

People with moderate to severe Alzheimer's disease may:

- **Demonstrate poor judgment and deep confusion.** People lose track of where they are, the day of the week or the season. They may confuse family members or close friends or confuse strangers as a family. They may be wandering about, perhaps looking for surroundings that they feel are more familiar. These difficulties make it unsafe to leave those in the middle stage of dementia alone.
- **Even greater memory loss.** People may forget details about their personal history, such as their address or phone number, or where they go to school. They repeat their favorite stories or create stories to fill in the gaps in memory.
- **You need help with certain daily activities.** Help may be needed in choosing the right clothes for the occasion or the weather as well as bathing, grooming, using the toilet, and other personal matters. Some people sometimes lose control of their bladder or intestinal movements.
- **Make significant changes in personality and behavior.** It is not uncommon for people with moderate dementia to develop baseless suspicions - for example, to make sure that friends, family or trained caregivers steal from them or that a marriage partner is dating. Some may see or hear things that do not really exist.
People often grow up restless and restless, especially during the day. Some people may experience a severe physical breakdown.

5) Severe dementia due to Alzheimer's disease

In the later stage of the disease, called severe dementia due to Alzheimer's disease, brain function continues to decline, and the disease has a profound effect on movement and physical strength.

In the latest phase, the worst-case scenario for Alzheimer's disease, people in general:

- **Loss of ability to communicate consistently**
A person can no longer communicate or speak logically, though he may sometimes say words or phrases.
- **Need daily help with personal care**
This includes complete assistance with food, clothing, toilet use and all other daily chores.
- **Feeling weak**
The person may not be able to walk without assistance, and may not be able to sit or raise their head without support. Muscles may tighten and the brain may appear abnormal. Eventually, the person loses the ability to swallow and control the function of the bladder and intestines

2.4 DIAGNOSIS:

An important part of diagnosing Alzheimer's disease involves being able to describe your symptoms, as well as the opinion of a close family member or friend about symptoms and their impact on daily life. Additionally, a diagnosis of Alzheimer's disease is based on a controlled examination by your doctor to test your memory and thinking skills.

Tests: A diagnostic work-up would likely include the following tests:

1) Physical and neurological exam

Your doctor will perform a physical exam and likely assess overall neurological health by testing the following:

- Reflexes
- Muscle tone and strength
- Ability to get up from a chair and walk across the room
- Sense of sight and hearing
- Coordination
- Balance

2) Lab tests

A blood test can help your doctor diagnose other causes of memory loss and confusion, such as thyroid disorders or vitamin deficiencies.

❖ Mental status and neuropsychological testing

❖ Brain imaging

- Magnetic resonance imaging (MRI)
- Computerized tomography (CT)
- PET scanning
- Amyloid PET imaging
- Tau PET imaging
- Future diagnostic tests

Researchers are working to develop tests that can measure the biological symptoms of brain disease processes. These tests, which include blood tests, may improve diagnostic accuracy and enable early diagnosis before symptoms start. Plasma A β blood tests are currently available and have recently received a certificate in the U.S. by Centers for Medicare & Medicaid Services to allow for market distribution. Genetic testing is usually not recommended for tests for Alzheimer's disease. The exception is people with a family history of Alzheimer's disease that has just started. Consultation with a genetic counselor to discuss the risks and benefits of genetic testing is recommended prior to any test.

2.5 CAUSES:

Alzheimer's disease is thought to be caused by the abnormal build-up of proteins in and around brain cells.

Increased risk: Although it's still unknown what triggers Alzheimer's disease, several factors are known to increase your risk of developing the condition.

- Age
- Family history
- Down's syndrome
- Head injuries
- Cardiovascular disease.
- smoking
- obesity
- diabetes
- high blood pressure
- high cholesterol

2.6 TREATMENTS:

There is no known cure for Alzheimer's disease. It is not possible to reverse the death of brain cells.

Treatment can however, relieve its symptoms and improve quality of life for the person and their family and caregivers.

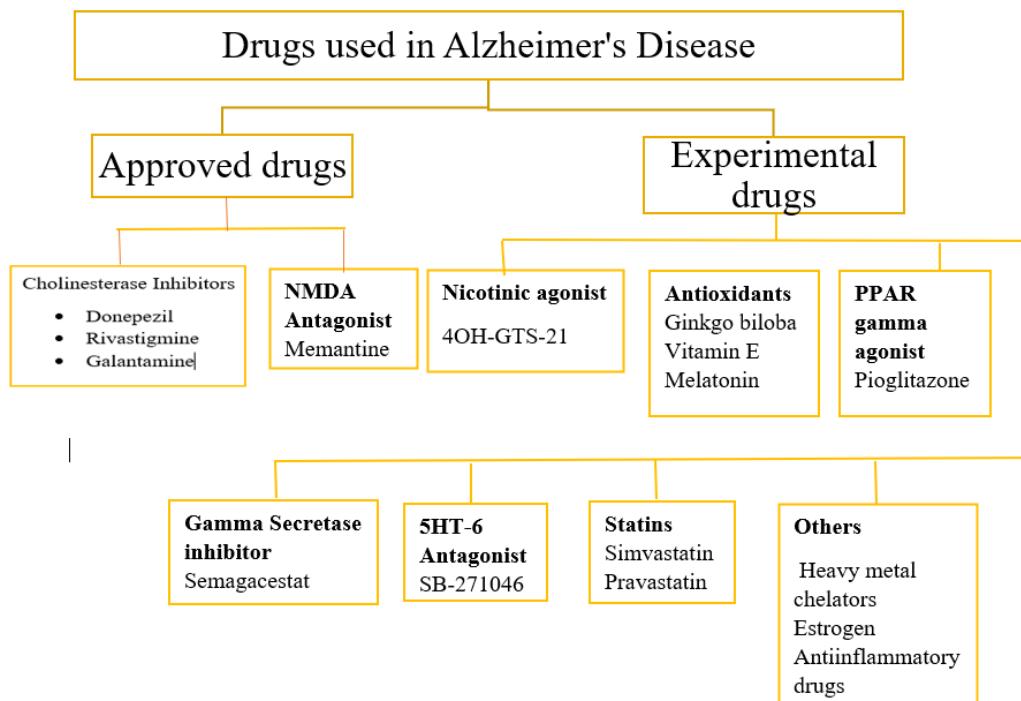


Figure 2: Classification of Drugs used in Alzheimer's Disease

The drugs in current use for treatment of AD are given orally. Therefore, the bioavailability of drug as well as their transport to brain crossing BBB is limited and thus may not achieve effective concentration to treat AD effectively. Delivering drugs through intranasal route to achieve desired concentration at the site of action rapidly and for prolonged period could be an effective strategy for managing AD. Today, the most common ChEIs used to treat cognitive symptoms in mild to moderate AD are rivastigmine, galantamine, and donepezil.

Donepezil has several side effects after oral administration such as diarrhoea, vomiting, insomnia, fatigue, muscle cramps, nausea, and anorexia due to increased gastric secretion caused by enhanced cholinergic activity through the gastrointestinal tract. The drug is administered by orally disintegrating tablets (5 or 10 mg) and formulations for sustained release (23 mg), all of which are intended for the oral route. To avoid these side effects, we previously proposed lipid-based formulations (LBFs) composed of monoolein, oleic acid, and water for the sustained release of donepezil.

Thus, in this study attempt has been made to deliver Donepezil HCl to brain through nasal route by formulating Donepezil HCl loaded NLC.

3. NANOSTRUCTURED LIPID CARRIERS:

Nanostructured lipid nanoparticles are modified version and next generation solid lipid nanoparticles. These drug delivery systems were introduced in order to overcome the possible difficulties of SLN's. NLC's have the major advantage over SLN's such as increased loading capacity, stability during storage, also prevents drug expulsion during long term storage and less water content⁵⁶.

3.1 COMPOSITION OF NANOSTRUCTURED LIPID CARRIER'S

NLCs are a binary mixture of solid lipids (fats) and liquid lipids (oils) at room temperature. Concentrations of solid lipids and liquid lipids in formulations typically range between 50:50 up to 90:10. The surfactants present in the composition are about 15% (w/v). Surfactants have a major role in the stability of NLC as well as in the preparation of a stable formulation by reducing surface tension between the lipid phase and the aqueous phase^{56,57}. The drug is loaded into the liquid lipid and liquid lipids are then loaded onto solid lipids and thus dual protection is provided in the form of central structure of the external degradation factor. Solid fat and liquid fat selection set an important role in the stability of NLCs for long-term use. All ingredients are used for manufacturing of nanostructured lipid carriers and subject to regulatory authorities such as GRAS (Generally recognized as safe) list⁵⁸.

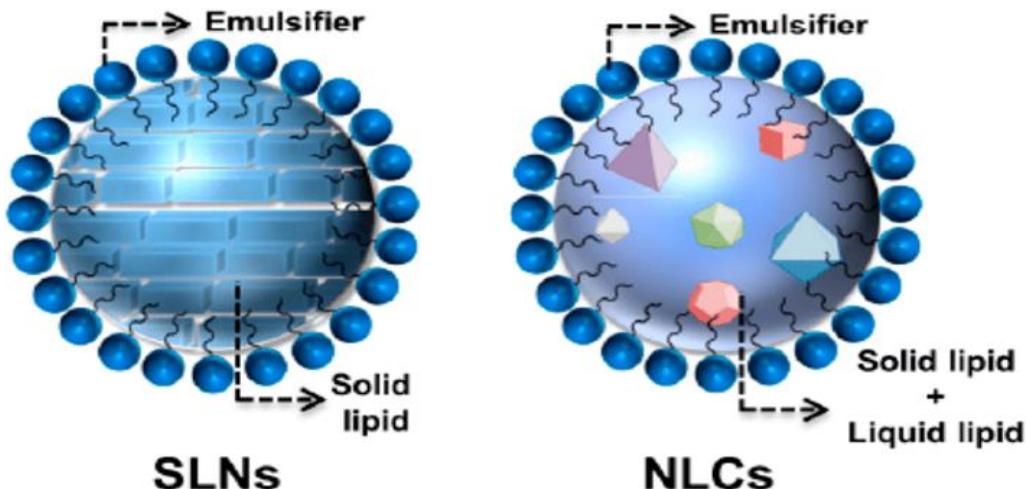


Figure 3: SLN & NLC Structure

3.2 ADVANTAGES OF NLC'S:

- 1) Physical stability is improved as compared to SLN.
- 2) Dispersion in aqueous phase is increased and hence observed high entrapment efficiency of hydrophilic drugs and lipophilic drugs.
- 3) Particle sizes are controlled and the NLC showed better penetration ability.
- 4) Use of organic solvents in production of NLC is avoided as in the case of preparation of other nanoparticulate systems.
- 5) NLC's are prepared with lipids which are biodegradable, well tolerated and easily thrown out of the body.
- 6) Depending on the different manufacturing methods available and different concentrations of obtained different types of lipids and NLCs⁵⁹.

3.3 TYPES OF NLC:

Based on the variation in the composition of lipid and oil mixtures in addition to the various fabrication methods, NLC can be categorized into three types:

- a. The imperfect type
- b. The amorphous type
- c. Multiple oil-in-solid fat-in-water (O/F/W) type

I. Imperfect type NLC (TYPE I)

Imperfect type NLC involves mixing of spatially different lipids such as glycerides, composed of a number of fatty acids, which introduce imperfections in the crystal order. The drug loading can be further increased by increasing imperfections by using a mixture of various glycerides, varying saturation and length of carbon chains^{58,60}.

II. Amorphous type NLC (TYPE II)

In the amorphous type, the unstable amorphous matrix is formed by mixing special lipids such as hydroxyoctacosanyl hydroxy stearate or iso-propyl myristate with a strong lipid.

As a result the NLC exists in an amorphous state rather than an ordered state which prevents the drug expulsion resulting from β -modification during storage⁶¹.

III. Multiple O/F/W type NLC (TYPE III)

Multiple O/F/W type NLC contains numerous nanosized liquid oil compartments distributed in the solid matrix. Drug resistance is high in these nanosized compounds which leads to increased drug loading. In addition, the release is extended because the rooms are surrounded by a solid lipid matrix⁶².

3.4 METHODS OF FABRICATION:

Following are the various production techniques explained briefly:

34.1 HIGH PRESSURE HOMOGENIZATION (HPH)

a. Hot homogenization:

In hot HPH, the drug - lipid soluble is applied to an aqueous stabilizer solution stored at the same temperature under high shear leading to pre-emulsion heating. The pre-emulsion is then processed in a HPH maintaining the temperature above lipid melting point. The nano-emulsion formed recrystallizes to form NLC when cooled at room temperature⁶³.

b. Cold homogenization:

In cold HPH, drug-lipid soluble is stabilized and digested rapidly under liquid nitrogen leading to microparticles. The formed microparticles are dispersed in a cold surfactant solution before subjecting it to HPH at or below room temperature. Dispersion rate is usually compromised by the presence of small particles. Usually, 3-5 homogenization cycles at 500-1500 bar are sufficient for nano-emulsion formulation⁶⁴.

3.4.2 EMULSIFICATION- ULTRASONICATION METHOD

In this method drug, solid lipid, liquid lipid is mixed and melted 5-10°C temperature above melting point of solid lipid. The surfactant is dissolved in distilled water and heated at same temperature. Aqueous phase is added to lipid phase then this pre-emulsion is homogenized and ultrasonicated for specific time and volume is made by distilled water⁶⁵.

3.4.3 SOLVENT DIFFUSION METHOD:

This technique utilizes water miscible organic solvents like methanol, ethanol & acetone. Drug and lipids are added in single or mixture of organic phases then added to aqueous phase⁶⁶.

3.4.4 SOLVENT EMULSIFICATION EVAPORATION METHOD:

This method uses water immiscible organic solvents like chloroform, cyclohexane to dissolve drug and lipid. The lipid solution is then emulsified in an aqueous surfactant solution under continuous stirring. The organic is then evaporated, resulting in lipid precipitation⁵⁶.

3.4.5 FILM-ULTRASONICATION METHOD:

This method adopted from preparation method of vesicular drug delivery system. Lipids and drug are dissolved in an organic solvent in ethanol & aqueous phase containing surfactant. By creating vacuum using rotary evaporator there is formation of thin film⁶⁷.

3.4.6 MICROEMULSION METHOD:

Melted lipid is added in preheated oil and drug is dissolved in mixture is added to Aqueous phase containing surfactant at elevated temperature. This warm microemulsion is added to cold water under stirring⁶⁸.

3.4.7 PHASE INVERSION TECHNIQUE:

A mix of lipid, drug, water and surfactant is formed under stirring and exposed to three heating and cooling cycles (85-60-85°C) after which the stock is induced by diluting with cold water (0°C) resulting in NLC formulation⁶⁹.

In this study *Nigella sativa* oil has been used as liquid lipid which having antioxidant activity and thus may give synergistic action with donepezil HCL for efficient management of AD.

4. NIGELLA SATIVA L.

Family-Ranunculaceae

Common Names: Black seed, Cumin seeds, Black cumin

Origin- The plant grows in Eastern Europe, the Middle East, and Western Asia

Constituents- *N. sativa* seed contains more than 30% fixed oil and 0.4 to 0.45% volatile oil.

The fixed oil (32-40 %) contains: unsaturated fatty acids which includes: arachidonic, eicosadienoic, linoleic, linolenic, oleic, almitoleic, palmitic, stearic and myristic acid as well as beta-sitosterol, cycloecalenol, cycloartenol, sterol esters and sterol glucosides.

The volatile oil (0.4-0.45 %) contains saturated fatty acids which includes: nigellone that is the only component of the carbonyl fraction of the oil, Thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citronellol, p-cymene volatile oil of the seed also contains: p-cymene, carvacrol, t-anethole, 4-terpineol and longifolene.

Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine. The nutritional compositions of *N. sativa* are vitamins, carbohydrates, mineral elements, fats and proteins that include eight or nine essential amino acids.

Functional benefits & description

Anti-Alzheimer action; liquid lipid; Antioxidant activity

N. sativa seeds and oil have been extensively used in treatment of different diseases throughout the world. N. sativa is included in the list of natural drugs in different medicines including Tibb-e-Nabavi (The medicine of Prophet Mohammad), Unani Tebb, and Indian traditional medicine. In traditional remedy, N. sativa seeds are commonly used as a spice and carminative. In addition, several properties such as liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, and anti-bacterial have been attributed to this plant. N. sativa has been investigated for its biological effects and therapeutic potential and shown to have broad spectrum of activities including antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, and antioxidant properties.

