MICROEMULSION AS A CARRIER FOR NOSE TO BRAIN TARGETING: A REVIEW AND UPDATE

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ABSTRACT
There are various approaches in delivering a therapeutic substance to the target site in a controlled release fashion. One such approach is using microemulsion as carriers for drugs. Recently, there has been a considerable interest for the microemulsion formulation, for the delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilization capacity, long shelf life, easy of preparation and improvement of bioavailability. Microemulsion is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microemulsion received much attention not only for prolonged release, but also for targeting of drugs to a particular site. The intent of the paper is focuses on use of microemulsion technology in drug targeting to the brain along with mechanism of nose to brain transport, formulation and formation of microemulsion and its characterization.

Key words: Controlled release, Microemulsion, Drug carrier, Target site, Nose to Brain transport

INTRODUCTION

The selection of an appropriate dosage form is critical because a dosage form with poor drug delivery can make a useful drug worthless. Bioavailability has important clinical implications as both pharmacologic and toxic effects are proportional to both dose and bioavailability[1].

Many approaches have been meticulously explored to brain targeting of such drugs i.e. Nanoparticles, liposomes; but these methods are not always practical. For example, Nanoparticles are difficult to prepare and their preparation requires an additional step in order to attach or loading the drug to the particles. Also the prepared
Nanoparticles show often shows a wide size distribution, as opposed to microemulsion, which is monodispersed. Another disadvantage is the limited solubility of drug in the Nanoparticles. Finally the polymer generally used in Nanoparticles are not usually biocompatible, thus toxicity is another problem\(^2\).

Of late lipid-based formulations have attracted great deal of attention in Drug targeting and to improve the oral bioavailability of poorly water soluble drugs. In fact, the most favored approach is to incorporate lipophilic drugs into inert lipid vehicles such as oils, surfactant dispersions, microemulsions, self-emulsifying formulations and self microemulsifying formulations\(^3-6\).

Microemulsions are liquid dispersions of water and oil that are made homogenous, transparent or translucent and thermodynamically stable by the addition of relatively large amounts of a surfactant and a co-surfactant and having diameter of the droplets in the range of 10 – 100 nm\(^7, 8, 9-11\). Microemulsions have been widely studied for drug targeting to the brain and to enhance the bioavailability of the poorly soluble drugs\(^12\). They offer a cost effective approach in such cases. Microemulsions have very low surface tension and small droplet size which results in high absorption and permeation. Interest in these versatile carriers is increasing and their applications have been diversified to various administration routes in addition to the conventional oral route. This can be attributed to their unique solubilization properties and thermodynamic stability which has drawn attention for their use as carrier for drug targeting to the brain. Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation.

Literature survey revealed that intranasal administration of microemulsion offers a practical, noninvasive, alternative route of administration for drug delivery to the brain\(^13,14\). Intranasal administration allows transport of drugs to the brain circumventing BBB, thus providing better option to target drugs to the brain\(^15-19\). Therefore particularly intranasal microemulsions are promising carrier for achieving the goals in drug targeting to brain.

**INTRANASAL DELIVERY FOR BRAIN TARGETING**
Many drugs are not being effectively and efficiently delivered using conventional drug delivery approach to brain due to its complexity. Intranasal drug delivery is one of the focused delivery options for brain targeting, as the brain and nose compartments are connected to each other via the olfactory route and via peripheral circulation. Realization of nose-to-brain transport and the therapeutic viability of this route can be traced from the ancient times and has been investigated for rapid and effective transport in the last two decades. The development of nasal drug products for brain targeting is still faced with enormous challenges. A better understanding in terms of properties of the drug candidate, nose-to-brain transport mechanism, and transport to and within the brain is of utmost importance.

For some time the BBB has impeded the development of many potentially interesting CNS drug candidates due to their poor distribution into the CNS. Owing to the unique connection of the nose and the CNS, the intranasal route can deliver therapeutic agents to the brain bypassing the BBB. Absorption of drug across the olfactory region of the nose provides a unique feature and superior option to target drugs to brain. Many scientists have reported evidence of nose-to-brain transport. Many previously abandoned potent CNS drug candidates promise to become successful CNS therapeutic drugs via intranasal delivery. Recently, several nasal formulations, such as ergotamine (Novartis), sumatriptan (GlaxoSmithKline), and zolmitriptan (AstraZeneca) have been marketed to treat migraine. Scientists have also focused their research toward intranasal administration for drug delivery to the brain especially for the treatment of diseases, such as epilepsy\textsuperscript{[15,20-24]}, migraine\textsuperscript{[13,14,18,25-27]}, emesis, depression\textsuperscript{[28]}, angina pectoris\textsuperscript{[29]} and erectile dysfunction\textsuperscript{[30]}.