LITERATURE SURVEY



1] Saleem et al (2020) developed almotriptan loaded nasal nanostructured lipid carriers for the treatment of migraine. The objective of this study was to develop novel transnasal NLC containing Almotriptan for treatment of Migraine. Being flexible and lipophilic in nature, nanostructured lipid carriers (NLCs) represent a promising tool in delivering therapeutic substances to the brain. This investigation is meant to explore the capability of mucoadhesive chitosan coated NLCs to efficiently deliver ALM to the brain through the nasal route as a non-invasive alternative route for targeting the central nervous system (CNS). D-optimal design was adopted and thirteen different formulae were prepared using hot homogenization and ultrasonication technique; where an accurate amount of the almotriptan was added to the molten lipid mixture followed by the addition of the heated aqueous phase under stirring, then the mixture was subjected to homogenization and ultrasonication. The prepared systems were then assessed for their particle size, PDI (polydispersity index), zeta potential (ZP), and entrapment efficiency (EE). The optimized selected formula; F1; composed of 50/50 Compritol/Labrafil and a co-mixture of 2:1 tween 80: Lauroglycol all coated in chitosan, showed a PS of 255 nm, PDI 0.27, ZP 34.1 mV, and 80% EE. A bi-phasic in-vitro drug release pattern was obtained, and enhanced mucoadhesive property as well as ex-vivo permeability through sheep nasal mucosa were attained. The In-vivo studies performed on rabbits showed significantly higher Cmax values in plasma of the optimized ALM-NLC (1.54 mg/mL) compared to those of IN ALM solution (0.25 mg/mL) and ALM oral tablet market product (0.58 mg/mL). Brain Cmax were found to be 3.64 mg/mL, 0.5 mg/mL and 0.48 mg/mL for IN ALM-NLC, oral ALM market product and, IN ALM solution, respectively⁷⁰.

2] Devkar et al (2014) developed Surface engineered nanostructured lipid carriers for efficient nose to brain delivery of ondansetron HCl using Delonix regia gum as a natural mucoadhesive polymer. The objective of this investigation was to fabricate ondansetron hydrochloride [OND] loaded mucoadhesive nanostructured lipid carriers [NLCs] for efficient delivery to brain through nasal route. NLCs were prepared by high pressure homogenization [HPH] technique using glycerol monostearate [GMS]; as solid lipid, Capryol 90; as liquid lipid, soya lecithin; as surfactant and poloxamer 188; as cosurfactant. In the fabrication of NLCs, Delonix regia gum [DRG], isolated from seeds of *D. regia* belonging to family fabiaceae was used as a mucoadhesive polymer. The NLCs were evaluated for particle size, morphology, drug-entrapment efficiency [%EE], mucoadhesive strength, in vitro drug release, histological examination, ex vivo permeation study, in vivo biodistribution and pharmacokinetic studies in the brain/blood following intravenous [IV] and intranasal [IN] administration. Particle size, PDI, Zeta potential was observed in the range of 92.28–135 nm, 0.32–0.46, and –11.5 to –36.2 respectively. Prepared NLCs achieved thermodynamic stability, control release pattern with minor histopathological changes in sheep nasal mucosa. The significantly [$P < 0.05$] higher values for selected batch, when administered by IN route showed higher drug targeting efficiency [506%] and direct transport percentage [97.14%] which confirms the development of promising OND-loaded NLC for efficient nose-to-brain delivery⁷¹.

3] Abourehab et al (2021) developed Sesame Oil-Based Nanostructured Lipid Carriers of Nicergoline, Intranasal Delivery System for Brain Targeting of Synergistic Cerebrovascular Protection. This work aimed to formulate and optimize sesame oil-based NIC-nanostructured lipid carriers (NIC–NLCs) for intranasal (IN) delivery with expected synergistic and augmented neuroprotective properties. The NIC–NLC were prepared using sesame oil as a liquid lipid. A three-level, three-factor Box–Behnken design was applied to statistically optimize the effect of sesame oil (%) of the total lipid, surfactant concentration, and sonication time on particle size, zeta potential, and entrapment efficacy as responses. Solid-state characterization, release profile, and ex vivo nasal permeation in comparison to NIC solution (NIC– SOL) was studied. In vivo bioavailability from optimized NIC–NLC and NIC–SOL following IN and IV administration was evaluated and compared. The optimized NIC–NLC formula showed an average particle size of 111.18 nm, zeta potential of -15.4 mV, 95.11% entrapment efficacy, and 4.6% loading capacity. The NIC–NLC formula showed a biphasic, extended-release profile (72% after 48 h). Permeation of the NIC–NLC formula showed a 2.3 enhancement ratio. Bioavailability studies showed a 1.67- and 4.57-fold increase in plasma and brain following IN administration. The results also indicated efficient direct nose-to-brain targeting properties with the brain-targeting efficiency (BTE%) and direct transport percentage of 187.3% and 56.6%, respectively, after IN administration⁷².

4] Cunha et al (2020) developed Double Optimization of Rivastigmine-Loaded Nanostructured Lipid Carriers (NLC) for Nose-to-Brain Delivery Using Quality by Design (QbD) Approach Formulation Variables and Instrumental Parameters. Rivastigmine is a drug commonly used in the management of Alzheimer's disease which have bioavailability problems. To overcome this, the use of nanosystems, such as nanostructured lipid carriers (NLC), administered through alternative routes seems promising. In this work, authors carried out performed a double optimization of a rivastigmine-loaded NLC formulation for direct drug delivery from the nose to the brain using the quality by design (QbD) approach, whereby the quality target product profile (Q TPP) was the requisite for nose to brain delivery. The experiments started with the optimization of the formulation variables (or critical material attributes—CMAs) using a central composite design. The rivastigmine-loaded NLC formulations with the best critical quality attributes (CQAs) of particle size, polydispersity index (PDI), zeta potential (ZP), and encapsulation efficiency (EE) were selected for the second optimization, which was related to the production methods (ultrasound technique and high-pressure homogenization). The most suitable instrumental parameters for the production of NLC were analyzed through a Box–Behnken design, with the same CQAs being evaluated for the first optimization. For the second part of the optimization studies, were selected two rivastigmine-loaded NLC formulation are selected one produced by ultrasound technique and the other by the high-pressure homogenization (HPH) method. Afterwards, the pH and osmolarity of these formulations were adjusted to the physiological nasal mucosa values and in vitro drug release studies were performed. The results of the first part of the optimization showed that the most adequate ratios of lipids and surfactants were 7.49:1.94 and 4.5:0.5 (% , w/w), respectively. From the second part of the optimization, the results for the particle size, PDI, ZP, and EE of the rivastigmine-loaded NLC formulations

produced by ultrasound technique and HPH method were, respectively, 114.0 ± 1.9 nm and 109.0 ± 0.9 nm; 0.221 ± 0.003 and 0.196 ± 0.007 ; -30.6 ± 0.3 mV and -30.5 ± 0.3 mV; $97.0 \pm 0.5\%$ and $97.2 \pm 0.3\%$. Herein, the HPH was selected as the most suitable production method, although the ultrasound technique has also shown effectiveness. In addition, no significant changes in CQAs were observed after 90 days of storage of the formulations at different temperatures. In vitro studies showed that the release of rivastigmine followed a non-Ficksian mechanism, with an initial fast drug release followed by a prolonged release over 48 h. This study has optimized a rivastigmine-loaded NLC formulation produced by the HPH method for nose-to-brain delivery of rivastigmine. The next step is for in vitro and in vivo experiments to demonstrate preclinical efficacy and safety. QbD was demonstrated to be a useful approach for the optimization of NLC formulations for which specific physicochemical requisites can be identified⁷³.

5] Madane et al (2014) developed Curcumin-loaded nanostructured lipid carriers (NLCs) for nasal administration for cancer treatment. Cancer nanotherapeutics is beginning to overwhelm the global research and viewed to be the revolutionary treatment regime in the medical field. This investigation describes the development of a stable nanostructured lipid carrier (NLC) system as a carrier for curcumin (CRM). The CRM-loaded NLC developed as a particle with the size of 146.8 nm, a polydispersity index of 0.18, an entrapment efficiency (EE) of 90.86%, and the zeta potential (ZP) of 21.4 mV. Increased cytotoxicity of CRM-NLC than that of CRM to astrocytoma-glioblastoma cell line (U373MG) was observed. Results of biodistribution studies showed higher drug concentration in brain after intranasal administration of NLCs than in drug solution. The results suggest that CRM-NLC is a promising drug delivery system for brain cancer therapy⁷⁴.

6] Jazuli et al (2019) developed Nanostructured Lipid Carriers of Lurasidone Hydrochloride Using Box-Behnken Design for Brain Targeting. Intranasal nanostructured lipid carrier (NLC) of lurasidone hydrochloride (LRD) for brain delivery was prepared by the solvent evaporation method. The effects of independent variables, X1-lipid concentration, X-2 surfactant, and X-3 sonication times on dependent variables, Y1-particle size, Y-2 polydispersity index, and Y-3% entrapment efficiency were determined using Box-Behnken design. Optimized LRD-NLC was selected from the Box-Behnken design and evaluated for their morphological, physiological, nasal diffusion, and in vivo distribution in the brain after intranasal administration. Particle size, polydispersity index, and entrapment efficiency of optimized LRD-NLC were found to be 207.4 ± 1.5 nm, 0.392 ± 0.15 , and $92.12 \pm 1.0\%$, respectively. Transmission electron microscopy and scanning electron microscopy was used to determine the particle size and surface morphology of LRD-NLC. The prepared LRD-NLC follows biphasic in vitro drug release. Prepared NLC showed a 2-fold increase in LRD concentration in the brain when compared with the drug solution following intranasal administration. Results showed that intranasal route can be a good and efficient approach for delivering the drug directly to the brain and enhancing the drug efficacy in the brain for the management of schizophrenia and is a good alternative to oral drug delivery⁷⁵.

7] Du et al (2019) studied Development of nose-to-brain delivery of ketoconazole by nanostructured lipid carriers against cryptococcal meningoencephalitis in mice. Cryptococcus neoformans-mediated meningoencephalitis is a critical infectious disorder of the human central nervous system. However, efficient treatment for the disease is limited due to the poor penetration across the blood brain barrier (BBB). Thus, authors have developed a nose-to-brain drug delivery system utilizing nanostructured lipid carriers (NLCs). We demonstrated that fluorescent-dye-loaded NLCs efficiently uptake into the cytoplasm of encapsulated C. neoformans cells. In comparison with current antifungal drugs, the ketoconazole (keto)-NLCs show significantly increased antifungal activity against C. neoformans in vivo under various growth conditions. The NLCs show enhanced tissue colonization properties. Importantly, using animal imaging analyses, NLCs are able to enter brain tissues via the olfactory bulb region by intranasal administration, bypassing the BBB. In addition, NLCs maintain prolonged residence in tissues. In mouse brain tissue, keto-NLCs showed significantly enhanced antifungal activity when administered intranasally, drastically dampening the C. neoformans burden. Taken together, NLCs not only improve the ketoconazole penetration efficiency against capsulated C. neoformans cells, but also boost the efficacy of antifungal drugs. Most importantly, keto-NLCs significantly contribute to the treatment of cryptococcal meningoencephalitis in mice by bypassing the BBB via the olfactory system⁷⁶.

8] Costa et al (2021) optimized diazepam-loaded nanostructured lipid carriers (NLC) for nose-to-brain delivery using the quality by design (QbD) approach. The aim of this work was to optimize two diazepam-loaded NLC formulations for nose-to-brain delivery, one with positive surface charge and one with negative surface charge. The quality by design (QbD) approach was used to design the experiments, where the quality target product profile (QTPP), the risk assessment and the critical quality attributes (CQAs) were defined to ensure safety, efficacy and quality of the final formulations. The results of the optimization of the CMAs showed that the ratios of lipids and emulsifiers more adequate were 6.7:2.9 and 4.2:0.3 (% w/w), respectively. Regarding the CPPs, HPH was considered the most suitable production method, resulting in an optimized diazepam-loaded NLC formulation (F1C15) with negative surface charge, showing particle size of 69.59 ± 0.22 nm, polydispersity index (PDI) of 0.19 ± 0.00 , zeta potential (ZP) of -23.50 ± 0.24 mV and encapsulation efficiency (EE) of 96.60 ± 0.03 %. The optimized diazepam-loaded NLC formulation (F2A8) with positive surface charge had particle size of 124.40 ± 0.84 nm, PDI of 0.17 ± 0.01 , ZP of 32.60 ± 1.13 mV and EE of 95.76 ± 0.24 %. In addition, the incorporation of diazepam in NLC resulted in a sustained release of the drug. No significant changes in particle size, PDI, ZP and EE were observed for the formulation F1C15, after 3 months of storage, whereas for formulation F2A8, particle size increased significantly. Biocompatibility studies showed that the formulation F2A8 was more cytotoxic than the formulation F1C15. Thereby, we conclude that the formulation F1C15 is more suitable for targeting the brain, when compared with the formulation F2A8. From the results of these studies, it can be confirmed that the QbD approach is an adequate and central tool to optimize NLC formulations⁷⁷.

9] **Alam et al (2012)** studied Intranasal administration of nanostructured lipid carriers containing CNS acting drug. The study was aimed to investigate and compare the efficacy of duloxetine (DLX) loaded nanostructured lipid carriers (NLC) with DLX solution pharmacodynamically following intranasal administration. The study was further conducted to estimate DLX concentration in brain and blood. DLX was administered to Wistar rats either intranasally or orally in solution form (DLX solution) or encapsulated in NLC (DLX NLC). Formulation was evaluated in-vivo for pharmacodynamic studies for depression by forced swimming test and locomotor activity test. Intranasal DLX NLC treatment exhibited improved behavioural analysis results (swimming, climbing, and immobility) than the DLX solution after 24 h of study. Furthermore, DLX NLC significantly increased the total swimming and climbing time when compared with control and significantly reduced the immobility period. The intranasal DLX NLC demonstrated improved locomotor activity when compared with DLX solution. Amount of DLX was quantified in blood and brain after the forced swimming test. The intranasal DLX NLC demonstrated higher concentration in brain compared with DLX solution. Thus, intranasal DLX NLC was found to be a promising formulation for the treatment of depression⁷⁸.

10] **Sivadsu et al (2020)** developed ziprasidone hydrochloride loaded nanostructured lipid carriers (ncls) for intranasal delivery. The study was an attempt to systemically deliver the most desirable schizophrenia drug, ziprasidone hydrochloride (ZRS) via the intranasal route using nanostructured lipid carrier (NLC) approach. The desired ZRS loaded NLCs were developed using central composite statistical design and the developed formulation was monitored for improving ZRS bioavailability and their brain targeting efficacy. Pharmacokinetic studies revealed a 10-fold increase (ZRS blood-brain ratio) for NLCs administered through nasal route (in comparison to intravenous route). Similarly, the concentration of ZRS (in the brain) delivered via nasal route exhibits 4-fold increment at all-time points. Therefore, the obtained results suggest a potential nose to brain transport of loaded ZRS by effective bypassing of the Blood-Brain Barrier (BBB)⁷⁹.



AIM:

To prepare, evaluate & optimize Donepezil HCl & Nigella sativa oil loaded Nanostructured Lipid Carrier for Intranasal delivery to target brain & obtain of synergistic cerebrovascular protection and anti-oxidant agent.

OBJECTIVE:

- To formulate & evaluate nanostructured lipid carrier for efficient nose to brain delivery of DNZ HCL by obtaining rapid onset and prolonged duration of action
- Improve bioavailability by avoiding hepatic first pass metabolism.
- Increase concentration of drug in brain thus improves clinical performance of drug.
- Reduce the oxidative stress by adding antioxidants i.e. *Nigella sativa oil*
- To optimize the formulation on in-vivo parameters
- To carryout formulation study in blood & brain using suitable rats.

PLAN OF WORK:

- ✓ Literature survey
- ✓ Procurement of drug and polymer
- ✓ Pre-formulation studies
 - Melting point
 - Infrared spectroscopy
 - DSC
 - DPPH in-vitro assay for anti-oxidant activity.
- ✓ Formulation of NLC
- ✓ Characterization of NLC
 - Particle size, PDI, Zeta potential
 - Entrapment Efficiency
 - Drug Loading capacity
 - Drug content
- ✓ In-vitro Characterization
- ✓ Biodistribution study in blood & brain
- ✓ Stability studies

NEED OF WORK



NEED OF WORK:

BBB is major obstacle in treating CNS disorder after oral administration. Alternate route to treat neurological disorders is bypassing the BBB through intranasal delivery.

The prevailing cause for Anti-Alzheimer disease is due to plaque formation, death of brain cells and oxidative stress. This results into lack of performance in efficiency in day-to-day activity of the patients. Along with administration of currently available anti-Alzheimer's agent are of anti-oxidant activity can be useful.

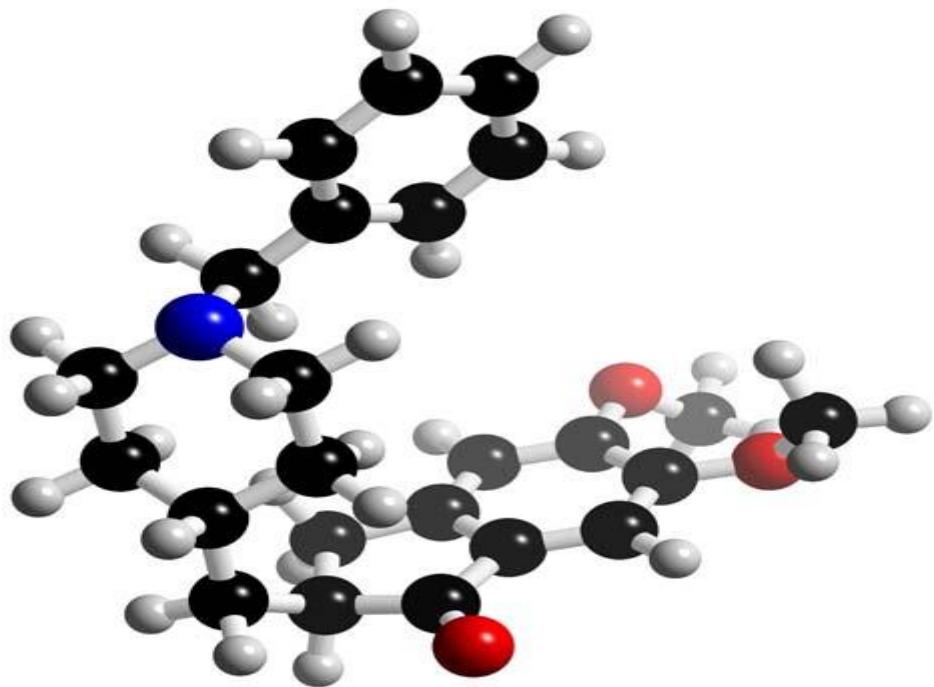
The oxidative stress can be managed by incorporating anti-oxidant substance in the formulation.

The present research work is focused on evaluating the anti-oxidant property of Nigella sativa oil and further attempt has been made to incorporate Nigella sativa oil in the nanostructured lipid carrier along with FDA approved Anti-Alzheimer drug Donepezil hydrochloride to obtain synergistic action.

To overcome these problems, intranasal approach is used to deliver the drug from nasal cavity to brain to target the CNS.

Though oral and parenteral techniques are used for decades it shows many limitations such as poor drug absorption, limited brain uptake, peripheral effects, low permeability, and inconvenient administration etc.

**DRUG
AND
EXCIPIENT
PROFILE**



DRUG AND EXCIPIENT PROFILE:**DONEPEZIL HCL (DNZ):**

Chemical structure	
Molecular formula	C ₂₄ H ₃₀ ClNO ₃
IUPAC name	2-[(1-benzylpiperidin-4-yl) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one; hydrochloride
Appearance	White amorphous powder
Category	Anti-Alzheimer
Molecular weight	416.0g/mol
Protein binding	96%
Melting point	223-227°C
Elimination half life	Upto 70 hours
Solubility	Water:31mg/ml Freely soluble in methyl alcohol, DMSO, ethanol Slightly soluble in DMF Insoluble in ether
Log P	4.7
Log pKa	8.9
BCS class	Class 1 (High permeability & high solubility)
Mechanism of Action	Donepezil binds and reversibly inactivates the cholinesterase's, thus inhibiting hydrolysis of acetylcholine. This increases acetylcholine concentrations at cholinergic synapses. The precise mechanism of action of donepezil in patients with Alzheimer's disease is not fully understood. Certainly, Alzheimer's disease involves a substantial loss of the elements of the cholinergic system and it is generally accepted that the symptoms of Alzheimer's disease are related to this cholinergic deficit, particularly in the cerebral cortex and other areas of the brain. In addition to its actions as an acetylcholinesterase inhibitor, donepezil has been found to act as a potent agonist of the σ1 receptor (Ki = 14.6 nm), and has been shown to produce specific anti-amnestic effects in animals mainly via this action.

	<p>Some noncholinergic mechanisms have also been proposed. Donepezil upregulates the nicotinic receptors in the cortical neurons, adding to neuroprotective property. It inhibits voltage-activated sodium currents reversibly and delays rectifier potassium currents and fast transient potassium currents, although this action is unlikely to contribute to clinical effects.</p>
Adverse effects	<p>In clinical trials the most common adverse events leading to discontinuation were nausea, diarrhoea, and vomiting. Other side effects included difficulty sleeping, muscle cramps and loss of appetite. Most side effects were observed in patients taking the 23 mg dose compared to 10 mg or lower doses. Side effects are mild and transient in most patients, lasting up to three weeks and usually improved even with continued use.</p> <p>Donepezil, like other cholinesterase inhibitors, can cause nightmares due to enhanced activation of the visual association cortex during REM sleep. Dosing donepezil in the morning can reduce the frequency of nightmares.</p>
Precaution	<p>Donepezil should be used with caution in people with heart disease, cardiac conduction disturbances, chronic obstructive pulmonary disease, asthma, severe cardiac arrhythmia and sick sinus syndrome.</p> <p>People with peptic ulcer disease or taking NSAIDs should use with caution because increased risk of gastrointestinal bleeding was noted. Slow heart beat and fainting in people with heart problems were also seen. These symptoms may appear more frequent when initiating treatment or increasing the donepezil dose. Although occurrence of seizures is rare, people who have a predisposition to seizures should be treated with caution.</p> <p>If daily donepezil has suspended for 7 days or less, restarting at the same dose is recommended, while if the suspension lasts longer than 7 days, retitrate from 5 mg daily is suggested.</p>

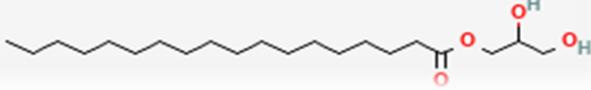
PROFILE OF EXCIPIENTS

The selection of excipients depends upon the nature of drug & desired properties to be achieved in that final dosage form. The detailed profiles of excipients used in present study are given as:

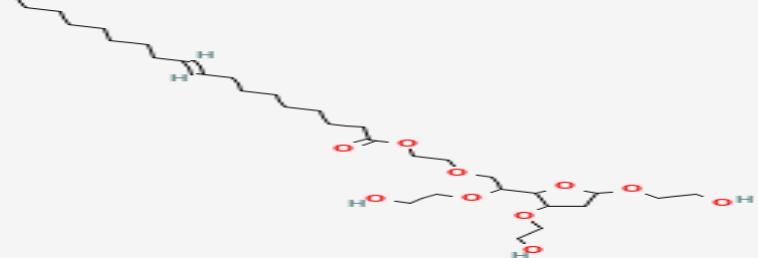
NIGELLA SATIVA OIL

Scientific Name	Nigella sativa L. (family-Ranunculaceae)
Common Name	Black seed, Cumin seeds, Black cumin
Origin	The plant grows in Eastern Europe, the Middle East, and Western Asia
Chemical composition	<p>N. sativa seed contains more than 30% fixed oil and 0.4 to 0.45% volatile oil.</p> <p>The fixed oil (32-40 %) contains: unsaturated fatty acids which includes: arachidonic, eicosadienoic, linoleic, linolenic, oleic, almitoleic, palmitic, stearic and myristic acid as well as beta-sitosterol, cycloecalenol, cycloartenol, sterol esters and sterol glucosides.</p> <p>The volatile oil (0.4-0.45 %) contains saturated fatty acids which includes: nigellone that is the only component of the carbonyl fraction of the oil, Thymoquinone (TQ), thymohydroquinone (THQ), dithymoquinone, thymol, carvacrol, α and β-pinene, d-limonene, d-citronellol, p-cymene volatile oil of the seed also contains: p-cymene, carvacrol, t-anethole, 4-terpineol and longifolene.</p> <p>Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine. The nutritional compositions of N. sativa are vitamins, carbohydrates, mineral elements, fats and proteins that include eight or nine essential amino acids.</p>
Functional Benefits	Anti-Alzheimer action; liquid lipid; Antioxidant activity
Description	N. sativa seeds and oil have been extensively used in treatment of different diseases throughout the world. N. sativa is included in the list of natural drugs in different medicines including Tibb-e-Nabavi (The medicine of Prophet Mohammad), Unani Tebb, and Indian traditional medicine. In traditional remedy, N. sativa seeds are commonly used as a spice and carminative. In addition, several properties such as liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, and anti-bacterial have been attributed to this plant. N. sativa has been investigated for its biological effects and therapeutic potential and shown to have broad spectrum of activities including antidiabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, renal protective, gastro-protective, and antioxidant properties.

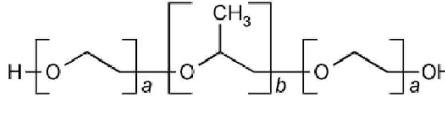
GLYCERYL MONOSTEARATE

Structure	
Molecular formula	C ₂₁ H ₄₂ O ₄
IUPAC Name	2,3-dihydroxypropyl octadecanoate
Molecular weight	358.6g/mol
Melting point	75°C
Solubility	Insoluble in water, soluble in hot oils, organic solvents; soluble in hot alcohol (in alcohol)
Use	Solid lipid; Emulsifier

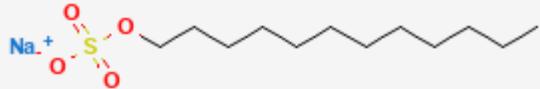
TWEEN 80

Chemical structure	
Common Name	Polyoxyethylene (20) sorbitan monooleate
IUPAC Name	2-[2-[3,5-bis(2-hydroxyethoxy)oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl (E)-octadec-9-enoate
Molecular formula	C ₃₂ H ₆₀ O ₁₀
Molecular weight	604.8g/mol
Category	Nonionic surfactant and emulsifier

POLOXAMER 407

Chemical structure	 a=100 & b=65
IUPAC Name	2-methyloxirane;oxirane
Molecular formula	C ₅₇₂ H ₁₁₄₆ O ₂₅₉
Molecular weight	12600g/mol
Melting point	53–57 °C
Category	hydrophilic non-ionic surfactant

SODIUM LAURYL SULFATE

Chemical structure	
IUPAC Name	sodium;dodecyl sulfate
Molecular formula	NaSO ₄ C ₁₂ H ₂₅
Molecular weight	288.38
Melting point	205.5 °C
Category	Anionic Surfactant

MATERIALS AND EQUIPMENT'S



MATERIALS

Donepezil Hydrochloride was received as a gift sample from Lupin Limited, Verna, Goa, India.

Nigella sativa oil was received as a gift sample from Rmayra Naturals Impex, Delhi.

Glyceryl monostearate, Tween 80 and stearic acid was received as a gift sample from Mohini Organics Pvt. Ltd. Mumbai.

Compritol 888 ATO and Precirol ATO 5 was received as a gift sample from Gattefosse, Mumbai. All other chemicals and reagents used were of analytical reagent grade.

Table 1: List of chemicals and its use

Sr No.	Name of chemicals	Use	Source
1	Donepezil Hydrochloride	Anti-Alzheimer drug	Lupin Limited
2	Glyceryl monostearate, Stearic acid, Compritol 888ATO, Precirol ATO5, Gelucire 43/01	Solid Lipid	Mohini organics pvt. Ltd. & Gattefose
3	Transcutol P	Liquid lipid	Gattefose
4	Nigella sativa oil	Liquid lipid	Rmayra Naturals Impex
5	Poloxamer 407	Co surfactant	Loba chem
6	Tween 80	Surfactant	Loba chem
7	SLS	Stabiliser	Loba chem
8	Benzalkonium chloride	Preservative	Loba chem
9	DPPH Reagent	Reagent	Loba chem

EQUIPMENTS:

Table 2: List of Equipment's

EQUIPMENT	MODEL/COMPANY
Digital balance	AUX 220, Shimadzu, Japan
UV visible spectrophotometer	1800, Shimadzu, Japan
FT-IR spectrophotometer	8400 IR Affinity 1-CE, Shimadzu, Japan
DSC	Mettler Toledo, SW STARe, USA
pH tester	pH tester 20, Eutech instruments, USA
Melting point apparatus	VMP-D, VEEGO, India
Hot air oven	Universal India
Stability chamber	CHM-6 Plus, Remi, India
Ultra Turrax	IKA, T18 Digital Ultra turrax
Ultra-Centrifuge	Beckman Coulter, USA, Optima MAX_XP
Franz diffusion cell apparatus	EM FDC-06, Orchid Scientific, India
Probe sonicator	VCX-750, sonics vibra cell, India
High Pressure homogenizer	Panda Plus 2000, Italy
HPLC	LC 100, Cyberlab, India

METHODOLOGY



METHODOLOGY:

❖ PRE-FORMULATION STUDY:

Pre-formulation study plays very important role in rational development of dosage forms. It is defined as evaluation and investigation of physical as well as chemical properties of drug and when mixed with other excipients. It is method to optimize the delivery of drug by determining physicochemical properties of the new compound that may effect on drug performance and development of the efficacious, stable and safe dosage form. In development of any drug delivery system the preformulation study is one of the important pre-requisites. The pre-formulation study provides the information required to defined the physicochemical properties of the drug substance and provide a structure for drug combination with pharmaceutical excipients in the dosage form. Hence, pre-formulation studies are carried out on the obtained sample of drug and polymer.

❖ DRUG CONFIRMATION:

Identification of drug was done by using DSC, IR and Melting point determination.

1.MELTING POINT DETERMINATION BY CAPILLARY METHOD

Melting point of the drug was determined by capillary method. Glass capillary was taken and sealed at one end when other is kept open. Drug sample was introduced by open end and that ensure it should reach at bottom of capillary. The capillary was subjected to melting point apparatus (VEEGO Model- VMP-D, India). The temperature was increased slowly. The point at which drug started to melt was taken as a melting point within range and ensure the complete liquefaction of drug.

2.FT-IR SPECTROSCOPY

The conformation for structure of Donepezil HCl was determined using FTIR study. IR affinity 8400 Shimadzu instrument was used. The samples were selected i.e., Drug, GMS, Nigella sativa oil, P407, Tween 80 & optimized formulation. The sample compartment was cleaned with ethanol & set for background clearance then sample was placed on sample compartment & swivel pressure tower was fitted & samples was analysed. pH of liquid samples was measured prior to IR study because of sensor sensitivity.

3. DSC STUDY:

Thermal analysis was performed for Drug using DSC. Sample was weighed 5-6mg the bottom pan was used & hermatically sealed. The heating rate was 30-300°C with constant rate of temperature at 10°C/min under nitrogen atmosphere(50-60ml/min).

❖ DRUG EXCIPIENT INTERACTION STUDY:

The drug- excipient interaction study was carried out using DSC & FTIR.

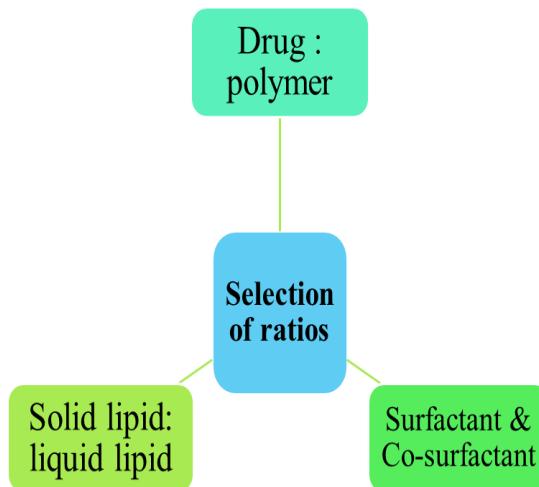
DSC STUDY:

Thermal analysis was performed for DNZ HCl, GMS, Nigella sativa oil and physical mixture of them using DSC (Mettler Toledo, SW STARE, USA). Samples were weighed (5-6mg), the bottom pans were used and hermetically covered with lead. The heating range was 30-300 °C for all samples with constant increasing rate of temperature at 10°C/min under the nitrogen atmosphere (50-60ml/min). The resulted thermogram of drug were compared with thermogram obtained for physical mixture and confirm any changes that occurs in the principal peaks.

FT-IR SPECTROSCOPY

The conformation for structure of Donepezil HCl was determined using FTIR study. IR affinity 8400 Shimadzu instrument was used. The samples were selected i.e., physical mixture of GMS & Drug, Drug & Nigella sativa oil, P407, Tween 80 & optimized formulation. The sample compartment was cleaned with ethanol & set for background clearance then sample was placed on sample compartment & swivel pressure tower was fitted & samples was analysed. pH of liquid samples was measured prior to IR study because of sensor sensitivity.

❖ SELECTION OF RATIOS



SCREENING OF LIPIDS:

Solid lipids are one of the main constituents of NLC. Glyceryl monostearate, stearic acid, Compritol 888 ATO, Gelucire 43/01 and Precirol ATO 5 were among the solid lipids used for screening. The lipids were heated +5°C above the melting point of respective lipids and slowly the drug was added and gently shaken to solubilize the drug. The confirmation of solubility of drug was done by visual inspection and checked for no visual remaining of drug particulates in the molten lipid phase. The drug showing maximum solubility in solid lipid was then finalized for further study.

SCREENING OF SURFACTANTS:

NLC formulations should include two surfactants that promote steric and electrostatic stabilization, avoiding nanoparticle aggregation and ensuring long term stability. Surfactant should be selected according to their charge, molecular weight, and adequacy for the desired route of administration for the formulation. Phase diagram are essential in optimizing the Smix ratio were constructed using aqueous titration technique. Wherein the selected oil phase was heated and maintained at a temperature of 75°C, to which selected Smix was added in varying ratios so as to form homogenous mixtures. The mixture so obtained were then titrated against the desired aqueous phase, and the changes observed were recorded visually.

DRUG TO TOTAL LIPID RATIO:

NLC formulations consist of various Drug: Total lipid ratio depending upon nature of lipids used. According to literature various ratios was taken like 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:10, 1:15, 1:20, 1:25. The most stable and clear physical mixture was selected.