**MECHANISM OF NOSE TO BRAIN DRUG TRANSPORT**

It is important to examine the pathway/mechanisms involved prior to addressing the possibilities to improve transnasal uptake by the brain\textsuperscript{[31-33]}. The olfactory region is known to be the portal for a drug substance to enter from nose-to-brain following nasal absorption. Thus, transport across the olfactory epithelium is the predominant concern for brain targeted intranasal delivery. Nasal mucosa and subarachnoid space; lymphatic plexus located in nasal mucosa and subarachnoid space along with perineural sheaths in olfactory nerve filaments and subarachnoid space appears to have communications.
between them. The nasal drug delivery to the CNS is thought to involve either an intraneuronal or extraneuronal pathway\textsuperscript{[34, 35]}.

A drug can cross the olfactory path by one or more mechanism/pathways. These include \textit{paracellular transport} by movement of drug through interstitial space of cells \textit{transcellular} or simple diffusion across the membrane or receptor / fluid phase mediated endocytosis and \textit{transcytosis} by vesicle carrier\textsuperscript{[36]} and neuronal transport. The paracellular transport mechanism/route is slow and passive. It mainly uses an aqueous mode of transport. Usually, the drug passes through the tight junctions and the open clefts of the epithelial cells present in the nasal mucosa. There is an inverse log-log correlation between intranasal absorption and the molecular weight of water soluble compounds. Compounds, which are highly hydrophilic in nature and/or of low molecular weight, are most appropriate for paracellular transport. A sharp reduction in absorption and poor bioavailability was observed for the drugs having molecular weight greater than 1000 Da. Moreover, drugs can also cross cell membranes by a carrier – mediated active transport route. For example, chitosan, a natural biopolymer from shellfish, stretches and opens up the tight junctions between epithelial cells to facilitate drug transport. The transcellular transport mechanisms / pathways mainly encompass transport via a lipoidal route\textsuperscript{[37, 38]}. The drug can be transported across the nasal mucosa/epithelium by either receptor mediated endocytosis or passive diffusion or fluid phase endocytosis transcellular route. Highly lipophilic drugs are expected to have rapid/complete transnasal uptake. The olfactory neuron cells facilitate the drug transport principally to the olfactory bulb.

![Nose to brain transport routes](image)

\textbf{Figure 1}

Nose to brain transport routes
ADVANTAGES OF INTRANASAL DRUG DELIVERY

✓ Non-invasive, rapid and comfortable.
✓ Bypasses the BBB and targets the CNS, reducing systemic exposure and thus systemic exposure and thus systemic side effects.
✓ Does not require any modification of the therapeutic agent being delivered neurological and psychiatric disorders.
✓ Rich vasculature and highly permeable structure of the nasal mucosa greatly enhance drug absorption.
✓ Problem of degradation of peptide drugs in minimized up to a certain extent.
✓ Easy accessibility to blood capillaries.
✓ Avoid destruction in the gastrointestinal tract, hepatic first pass metabolism and increased bioavailability.

LIMITATIONS OF INTRANASAL DRUG DELIVERY

✓ Concentration achievable in different regions of the brain and spinal cord varies with each agent.
✓ Delivery is expected to decrease with increasing molecular weight of drug.
✓ Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.
✓ Nasal congestion due to cold or allergies may interfere with this method of delivery.
✓ Frequent use of this route may result in mucosal damage.

FACTORS AFFECTING BRAIN-TARGETED NASAL DELIVERY SYSTEMS

Some of the physicochemical, formulation and physiological factors are imperative and must be considered prior to designing intranasal delivery for brain targeting.