CALIBRATION CURVE FOR DNZ:

Standard calibration curve in PBS pH 6.4:

Accurately weighed 100 mg of DNZ was dissolved in 100mL of pH 6.4 buffer to obtain the working standard solution of 1000 µg/ml. 1mL was withdrawn from standard solution of 1000 and diluted to 100 mL to get the stock of 100 µg/mL. Aliquots of 0.2 ml to 1.2 ml representing 2µg/ml to 12µg/ml of drug was transferred to 10 ml volumetric flask and volumed to 10 ml with pH 6.4 and absorbance were taken at 231 nm. A graph of absorbance vs. concentration was plotted.

ANTI-OXIDANT ACTIVITY OF NIGELLA SATIVA OIL:

The radical scavenging activity of Nigella sativa oil was determined by using DPPH assay.

PRINCIPLE:

Diphenyl 2-Picryl Hydrazyl is a stable (in powder form) free radical with dark purple colour which turns yellow or colourless when scavenged. The DPPH assay uses this character to show radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the colour changes to yellow or colourless and also the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

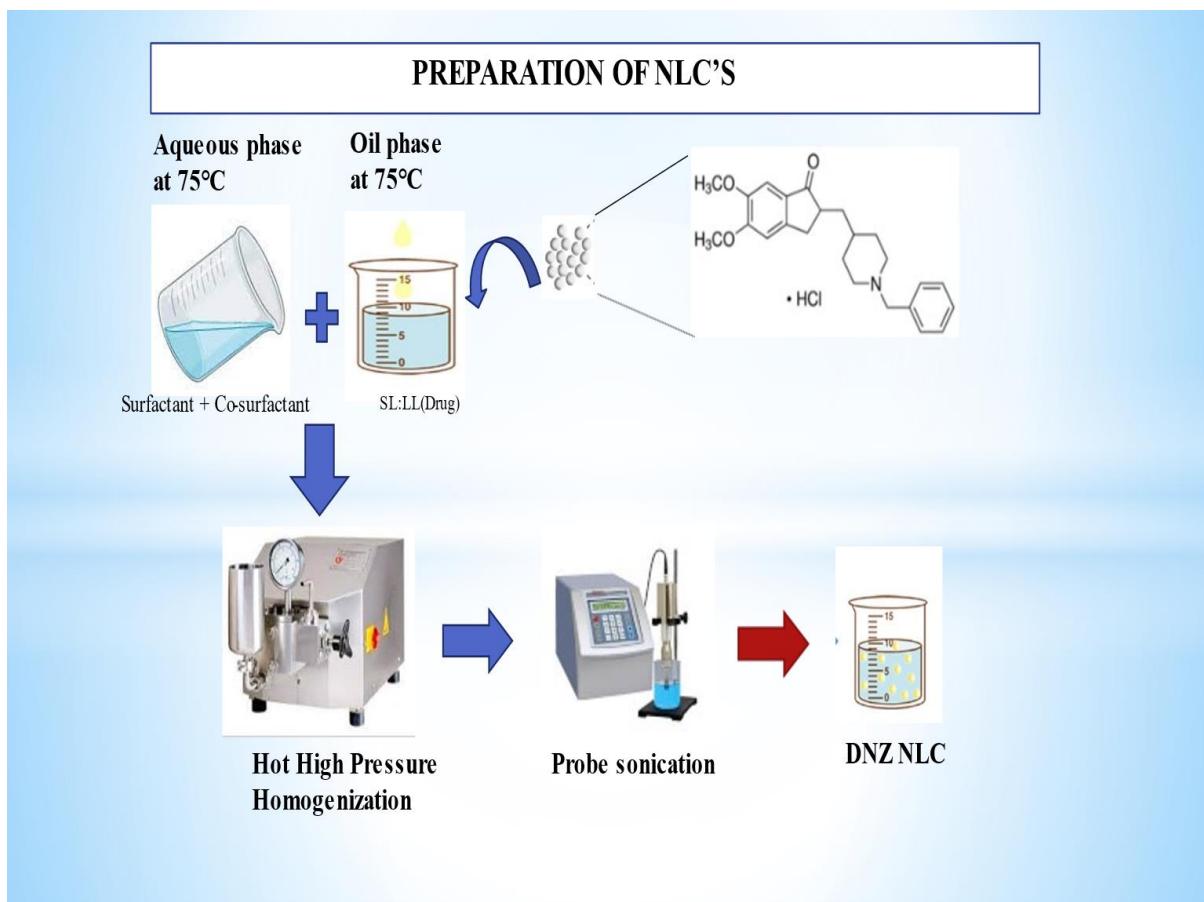
PROCEDURE:

Briefly, 5 ml of an oil was dissolved in 25 ml of toluene. To obtain various test concentrations, different volumes of these oils were adjusted to 2.7 ml. with toluene. Then 0.3 mL of a freshly prepared toluenic solution of DPPH (1mmol/l) was added, and the mixture was vortexed for 1 min at ambient temperature.

The samples were allowed to stand for 1 hour in the dark area avoiding sunlight. The colour of the test sample was compared with standard and observed for any colour changes.

PREPARATION OF NLC:

The desired NLC formulation were prepared using Hot High-Pressure Homogenization followed by Ultra-sonication. GMS and Nigella sativa oil were used as the solid and liquid lipid, respectively. The lipid phase contains solid lipid and liquid lipid which were melted +5°C above the melting point of solid lipid, to which the drug was added under continuous stirring for 5min. Aqueous phase contain surfactant at desired concentration. Both the phases were heated at same temperature. The aqueous phase was added dropwise to lipid phase with mechanical stirring. This pre-emulsion was then pass-through High-Pressure Homogenization at 750bar pressure with five homogenization cycle. Further, the solution was sonicated by using probe Sonicator for 15mins and 50% amplitude. A clear microemulsion was formed after undergoing cooling in ice bath. The liquid nanodroplets of melted lipid transformed into solid nanoparticles at low temperature and produced NLC dispersion. Sodium Lauryl Sulphate was added as a stabilizer into final formulation. The basic rule for the formulation of NLC is to maintain process temperature at least 5°C above the melting point of the solid lipid⁸⁰.



DESIGN OF EXPERIMENT:

Based on number of factors and their level, factorial design was used to systematically evaluate the effect of formulation parameter affecting the physicochemical properties of DNZ-NLC. The effect of three independent variables (drug to total lipid ratio, solid lipid to liquid lipid ratio and surfactant concentration) on dependent variable (particle size, polydispersity index, zeta potential, drug content, entrapment efficiency) was studied using Design expert software (version 8.0.7.1; M/s Stat-Ease, Minneapolis, USA). A total of 8 experimental batches were designed by the software.

Table 3 shows the coded independent variables and that levels.

Table 3: Formulation Batches by using Design of Expert Software

RUN	Factor A (Drug: Lipid ratio)	Factor B (SL: LL)	Factor C (Surfactant concentration)
1	-1	1	-1
2	1	1	1
3	-1	-1	1
4	-1	-1	-1
5	1	-1	1
6	1	-1	-1
7	-1	1	1
8	1	1	-1

Ingredients	Ratios /Concentrations
Drug : polymer ratio	1:4 ; 1:6
Solid lipid : Liquid lipid	70:30 ; 80:20
Surfactant	1% or 3%
Co-surfactant	0.5%

EVALUATION:

1] DETERMINATION OF PARTICLE SIZE, POLYDISPERSITY INDEX AND ZETA POTENTIAL:

HORIBA SZ-100 for Windows [Z Type] Ver2.20 was used to measure the size and zeta potential (ZP) of NLCs of all drug loaded samples. All samples were diluted with distilled water to make up a suitable concentration. The Z-average particle size, polydispersity index (PI), And Zeta potential (ZP) values were determined.

2] DETERMINATION OF TOTAL DRUG CONTENT:

The total amount of drug in the formulation was determined by dissolving 0.1 ml of the suspension in 10 ml of PBS 6.4. The amount of DNZ in each sample was determined using UV spectrophotometer (1800, Shimadzu, Japan) by measuring the absorbance at a λ_{max} value of 231nm. Each experiment was performed in triplicate. The total drug content was calculated using the following equation:

$$\text{Total Drug Content} = \frac{\text{Actual drug added}}{\text{Theoretical drug added}} \times 100$$

3] DETERMINATION OF %EE AND % DRUG LOADING:

Percent entrapment efficiency & drug loading of NLC were determined by taking 3ml of NLC formulation and centrifuging with the help of ultracentrifuge for 30mins at 60000rpm. The supernatant was collected and further diluted suitably. After dilution the absorbance was taken for all the batches and %EE was calculated by following formula.

$$\text{Entrapment Efficiency} = \frac{(\text{Amount of drug added} - \text{Amount of drug in supernatant})}{\text{Amount of drug added}} \times 100$$

$$\text{Drug loading Capacity} = \frac{\text{Amount of drug added} - \text{Amount of drug in supernatant}}{\text{Weight of NLCs}} \times 100$$

4] IN-VITRO RELEASE STUDY:

An invitro release study of the DNZ from NLCs solution was performed in simulated nasal fluid (SNF) pH 6.4 using Franz Diffusion cell with Dialysis membrane. For saturating the membrane, they were initially soaked in phosphate buffer solution pH6.4 for 24h before the experiment. Diffusion cell was filled with PBS pH 6.4 and dialysis membrane was mounted on cell. The temperature was maintained at 37°C. After a pre-incubation time of 20minutes, DNZ NLC formulation was placed in donor chamber.

At predetermined time points, 3ml sample from each batch were withdrawn from the receptor compartment, replacing the sampled volume with PBS pH 6.4 after each sampling, for a period of 360 minutes (6h). The amount of permeated drug was determined using a UV- spectrophotometer at 231nm.

5] STABILITY STUDY:

The stability study was performed on Optimized NLC formulation under at 4°C. Various parameters were evaluated to check the stability of NLC for 0, 15, 30, 60, 90 days. Changes in pH, particle size, zeta potential & drug content was measured.

6] IN-VIVO STUDY:

All animal experiments were approved and performed in accordance with the guidelines of Institutional Animal Ethics Committee of Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune[1379/PO/Re/S/10/CPCSEA].

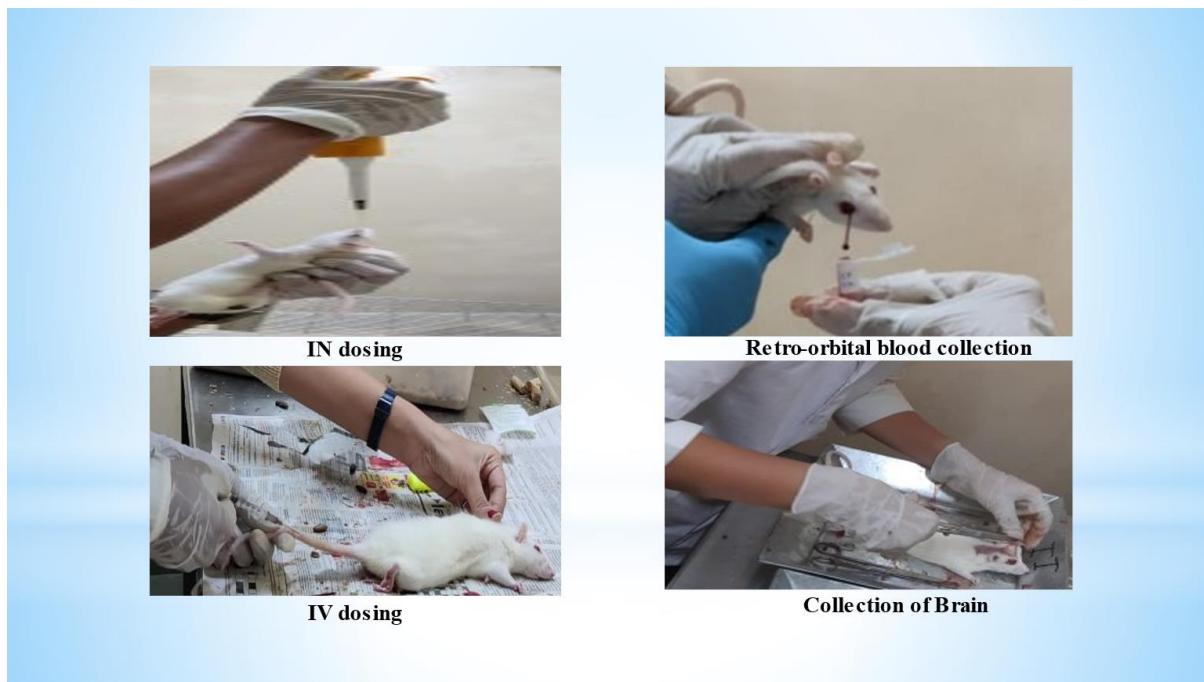
Pharmacokinetic & biodistribution study was performed on Male Sprague Dawley Rats weighing 200-250gms. The rats will be maintained on a 12-hour light and dark cycle at a temperature of 23-25°C and relative humidity of 30-70%. The animals were given food and water ad libitum and will be closely monitored for any kind of behavioural changes during the experiment. Before the start of the pharmacokinetic study, the animals will be fasted overnight. As per the protocol, the rats were divided into two groups. The animals in group I were administered 4.0 mg/kg body weight Donepezil Hydrochloride (DNZ) solution via intravenous route (1 ml/kg) & group II Donepezil Hydrochloride loaded NLC formulation (equivalent to 4.0mg of DNZ/kg body weight) was administered via intranasal route in each nostril in the group with the help of micropipette (10–100 μ L) with 0.1mm internal diameter. The rats were anaesthetized prior to nasal administration by isoflurane and held firmly from the back in a slanted position during nasal administration. The blood samples of 0.5ml will be collected by retro-orbital method at 15,60,120,180,300mins & stored in heparinized tubes. Blood was centrifuged to separate plasma. Animals was sacrificed by CO₂ Asphyxiation at 15,60,120,180,300mins. The brain tissues will be collected and will be washed thrice with saline, wiped with the soft fabric, weighed and stored at -20°C until analysis. The brain tissues were homogenized in phosphate buffer for extraction and analysed through HPLC for DNZ level. The homogenate was centrifuged at 5000 rpm for 20 min with the temperature of 4° C, and aliquots of the supernatant was separated and stored at -20°C until drug analysis by HPLC.

Pharmacokinetic parameters for formulations were calculated by pharmacokinetic software (Kinetica). The maximum plasma concentration of DNZ (C_{max}) and the time required to reach the Maximum concentration (T_{max}) was obtained directly from the actual plasma profiles. The degree of DNZ targeting to brain after I.N., administration can be evaluated by the drug targeting index (DTI) which can be described as the ratio of the value of AUC

brain/ AUC blood following I.N. administration to that of I.V. administration. The higher the DTI is, more the degree of DNZ targeting to brain can be expected after I.N. administration. Brain targeting efficiency i.e., Drug targeting efficiency (DTE %) that represents time average partitioning ratio was calculated.

Table 4: Biodistribution and Pharmacokinetic study in Rats

Sr. No.	Group Description	Number of Animals	Dose	Route of Administration
I.	Donepezil HCL solution	15	4.0mg of DNZ/kg body weight	IV
II.	Donepezil HCL Formulation (NLC)	15	Equivalent to 4.0mg of DNZ/kg body weight	IN



RESULTS AND DISCUSSION



RESULTS AND DISCUSSION

A) PREFORMULATION STUDY:

1) CONFIRMATION OF DRUG:

Confirmation or identification of drug was carried out by melting point determination, IR spectroscopy and DSC analysis.

MELTING POINT DETERMINATION:

Melting point of DNZ HCl was determined by capillary method. It was found to be 223-227°C. It was confirmed with the reported melting point of DNZ HCl (223-227°C).

FT-IR SPECTROSCOPY:

The FTIR spectrum of DNZ HCl was taken and the observed peaks are shown in figure-4 and table-4.

Figure 4: IR spectra of Donepezil drug

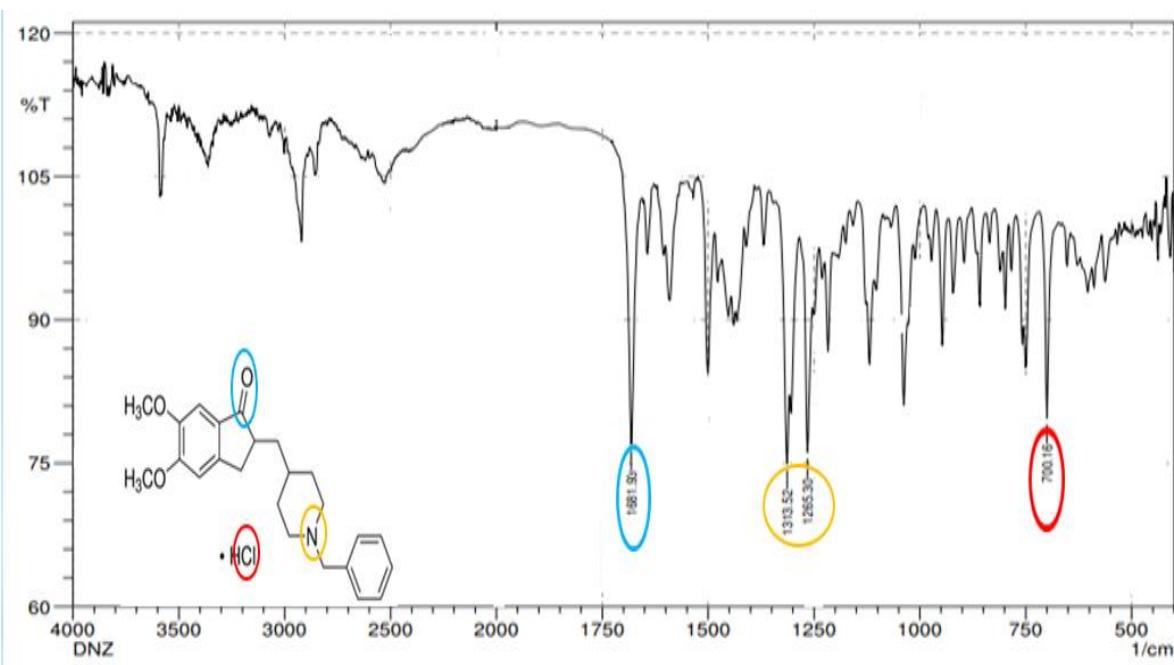


Table 5: Peaks & principal groups present in IR-spectrum of Donepezil HCl

Sr. No.	Standard value range	Observed value	Interpretation
1.	1020-1330	1265	C-N stretch
2.	3000-2800	2956	C-H stretch
3.	1850-1650	1681	C=O stretch
4.	550-730	700	H-Cl

The FTIR spectra of donepezil exhibited strong intense peaks at 1681cm^{-1} (due to C=O carbonyl stretching vibrations), 1593cm^{-1} (due to C=C aromatic ring stretching and 1313 & 1265 cm^{-1} (due to aromatic amine C-N stretching), 700cm^{-1} (due to presence of halogen atom i.e. chlorine). These peaks represent main functional groups in the chemical structure of Donepezil HCl.

DIFFERENTIAL SCANNING CALORIMETRY:

DSC is a basic method to determine crystallinity and amorphous state of drug in compound.

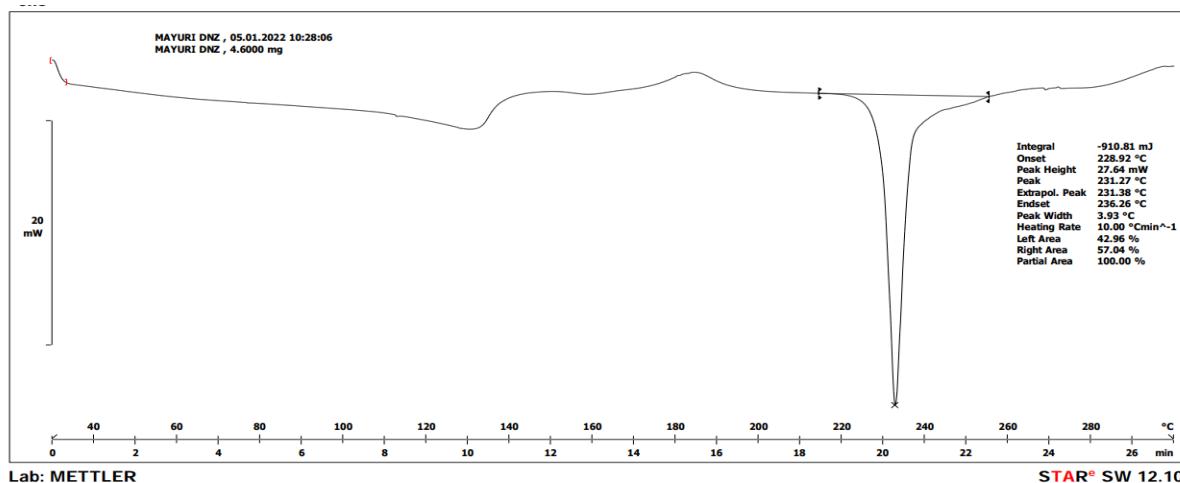


Figure 5: DSC graph of Donepezil Drug

DSC thermogram of donepezil exhibited sharp endothermic peak at 231.27°C indicating crystalline nature of drug.

STANDARD CALIBRATION CURVE:

Standard calibration curve in pH 6.4 buffer

Scanning of DNZ HCL solution in pH 6.4 Phosphate buffer by UV spectrophotometer showed the λ_{max} at 231nm. On this wavelength the standard curve followed the Beer-Lambert's Law in the concentration range of 0 to $12\mu\text{g/ml}$ with $R^2 = 0.9989$.

Table No 6: UV calibration data

Sr no	Concentration ($\mu\text{g/ml}$)	Absorbance
1	2	0.082
2	4	0.167
3	6	0.248
4	8	0.339
5	10	0.426
6	12	0.528

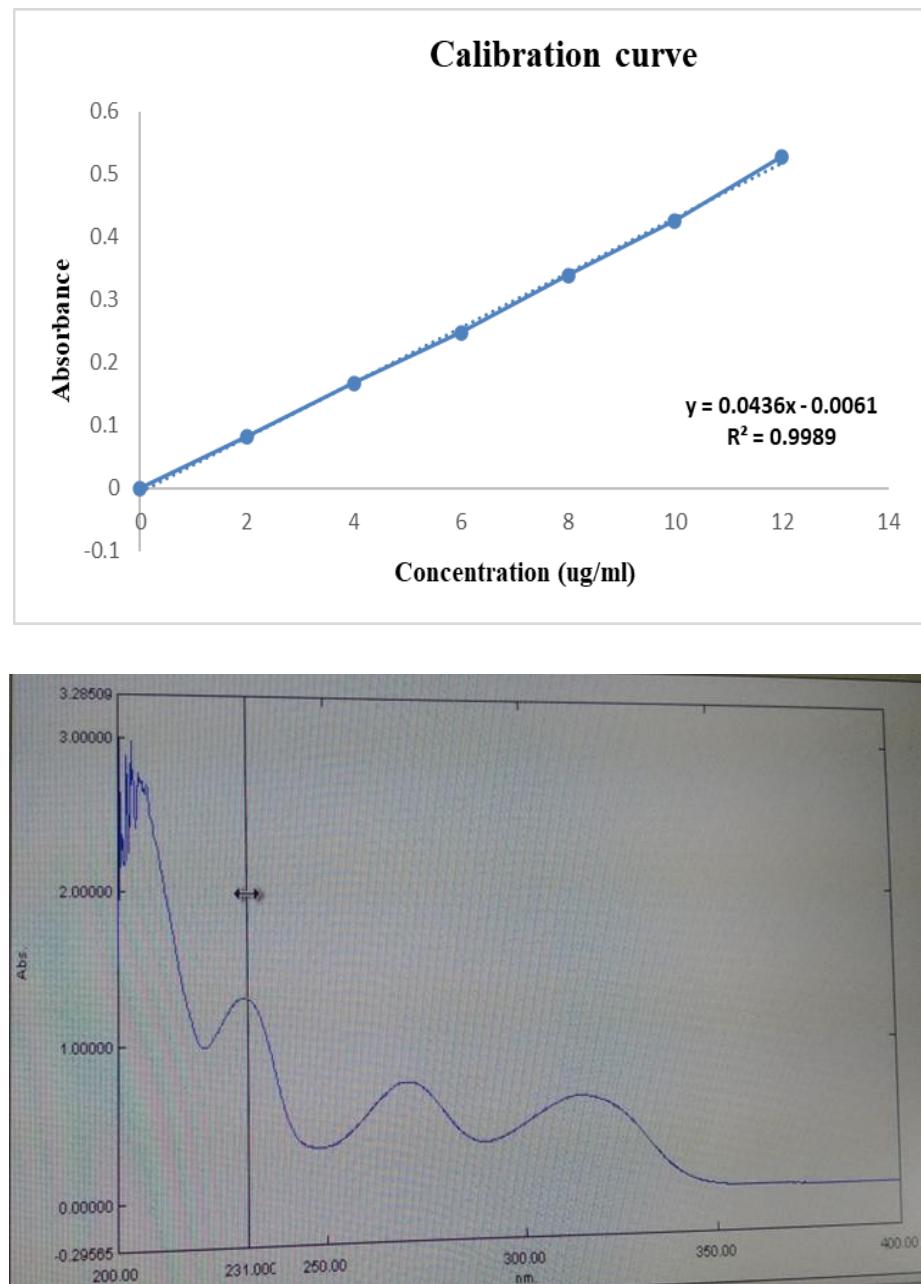


Figure 6: UV Calibration curve of DNZ HCL in PBS pH6.4

DRUG-EXCIPIENT COMPATIBILITY STUDY:

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Drug-polymer compatibility studies were performed by FTIR spectroscopy. FTIR spectrum of DNZ HCL, Nigella sativa oil and excipients (Solid lipids & surfactants) are shown in figure-7. The functional group retained in the spectra of physical mixture of donepezil & excipients. Hence, there was no interaction between the drug and excipients (GMS, NS oil, P407 & tween 80)

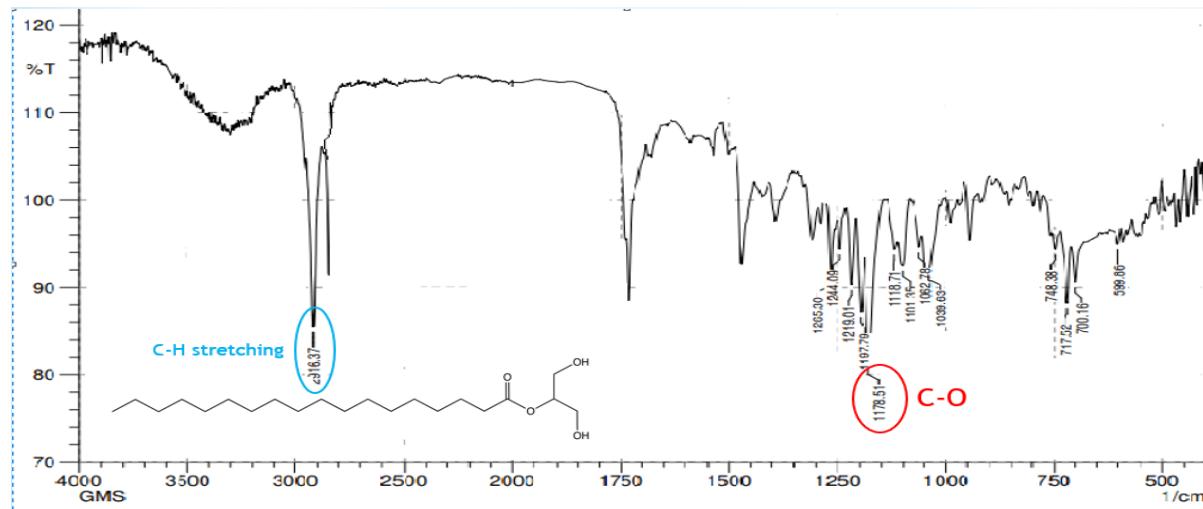


Figure 7 (a): FTIR spectra of GMS

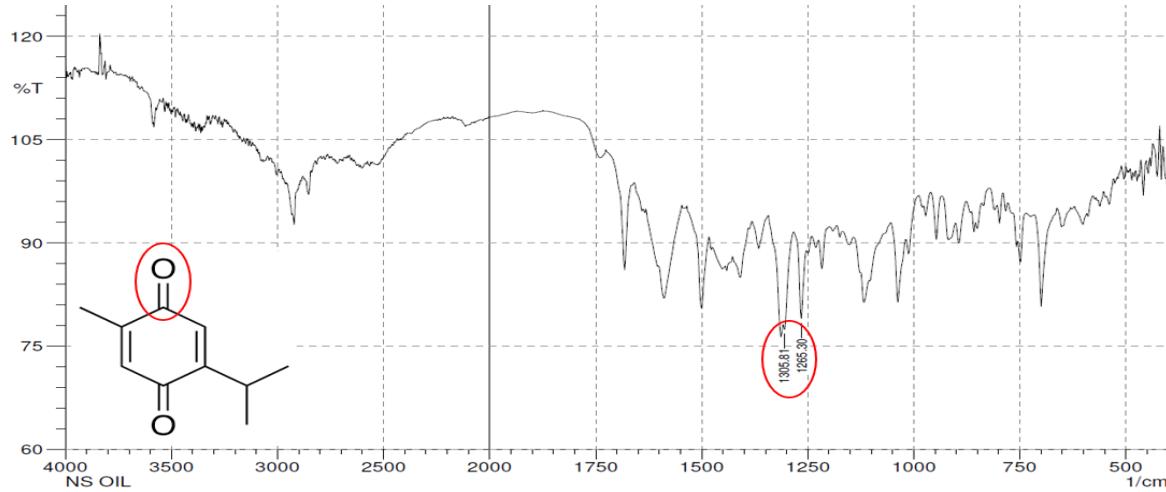


Figure 7 (b): FTIR spectra of Nigella sativa oil (Thymoquinone)

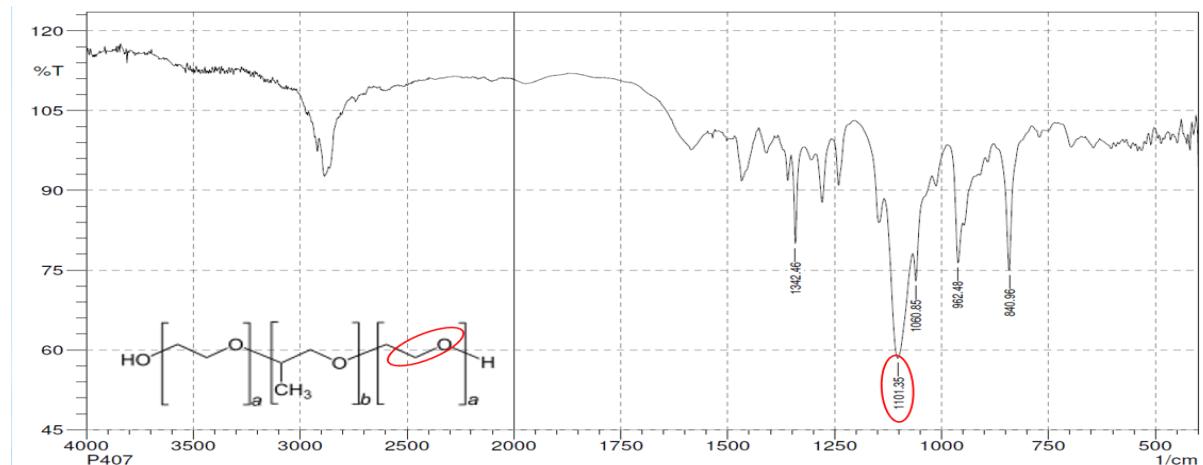


Figure 7 (c): FTIR spectra of P407

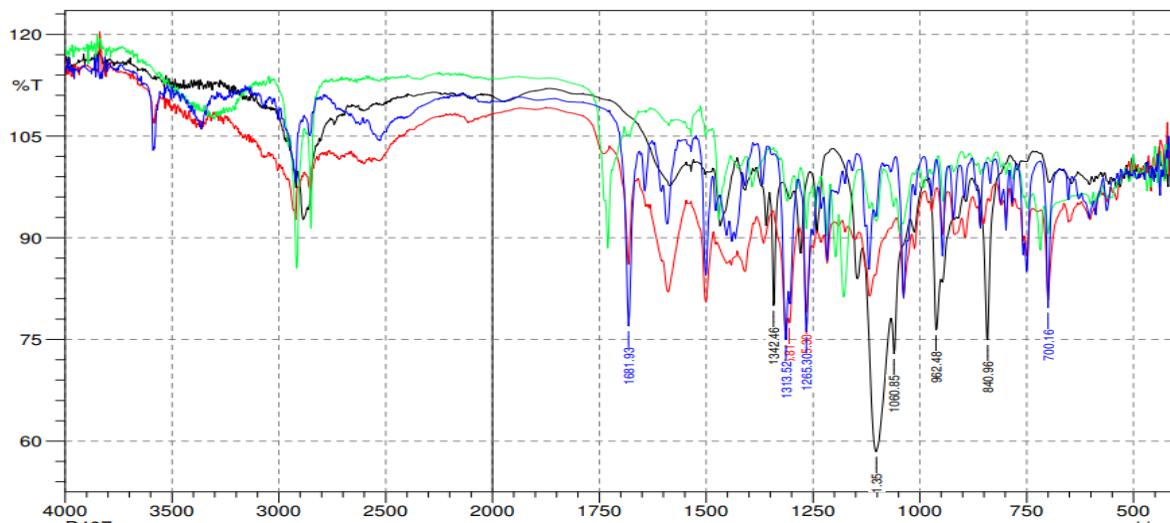


Figure 7(d): Overlay for FTIR spectra's

DSC STUDY:

DSC analysis was performed on pure drug, drug lipids physical mixture and optimized drug loaded NLC formulation sample. Thermograms were obtained by DSC (Mettler Toledo, SW STARe, USA) at a heating rate of 10°C/min from 30°C to 300°C under nitrogen purge of 50ml/min.