1. Physicochemical properties of drugs:
   
i) Chemical form: The chemical form of a drug is important in determining absorption. For example, conversion of the drug into a salt or ester form can also alter its absorption. Huang et al 1985 studied the effect of structural modification of drug on absorption\[^{39}\]. It was observed that in-situ nasal absorption of carboxylic acid esters of L-Tyrosine was significantly greater than that of L-Tyrosine.
ii) **Polymorphism**: Polymorphism is known to affect the dissolution rate and solubility of drugs and thus their absorption through biological membranes.

iii) **Molecular Weight**: A linear inverse correlation has been reported between the absorption of drugs and molecular weight up to 300 Da. Absorption decreases significantly if the molecular weight is greater than 1000 Da except with the use of absorption enhancers. The apparent cut-off point for molecular weight is approximately 1,000 with molecules less than 1,000 having better absorption. Shape is also important. Linear molecules have lower absorption than cyclic – shaped molecules.

iv) **Particle Size**: It has been reported that particle sizes greater than 10μm are deposited in the nasal cavity. Particles that are 2 to 10 μm can be retained in the lungs and particles of less than 1 μm are exhaled.

v) **Solubility & dissolution Rate**: Drug solubility and dissolution rates are important factors in determining nasal absorption from powders and suspensions. The particles deposited in the nasal cavity need to be dissolved prior to absorption. If a drug remains as particles or is cleared away, no absorption occurs.

2. **Formulation factors**:

i) **pH of the formulation**: Both the pH of the nasal cavity and pKa of a particular drug need to be considered to optimize systemic absorption. Nasal irritation is minimized when products are delivered with a pH range of 4.5 to 6.5. Also, volume and concentration are important to consider. The delivery volume is limited by the size of the nasal cavity. An upper limit of 25 mg/dose and a volume of 25 to 200 μL/ nostril have been suggested.

- To avoid irritation of nasal mucosa;
- To allow the drug to be available in unionized form for absorption;
- To prevent growth of pathogenic bacteria in the nasal passage;
- To maintain functionality of excipients such as preservatives; and
- To sustain normal physiological ciliary movement.

Lysozyme is found in nasal secretions, which is responsible for destroying certain bacteria at acidic pH. Under alkaline conditions, lysozyme is inactivated and the nasal tissue is susceptible to microbial infection. It is therefore advisable to keep the
formulation at a pH of 4.5 to 6.5 keeping in mind the physicochemical properties of the drug as drugs are absorbed in the unionized form.

ii) **Buffer Capacity:** Nasal formulations are generally administered in small volumes ranging from 25 to 200μL. Hence, nasal secretions may alter the pH of the administrated dose. This can affects the concentration of unionized drug available for absorption. Therefore, an adequate formulation buffer capacity may be required to maintain the pH in-situ.

iii) **Osmolarity:** Drug absorption can be affected by tonicity of formulation. Shrinkage of epithelial cells has been observed in the presence of hypertonic solutions. Hypertonic saline solutions also inhibit or cease ciliary activity. Low pH has a similar effect as that of a hypertonic solution.

iv) **Gelling / Viscosity building agents or gel-forming carriers:** Pennington et al 1988 studied that increase in solution viscosity may provide a means of prolonging the therapeutic effect of nasal preparations. Suzuki et al 1999 showed that a drug carrier such as hydroxypropyl cellulose was effective for improving the absorption of low molecular weight drugs but did not produce the same effect for high molecular weight peptides. Use of a combination of carriers is often recommended from a safety (nasal irritancy) point of view.

v) **Solubilizers:** Aqueous solubility of drug is always a limitation for nasal drug delivery in solution. Conventional solvents or co-solvents such as glycols, small quantities of alcohol, Transcutol (diethylene glycol monoethyl ether), medium chainnglycerides and Labrasol can be used to enhance the solubility of drugs. Other options include the use of surfactants or cyclodextrins such as HP-β-cyclodextrin that serve as a biocompatible solubilizer and stabilizer in combination with lipophilic absorption enhancers.

vi) **Preservatives:** Most nasal formulations are aqueous based and need preservatives to prevent microbial growth. Parabens, benzalkonium chloride, phenyl ethyl alcohol, EDTA and benzoyl alcohol are some of the commonly used preservatives in nasal formulations. Van De Donk et al 1980 have shown that mercury containing preservatives have a fast and irreversible effect on ciliary movement and should not be used in the nasal systems.
vii) **Antioxidants:** Usually, antioxidants do not affect drug absorption or cause nasal irritation. Chemical / physical interaction of antioxidants and preservatives with drugs, excipients, manufacturing equipment and packaging components should be considered as part of the formulation development program. Commonly used antioxidants are sodium metabisulfite, sodium bisulfite, butylated hydroxytoluene and tocopherol.