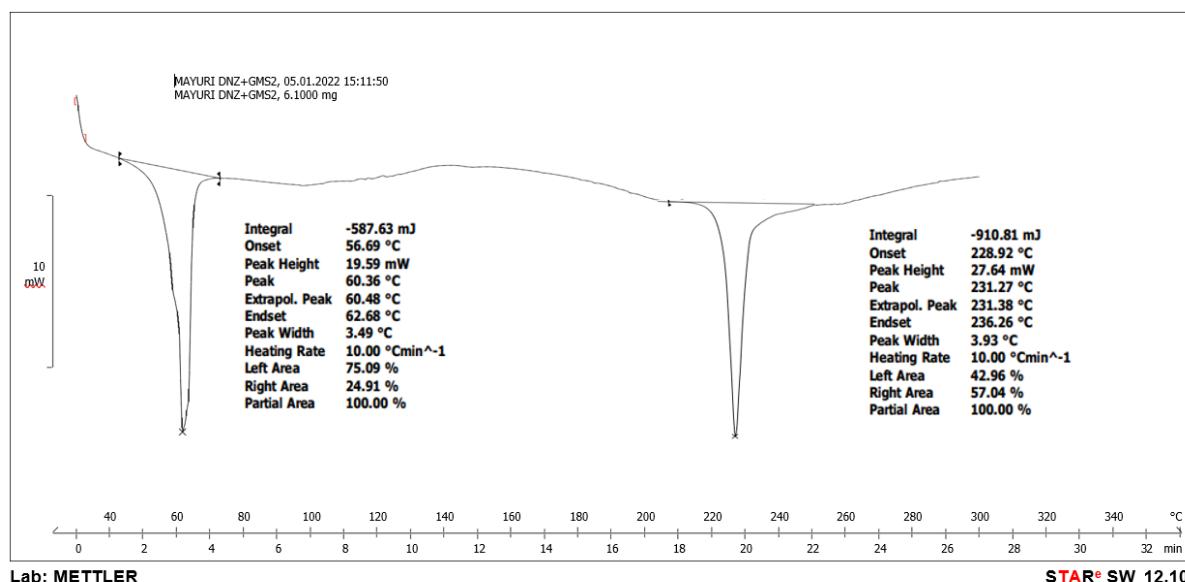


Figure 8: DSC curve for DNZ and GMS

Thermogram of physical mixture showed the drug melting peak at 231.27°C & whereas GMS exhibited endothermic peak at 60.36°C is sharp and not much shifted indicating compatibility of the drug and GMS.

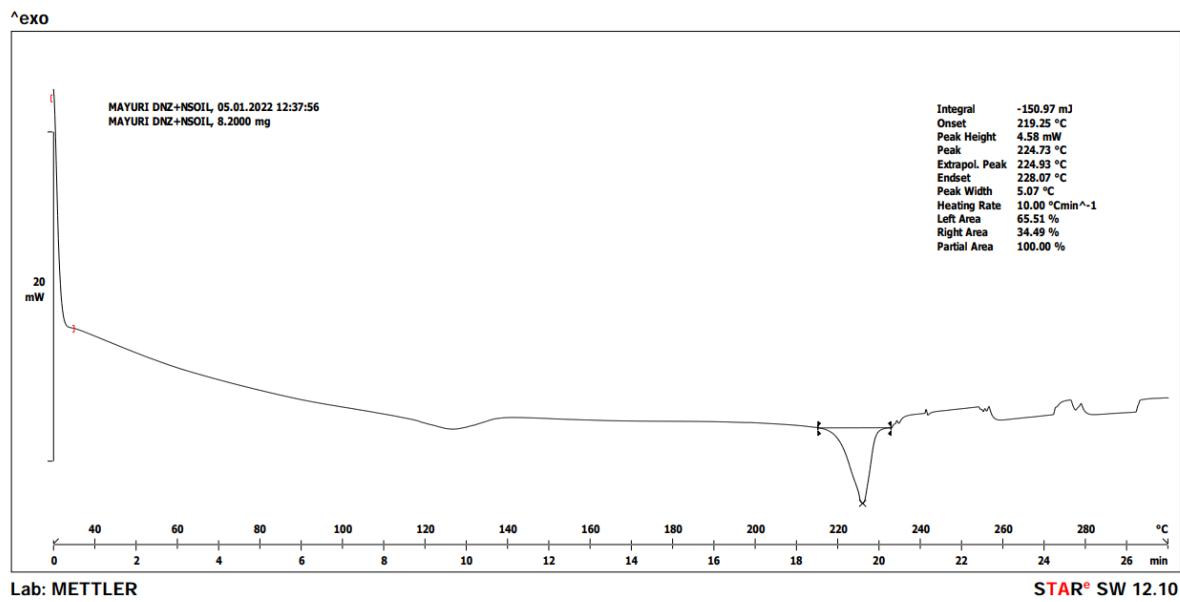
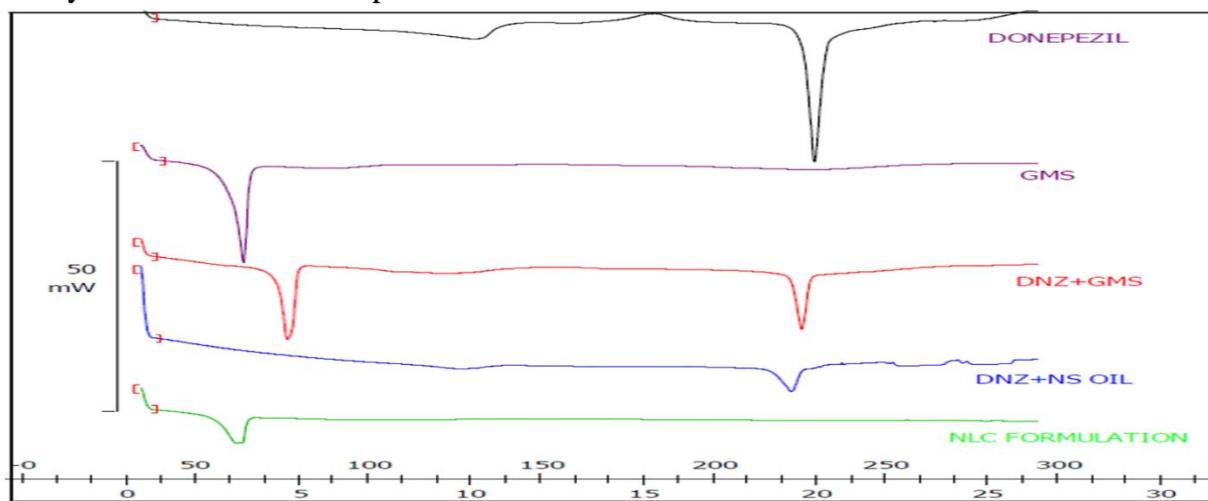


Figure 9: DSC curve for DNZ and Nigella sativa oil

Thermogram of physical mixture showed the drug melting peak at 224.73°C & whereas NS oil doesn't show any peak and parent peak of drug is not much shifted indicating compatibility of the drug and oil.

In case of formulation , one endothermic peak is observed there is conversion of crystalline DNZ to amorphous form.

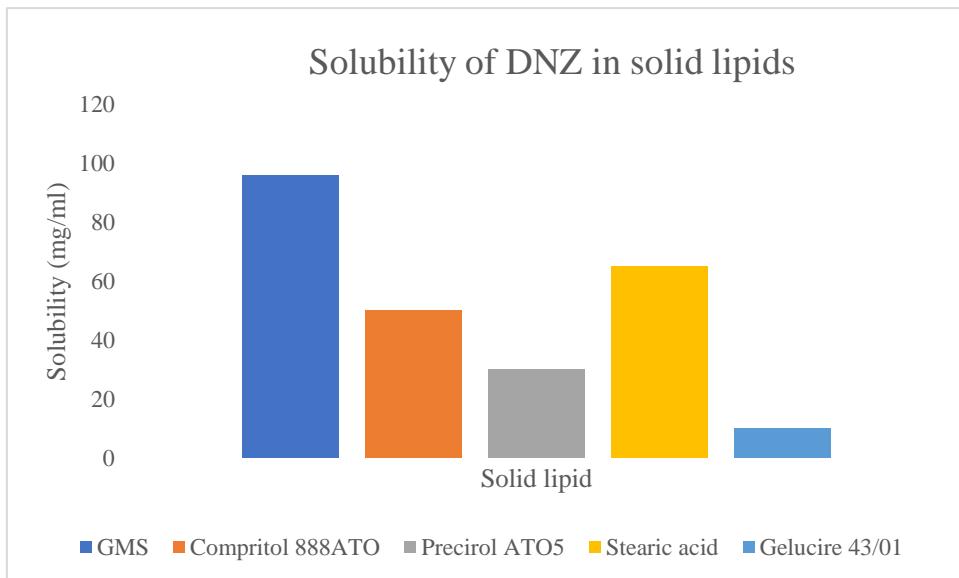


**Figure 10: Overlay Thermograms of a) Donepezil b) GMS c) DNZ+GMS
d) DNZ+ NS oil e) NLC formulation**

FORMULATION DEVELOPMENT AND EVALUATION:

SCREENING OF LIPIDS:

Among the five solid lipids i.e., GMS, stearic acid, compritol 888ATO, precirol ATO 5 and Gelucire 43/01 screened for solubility study with DNZ, the GMS showed maximum solubility of DNZ. Hence GMS was finalized for further formulation of NLC. Nigella sativa oil was used as liquid lipid.



SCREENING OF SURFACTANT:

Smaller particle sizes have been observed when a higher surfactant/lipid ratio was used. Accordingly, polysorbate 80 (Tween® 80), a non-ionic surfactant containing a polyoxyethylene chain tetrahydrofuran ring that provides steric stabilization and a hydrophobic tail that prevents particle aggregation, was selected based on previous works that showed its compatibility with the lipids used. Poloxamer 407, was selected on its emulsification capacity for selected lipid mixture, its non-irritating effect on the nasal mucosa, and its ability to minimize the polymorphic state transitions of lipids.

ANTI-OXIDANT ACTIVITY:

Following images shows change in colour from purple to slightly yellow or colourless. This indicated that Nigella sativa oil has Anti-oxidant activity.



Figure 11: Toluenic DPPH Solution (Standard)

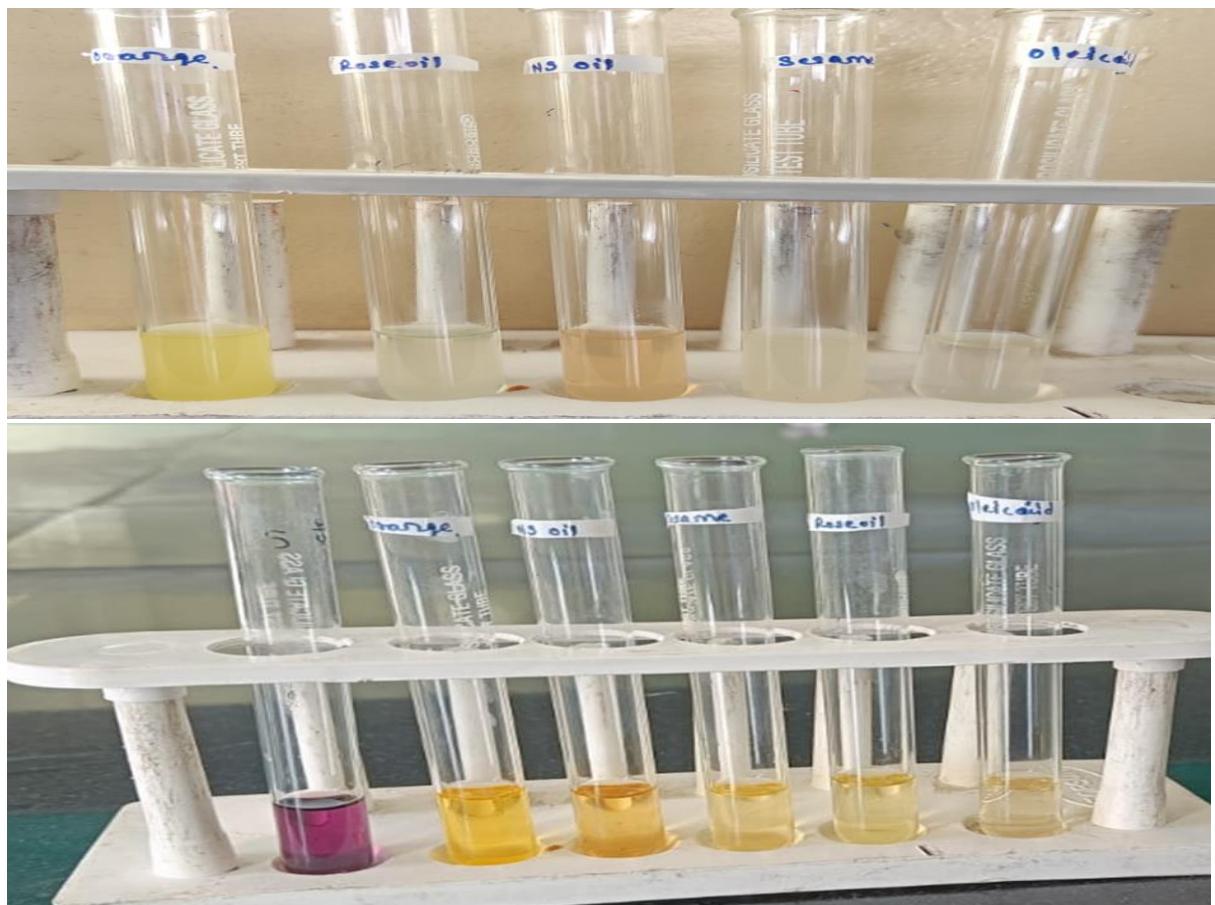


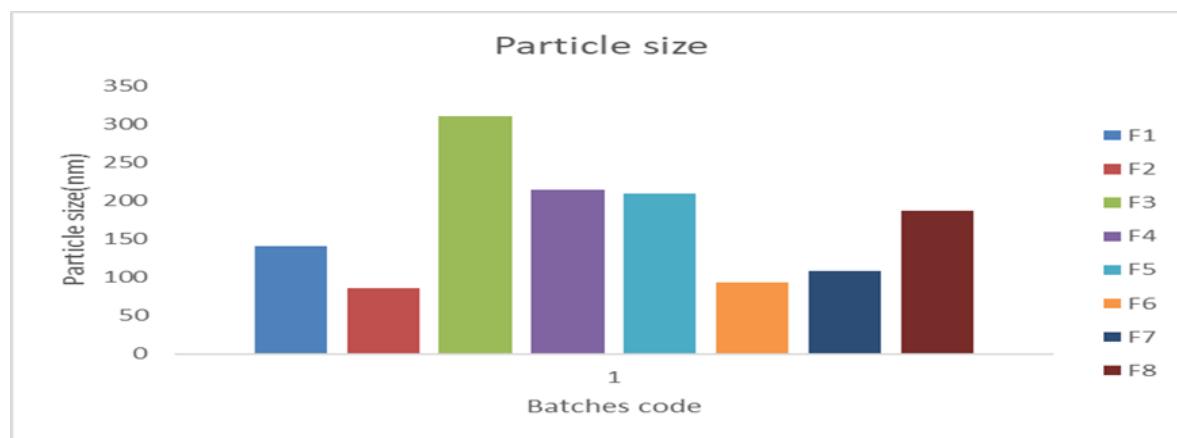
Figure 12: Comparison of Different oils for their anti-oxidant activity

DETERMINATION OF PARTICLE SIZE PDI AND ZETA POTENTIAL:

Particle size, PDI and Zeta potential for the 8 batches was determined by HORIBA SZ-100 for Windows [Z Type] Ver2.20.

Table 7: Results for particle size, PDI and zeta potential

Batch	Particle size (nm)	PDI	ZP (mV)
F1	139.4±0.9	0.353±0.03	-23.4
F2	85.3±2.23	0.363±0.04	-38.6
F3	331.1±18.30	0.344±0.04	-34.2
F4	217.2±7.95	0.357±0.04	-41.1
F5	201.4±12.45	0.329±0.04	-33.4
F6	94.1±2.90	0.271±0.04	-31.9
F7	107.4±2.64	0.266±0.04	-41.7
F8	181.5±8.75	0.353±0.00	-39.1



The particle size was found to be significantly affected with increase in total lipid amount at lower surfactant concentration. This may be attributed to inability of the surfactant solution to stabilize the emulsion at very low concentrations. Further higher concentrations of surfactant are sufficient enough to stabilize the emulsion with consistent particle size. Different concentrations of liquid lipid showed significant effect on particle size. As concentration of liquid lipid increases there observed decrease in particle size. The zeta potential value is a crucial parameter that influences the stability of nanocarriers, since it is related to the surface charge of the nanoparticles and indicates the degree of repulsion between closely positioned and similarly charged particles in dispersion. This repulsive force prevents the aggregation of particles. As presented in Table 7. the NLCs exhibited a negative zeta potential value. The particle size, PDI & Zeta potential was found to be between 85.3 to 331.1nm, 0.238 to 0.365 & -23.4 to -41.7 respectively.

Measurement Results

Date : 21 April 2022 05:19:57
 Measurement Type : Particle Size
 Sample Name : F7
 Scattering Angle : 90
 Temperature of the Holder : 25.1 deg. C
 Dispersion Medium Viscosity : 0.893 mPa.s
 Transmission Intensity before Meas. : 9684
 Distribution Form : [Standard]
 Distribution Form(Dispersity) : Monodisperse
 Representation of Result : Scattering Light Intensity
 Count Rate : 787 kCPS

Calculation Results

Peak No.	S.P. Area Ratio	Mean	S. D.	Mode
1	1.00	125.0 nm	47.8 nm	103.6 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	125.0 nm	47.8 nm	103.6 nm

Cumulant Operations

Z-Average : 107.4 nm
 PI : 0.254

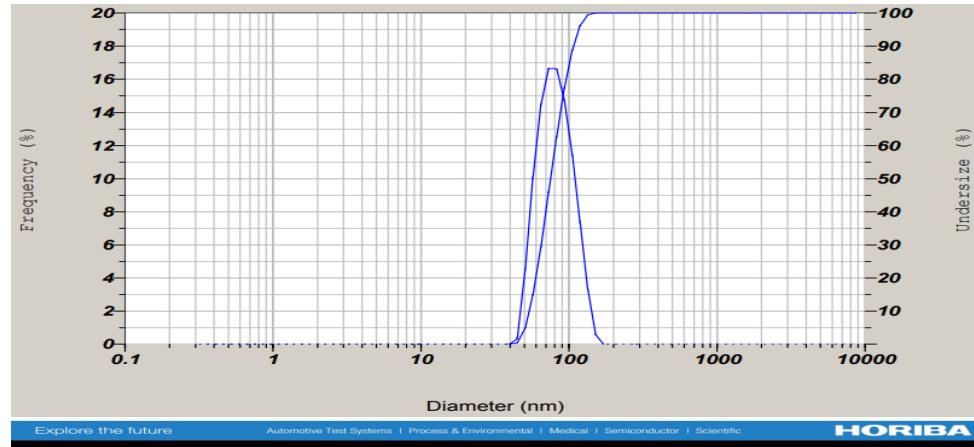


Figure 13: Particle Size & Polydispersity index graph of batch(F7)

Measurement Results

Date : 21 April 2022 06:17:33
 Measurement Type : Zeta Potential
 Sample Name : F7
 Temperature of the Holder : 25.2 deg. C
 Dispersion Medium Viscosity : 0.891 mPa.s
 Conductivity : 0.285 mS/cm
 Electrode Voltage : 3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-31.9 mV	-0.000248 cm ² /Vs
2	— mV	— cm ² /Vs
3	— mV	— cm ² /Vs

Zeta Potential (Mean) : -31.9 mV
 Electrophoretic Mobility Mean : -0.000248 cm²/Vs

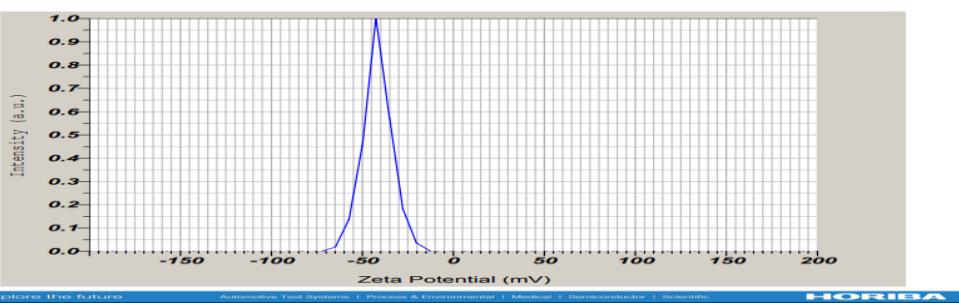


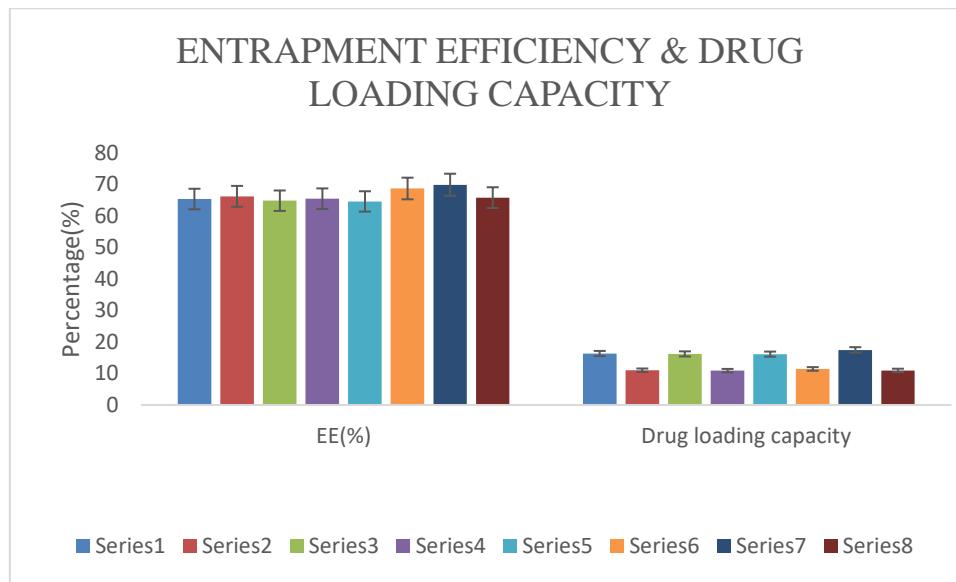
Figure 14: Zeta potential vs Intensity graph of optimized batch

ENTRAPMENT EFFICIENCY AND DRUG LOADING:

EE of drug is depended upon its solubility in lipid melt which is generally more than in the solidified lipid. Long chain triglycerides with higher melting point exhibit higher entrapment efficiency due to their lipophilic nature. Percent EE and % DL was found to be 64.81 to 70% & 10 to 17% respectively and the results are mentioned in table-8.

Table 8(a): Results for % EE & %DL

Batches	EE (%)	Drug loading capacity
F1	65.32±0.999	16.33±1
F2	66.52±0.577	11.03±1
F3	64.47±0.577	16.20±0.5
F4	65.76±0.577	10.90±0.5
F5	64.90±0.577	15.14±0.5
F6	69.00±0.577	11.44±0.5
F7	70.20±0.577	17.46±0.5
F8	65.11±1.154	10.96±0.5



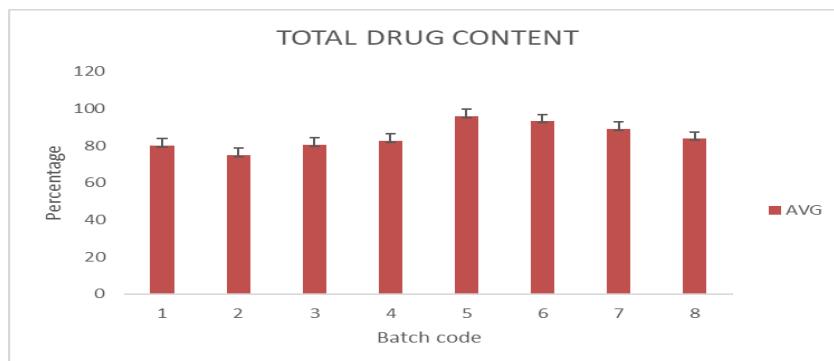
DRUG CONTENT:

The Drug content was used to determine the actual drug content present in formulation.

Average drug content was found to be in range **75 to 90%**.

Table No 8(b): Table for % Drug content

Batch code	Drug content (%)
1	81.74±1.34
2	76.35±0.52
3	79.15±0.86
4	81.10±0.77
5	91.74±1.36
6	90.07±0.65
7	88.40±1.12
8	84.55±0.95

**PH OF NLC FORMULATION:**

As this is intranasal formulation therefore pH of NLC is very important to travel a drug around nasal mucosa. The pH of Nasal mucosa lies in between 4.5 to 6.5. The pH of formulation batches was found to be in range of 6.4 to 6.5

Table 8(c): pH of formulation

Batches	pH of formulation
F1	6.4
F2	6.3
F3	6.4
F4	6.5
F5	6.4
F6	6.4
F7	6.4
F8	6.3

OPTIMIZATION OF NLC:

Experimental design:

2³ factorial designs have generated 8 experimental runs for the prepared formulations. Responses obtained from these runs were shown in table 8. The values of 3 independent variables are Y1: particle size (nm), Y2: Zeta potential(mV), Y3: Entrapment Efficiency(%). The main effect process order was suitably fitted. The values of R², SD, % coefficient of variation of each of the 3 responses are shown in table 8. The effect of independent variables on drug: total lipid, solid lipid: liquid lipid & surfactant concentration is presented on a #D surface and contour plots (fig 15). Moreover, a quantitative comparison that is resulted from experimental values of the responses with that of the predicted values can be analysed from fig.15.

Table 9(a): Experimental designed by software and their responses

RUN	X1 (Drug: Lipid ratio)	X2 (SL: LL)	X3 (Surfactant Conc)	Response 1 Particle size (nm)	Response 2 ZP (mV)	Response 3 EE (%)
F1	-1	1	-1	139.4±0.9	-23.4	65.32±0.999
F2	1	1	1	85.3±2.23	-38.6	66.52±0.577
F3	-1	-1	1	331.1±18.30	-34.2	64.47±0.577
F4	-1	-1	-1	217.2±7.95	-41.1	65.76±0.577
F5	1	-1	1	201.4±12.45	-33.4	64.90±0.577
F6	1	-1	-1	94.1±2.90	-31.9	69.00±0.577
F7	-1	1	1	107.4±2.64	-41.7	70.20±0.577
F8	1	1	-1	181.5±8.75	-39.1	65.11±1.154

Table 9(b): Summary of results of regression analysis for measured responses

Responses	R ²	Adjusted R ²	Predicted R ²	SD	%CV
Y1: Particle size (nm)	0.9962	0.9933	0.9847	1.22	0.88
Y2: Zeta potential (mV)	0.9246	0.8680	0.6982	1.12	3.18
Y3: Entrapment Efficiency (%)	0.5489	0.2105	0.8045	1.88	2.86

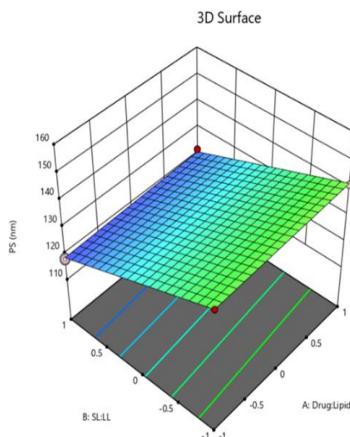
Table 9(c): Summary of results of ANOVA for measured responses

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	Significance
Particle size						
Model	1565.41	3	521.80	347.87	< 0.0001	significant
Residual	6.00	4	1.50			
Cor Total	1571.41	7				
Zeta potential						
Model	61.27	3	20.42	16.34	0.0104	significant
Residual	5.00	4	1.25			
Cor Total	66.27	7				
Entrapment Efficiency						
Model	17.27	3	5.76	1.62	0.03182	significant
Residual	14.19	4	3.55			
Cor Total	31.46	7				

EFFECT OF FORMULATION VARIABLES:

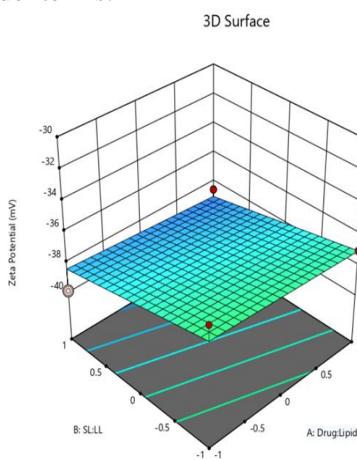
Response 1(Y1): Effect of Independent variables on particle size

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 347.87 and R² value of 0.9962 which implies model was significant ($P<0.0001$). And there was only chance that 0.01% a “model F value” this large occur due to noise. Values of probe P-Value less than 0.0500 indicate model terms are significant. In this case X1, X2 were significant model terms.



Response 2(Y2): Effect of Independent variables on Zeta potential

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 16.34 and R² value of 0.9246 which implies model was significant ($P<0.03182$). And there was only chance that 0.052% a “model F value” this large occur due to noise. Values of probe P-Value less than 0.0500 indicate model terms are significant. In this case X1, X2 were significant model terms.



Response 3(Y3): Effect of Independent variables on EE

On applying factorial design, the linear model was suggested by design expert software and found to be significant model with F value of 1.62 and R² value of 0.5489 which implies model was significant ($P<0.0104$). And there was only chance that 0.01% a “model F value” this large occur due to noise.

Values of probe P-Value less than 0.0500 indicate model terms are significant. In this case X1, X2 were significant model terms.

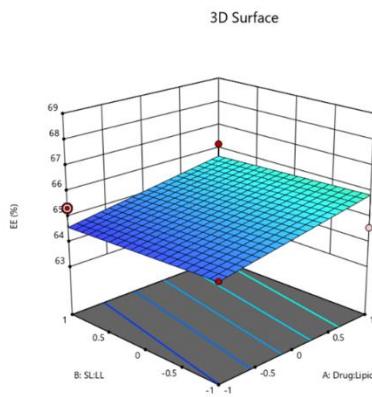


Figure 15(a): 3D surface plot for effect of independent variables on particle size, Zeta potential & EE

BATCHES SUGGESTED BY DOE:

Sr No.	Drug: Total Lipid	SL:LL	Surfactant Conc
1	0.733	-0.695	0.712
2	1.000	-1.000	1.000
3	-1.000	-1.000	1.000

Point Prediction

Solution 3 of 100 Response	Predicted Mean	Predicted Median	Observed	Std Dev	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
PS	140.175	140.175	145.39	1.22474	137.771	142.579	130.845	149.505
Zeta Potential	-36.375	-36.375	-41.4	1.11803	-38.57	-34.18	-44.8924	-27.8576
EE	63.8007	63.8007	67.54	1.88367	60.1026	67.4988	49.4505	78.1509

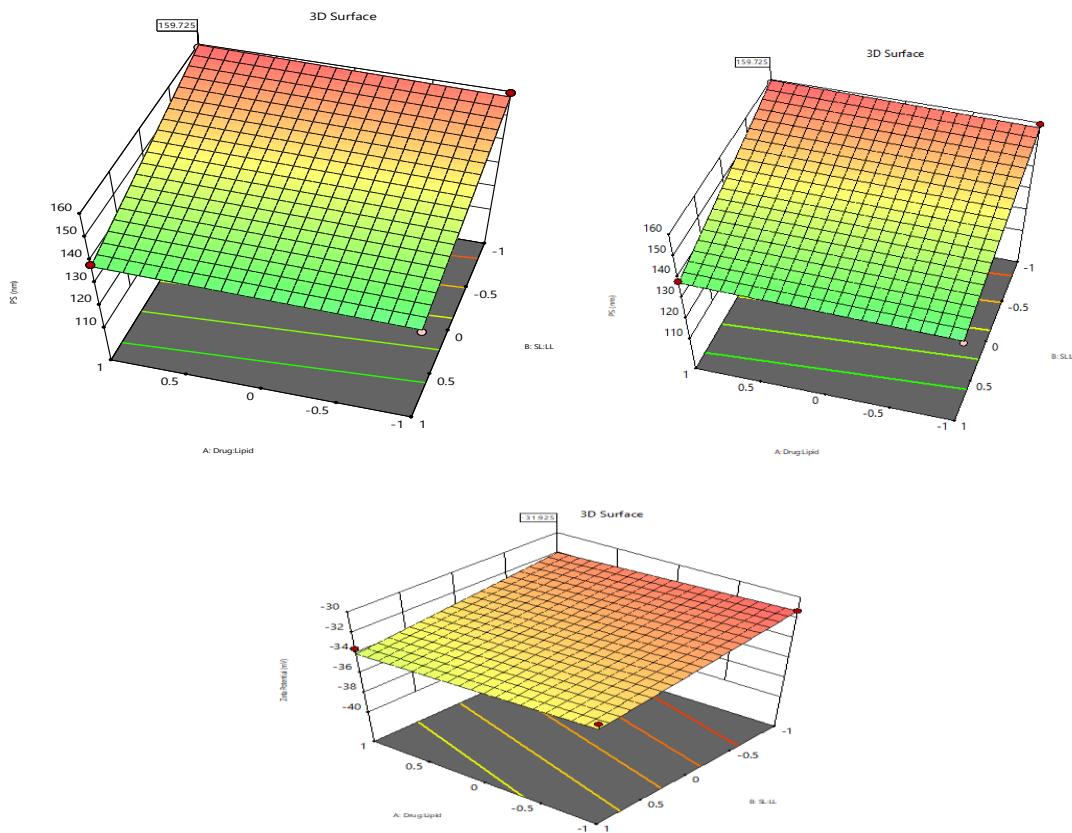


Figure 15(b): 3D surface plot for effect of independent variables on particle size, Zeta potential & EE of optimized formulation.

INVITRO DRUG RELEASE:

The in-vitro drug release studies were carried out on Franz diffusion cell apparatus using dialysis membrane using PBS 6.4. Optimized NLC showed the significant release from the prepared NLC, the initial burst release was observed at 60mins, followed by sustained release. This biphasic release pattern may be related to drug diffusion from the lipid matrix of NLCs, as has been evidenced for NLCs, related to the drug location in this lipid matrix. The rapid cooling of lipids during NLC preparation favours enrichment of the drug in the outer layers of the particles, leading to superficial entrapment, and consequently the initial burst release. The NLCs can also undergo partial erosion of the outer phospholipids layer, which results in fast diffusion of the hydrophobic drug. The release kinetic model that best fit the release data was evaluated by the correlation coefficient (r) value. The release data from DNZ-NLC followed the Korsmeyer-Peppas release kinetics model having $r = 0.9818$ with $n = 0.7257$ suggesting anomalous or non-Fickian diffusion, which is related to both the diffusion of the drug and dissolution of the NLC matrix. The goodness of models was evaluated with Akaike information criteria (AIC)=40.89 & Model Selection Criteria (MSC)=3.32. So, the sustained release pattern of DNZ over a period of 6h could be

attributed to drug diffusion through the lipid matrix of NLCs as well to the slow degradation of NLCs in the release medium.