viii) **Humectants:** Many allergic and chronic diseases are often connected with crusts and drying of mucous membrane. Adequate intranasal moisture is essential for preventing dehydration. Therefore humectants can be added especially in gel-based nasal products. Humectants avoid nasal irritation and are not likely to affect drug absorption. Common examples include glycerin, sorbitol and mannitol.

ix) **Drug Concentration, Dose & Dose Volume:** Drug concentration, dose and volume of administration are three interrelated parameters that impact the performance of the nasal delivery performance. Nasal absorption of L-Tyrosine was shown to increase with drug concentration in nasal perfusion experiments.

x) **Role of Absorption Enhancers:** Absorption enhancers may be required when a drug exhibits poor membrane permeability, large molecular size, lack of lipophilicity and enzymatic degradation by amino peptidases. Osmolarity and pH may accelerate the enhancing effect. Examples of enhancing agents are surfactants, glycosides, cyclodextrins, and glycols. Absorption enhancers improve absorption through many different mechanisms, such as increasing membrane fluidity, increasing nasal blood flow, decreasing mucus viscosity, and enzyme inhibition.

3. **Physiological factors:**

i) **Effect of Deposition on Absorption:** Deposition of the formulation in the anterior portion of the nose provides a longer nasal residence time. The anterior portion of the nose is an area of low permeability while posterior portion of the nose where the drug permeability is generally higher, provides shorter residence time.

ii) **Nasal blood flow:** Nasal mucosal membrane is very rich in vasculature and plays a vital role in the thermal regulation and humidification of the inhaled air. The blood flow and therefore the drug absorption will depend upon the vasoconstriction and vasodilatation of the blood vessels.
iii) **Effect of Mucociliary Clearance:** The absorption of drugs is influenced by the residence (contact) time between the drug and the epithelial tissue. The mucociliary clearance is inversely related to the residence time and therefore inversely proportional to the absorption of drugs administered. A prolonged residence time in the nasal cavity may also be achieved by using bioadhesive polymers or by increasing the viscosity of the formulation.

iv) **Effect of Enzymatic Activity:** Several enzymes that are present in the nasal mucosa might affect the stability of drugs. For example, proteins and peptides are subjected to degradation by proteases and amino-peptidase at the mucosal membrane. The level of amino-peptidase present is much lower than that in the gastrointestinal tract. Peptides may also form complexes with immunoglobulin (Igs) in the nasal cavity leading to an increase in the molecular weight and a reduction of permeability.

v) **Effect of Pathological Condition:** Intranasal pathologies such as allergic rhinitis, infections, or pervious nasal surgery may affect the nasal mucociliary transport process and/or capacity for nasal absorption. During the common cold, the efficiency of an intranasal medication is often compromised. Nasal clearance is reduced in insulin-dependent diabetes. Nasal pathology can also alter mucosal pH and thus affect absorption of drugs.

**MICROEMULSIONS AS INTRA NASAL DRUG DELIVERY**

Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multi component fluids composed of oil, water and surfactant (usually in conjunction with a cosurfactant). The droplets in a microemulsion are in the range of 1 nm-100 nm in diameter. The basic difference between emulsions and microemulsions is that emulsions exhibit excellent kinetic stability but they are thermodynamically unstable as compared to microemulsions. In recent years microemulsions have attracted a great deal of attention because of their biocompatibility, biodegradability, ease of preparation and handling and most importantly solubilization capacity for both water and oil soluble drugs.
Microemulsions have various textures such as oil droplets in water, water droplets in oil, bi continuous mixtures (Fig 2).

**WHY MICROEMULSION IS PREFERENCES OVER THE OTHER DOSAGE FORMS**

In recent years microemulsions have attracted a great deal of attention because of their following advantages;

1. Ease of manufacturing and scale-up.
2. Wide applications in colloidal drug delivery systems for the purpose of drug targeting and controlled release.
3. Helps in solubilization of lipophillic drug hence Increase the rate of absorption and bioavailability of drugs\[12\].
4. Eliminates variability in absorption.
5. Provides a aqueous dosage form for water insoluble drugs.
6. Various routes like tropical, oral and intravenous can be used to deliver the drugs\(^{[45]}\).

7. Rapid and efficient penetration of the drug moiety.

8. Helpful in taste masking.

9. Same microemulsions can carry both lipophilic and hydrophilic drugs\(^{[46]}\).

10. Provides protection from hydrolysis and oxidation as drug in oil phase in O/W microemulsion is not exposed