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
30	5.23	5.96	1.076	4.89	2.313	3.80	10.22	3.35
60	15.36	14.44	2.76	6.25	10.19	19.58	20.30	7.85
120	30.53	25.72	5.90	7.75	23.85	23.75	37.59	18.58
180	46.17	38.84	13.1	10.10	40.3	29.31	57.53	35.14
240	68.64	53.68	23.76	15.43	52.11	31.74	69.17	53.46
300	72.42	56.09	41.35	32.31	54.29	39.06	78.43	70.55
360	73.00	59.58	44.74	42.67	63.95	54.62	84.45	82.84

Table 10: Results for In-vitro drug release

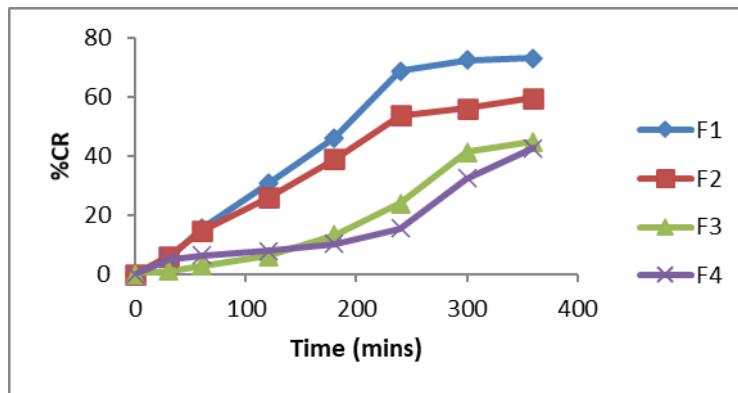


Figure 16(a): %CR Graph batches: F1-F4

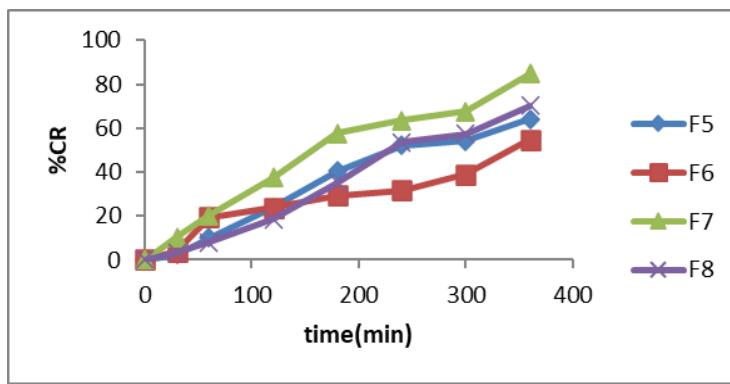


Figure 16(b): %CR Graph batches: F5-F8

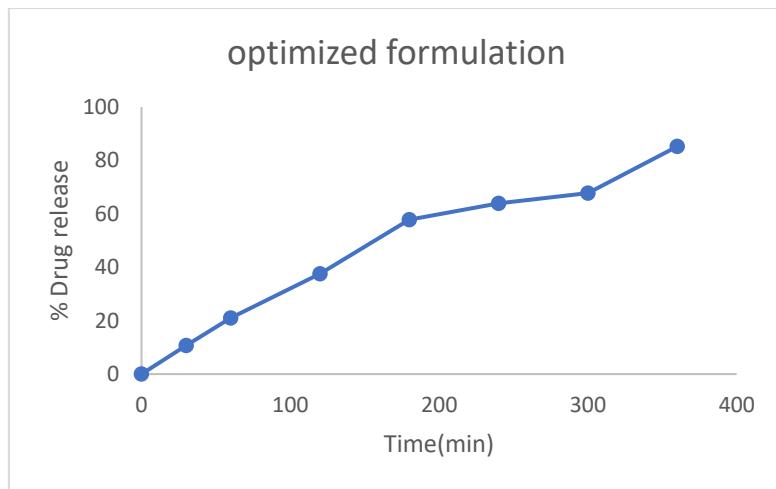


Figure 16(c): %CR Graph of optimized formulation

STABILITY STUDY:

The stability study was performed on Optimized NLC formulation for 0, 15, 30, 60 & 90 days at 4°C; the changes were observed & calculated as shown in table. There is significant decrease in pH towards acidic environment i.e., initially it was 6.4; after 90 days it changes to 5.2, particle size & zeta potential goes on increasing due to effect of solid lipids. The drug content was found to be 88.40% initially which reduces to 74.35%.

Table 11: Stability study of optimized NLC formulation.

Days	pH	Particle size	Zeta potential	Drug Content
0	6.4	107.4±2.64	-41.1	88.40±1.12
15	6.2	139.4±0.9	-39.1	86.90±1.12
30	6.2	158.9±0.67	-38.6	85.34±1.32
60	5.9	181.3±1.25	-35.9	82.98±1.78
90	5.2	228.8±2.58	-33.8	74.35±2.45

IN-VIVO STUDY:

Sample preparation

Briefly, brain homogenate (1 ml) and plasma samples (0.5 ml) were mixed with 0.2 ml of acetonitrile to precipitate out plasma proteins. The mixture was vortex mixed for 5 min; and centrifuged for 5min. The supernatant was taken and the volume was made up to 10 ml with mobile phase. Then 20µl of aqueous phase was injected directly into the HPLC column for drug content analysis.

Drug analysis in biological samples

RP-HPLC (Waters, USA) bio-analytical method was developed for the determination of donepezil HCl in plasma/tissue homogenate. Agilent C-18 column (150 4.6 mm, Agilent 1260 infinity) with a mobile phase of methanol, 0.02 M phosphate buffer and tri-ethyl amine (50:50:0.5 v/v) was used for analysis [43]. The pH of the mobile phase was adjusted to 3.2 by phosphoric acid in isocratic mode at a flow rate of 1 ml/min. Detection wavelength was set at 231 nm and 20 μ l injection volume was used. Calibration curve was prepared in the concentration range of 0.01 to 0.1 mg/ml by spiking known amount of donepezil HCl and 0.02 ml of fresh plasma obtained from rat. All data reported as mean \pm SD and the difference between the groups was tested using. [44] All data were dose and weight normalized. Pharmacokinetic parameters for donepezil HCl formulations were calculated using Kinetica 5.0 software. The Cmax and Tmax values after intranasal administration were read directly from the concentration–time profile. The AUC_{0–240} values obtained from curve were used to calculate the absolute bioavailability [45]. These findings are for intranasal administration of DNZ load NLC formulation of nose to brain direct pathway. The highest concentration was observed in plasma after IV administration, were Cmax was 1.0258(ug/ml) and 5.83305(ug/ml) at Tmax 2H and 15min in Brain and blood respectively whereas highest concentration after IN administration the Cmax was 4.597(ug/ml) and 2.2583(ug/ml) at Tmax 1hr in Brain and Blood respectively. In vivo study suggests that the high initial plasma concentration after IV administration may have caused lower transport of DNZ crossing BBB by passive diffusion. A statistically significant difference [P<0.05] between the two formulation was found from ANOVA analysis.

Fig17(a): Pharmacokinetic profiles of brain (concentration against time profile) for DNZ solution & DNZ-NLC delivered through IV & IN respectively.

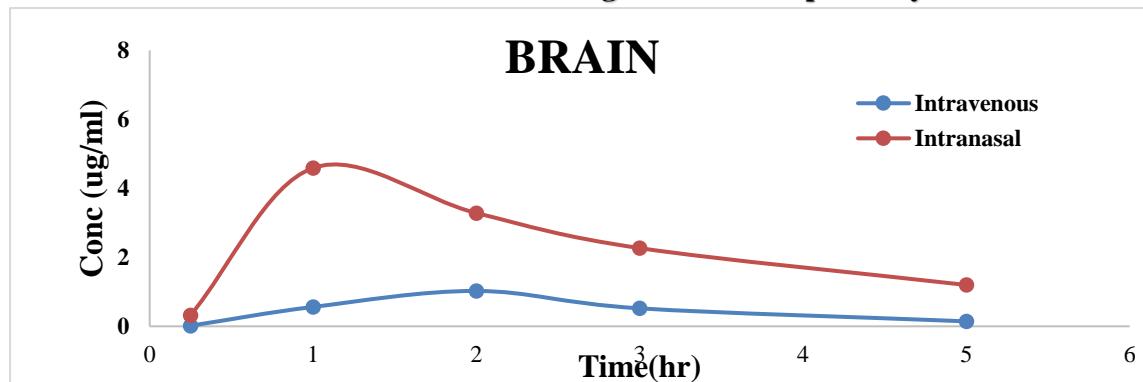
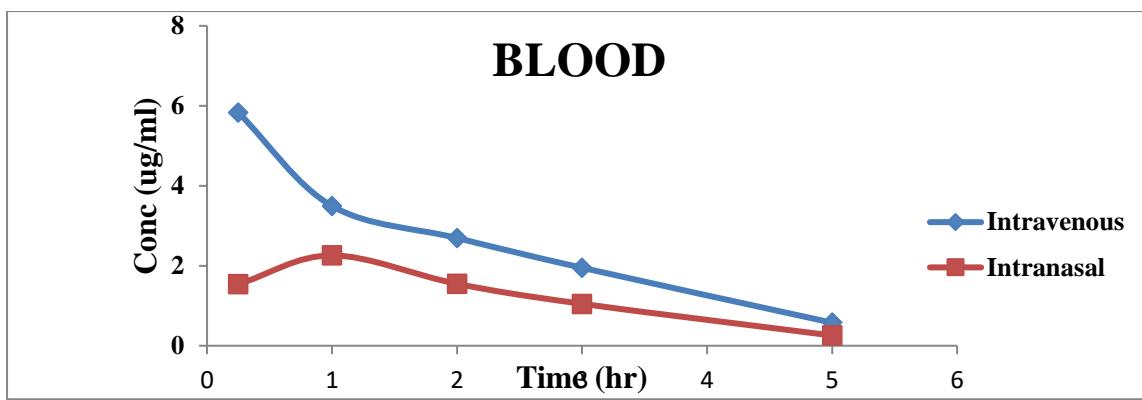


Fig17(b): Pharmacokinetic profiles of blood (concentration against time profile) for DNZ solution & DNZ-NLC delivered through IV & IN respectively

**Table 12 : Pharmacokinetic parameters of optimized Donepezil NLC formulation.**

Pharmacokinetic parameter	Tissue	Intravenous route	Intranasal route
Cmax(ug/ml)	Brain	1.0258	4.597
	Blood	5.83305	2.2583
Tmax(h)	Brain	2	1
	Blood	0.25	1
AUClast(ug/uL*h)	Brain	0.00178699	12.0606
	Blood	0.00964078	0.00481891
AUCextra(ug/uL*h)	Brain		3.51097
	Blood	0.00668131	0.00273574
MRT(h)	Brain		3.5128
	Blood	3.37226	2.93487

TIME(H)	BRAIN(IV) ug/ml	BRAIN(IN) ug/ml
0.25	0.017 ± 0.001	0.3310 ± 0.02
1	0.559 ± 0.08	4.414 ± 0.27
2	1.081 ± 0.07	3.25 ± 0.27
3	0.3526 ± 0.29	2.595 ± 0.06
5	0.1397 ± 0.058	1.179 ± 0.07
TIME	BLOOD(IV)	BLOOD(IN)
0.25	5.66 ± 0.11	1.816 ± 0.24
1	3.26 ± 0.15	1.160 ± 0.95
2	2.16 ± 0.30	1.291 ± 0.22
3	1.66 ± 0.16	1.027 ± 0.01
5	0.66 ± 0.06	0.353 ± 0.08

SUMMARY & CONCLUSION



SUMMARY:

Donepezil HCL is an Anti-Alzheimer drug which selectively and reversibly inhibits the acetylcholinesterase enzyme, which normally breaks down acetylcholine. As Donepezil HCl has oral bioavailability is 100%, but it needs to cross BBB, how much amount of Donepezil HCL reaches to the site of action is still unknown. Hence nose to brain delivery promises the direct availability at the site of action via nasal route in which drug will need not to cross BBB.

NLC are second generation & most stable nanoparticulate system which are used recently. NLC consist of solid lipid & liquid lipid, their imperfect matrix helps in greater entrapment of drug. NLC having capacity to overcome the poor loading capacity & tendency to particle growth. For formulating NLC the SL, LL, surfactant & co-surfactant are required. Here Glyceryl monostearate was selected as solid lipid as Donepezil HCl having highest solubility in GMS, Tween 80 & Poloxamer407 was used as surfactant & co-surfactant.

The Nigella sativa oil is selected as liquid lipid for its dual action as it is used in the formulation for antioxidant activity (oxidative stress is reduced) & itself act as Anti-Alzheimer's by inhibiting cholinesterase enzyme in the brain.

The antioxidant activity of Nigella sativa oil was studied using DPPH assay & comparing it with different oils to study the effect of oils. Amongst all (orange oil, rose oil, sesame oil, oleic acid & Nigella sativa oil) the Nigella sativa oil showed highest antioxidant activity according to assay. Thus, it was selected as major component of formulation.

Preformulation studies of Donepezil HCl were performed by determining melting point, solubility, FTIR, DSC & Drug-excipient interaction study. The drug was found to be compatible with selected excipients. UV spectroscopic study of drug was performed in phosphate buffer pH 6.4 which was suitable for nasal administration.

HPH (Hot High-Pressure Homogenizer) followed by ultrasonication method was used to formulate NLC. 2^3 factorial design was used to formulation & optimization of variables. The prepared NLC's were evaluated for physical properties, particle size, PDI, Zeta potential, drug content, entrapment efficiency, drug loading capacity, invitro diffusion study & in-vivo pharmacokinetic study.

The NLC formulations shown particle size, PDI & Zeta potential range in between 80-330nm, 0.2 to 0.4, -30 to -45mV respectively.

All formulation shown pH range in between 6.2 to 6.5 which is well suited to the nasal pH.

The maximum Entrapment efficiency & drug content was found to be 70.20% & 90.07%. Invitro release drug profile showed significant increase in % drug release also showing an initial burst release & sustained release action. The release kinetic model that best fit the release data from DNZ-NLC followed the Korsmeyer-Peppas release kinetics model having $r = 0.98$ with $n = 0.72$, suggesting anomalous or non-Fickian diffusion, which is related to both the diffusion of the drug and dissolution of the NLC matrix.

The highest concentration was observed in plasma after IV administration, where Cmax was 1.0258(ug/ml) and 5.83305(ug/ml) at Tmax 2H and 15min in Brain and blood respectively whereas highest concentration after IN administration the Cmax was 4.597(ug/ml) and 2.2583(ug/ml) at Tmax 1hr in Brain and Blood respectively. In vivo study suggests that the high initial plasma concentration after IV administration may have caused lower transport of DNZ crossing BBB by passive diffusion. A statistically significant difference [P<0.05] between the two formulation was found from ANOVA analysis.

CONCLUSION:

The Donepezil HCl loaded NLC were formulated for brain targeting through intranasal route where Nigella sativa oil was used as liquid lipid for its synergistic action & antioxidant activity. The GMS, tween 80 & P407 was used as solid lipid, surfactant & co-surfactant respectively. HPH followed by Ultrasonication method was used to prepare NLC dispersion. The 2^3 factorial design was used to formulation & optimization of variables, average particle size, PDI, Zeta potential was found to be 80-330nm, 0.2 to 0.4, -30 to -45mV respectively. The maximum Entrapment efficiency & drug content was found to be 70.20% & 90.07%. Invitro release drug profile showed significant increase in % drug release also showing a sustained release action. The release kinetic model that best fit the release data from DNZ-NLC followed the Korsmeyer-Peppas release kinetics model having $r = 0.98$ with $n = 0.72$, suggesting anomalous or non-Fickian diffusion, which is related to both the diffusion of the drug and dissolution of the NLC matrix. The highest concentration after IN administration was 4.597(ug/ml) and 2.2583(ug/ml) at Tmax 1hr in Brain and Blood respectively.

FUTURE PROSPECTS

FUTURE PROSPECTS:

- 1) Need to study on mucoadhesive nature of formulation by adding mucoadhesive agent for better adhering action

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ANNEXURE:

Sr no.	Achievements	Status
01	IAEC protocol [1379/PO/Re/S/10/CPCSEA]	Approved
02	Formulation & evaluation of Donepezil HCl loaded NLC for CNS targeting through intranasal delivery	Journal of Drug Delivery Science and Technology
03	Advances in nose to brain delivery of drugs to treat neurological disorders efficiently and effectively: A review	Advanced Pharma bulletin Status: Communicated
04	A review on microemulsion as novel approach for CNS targeting via nasal route	Advanced Pharma bulletin Status: Communicated

ERRATA:

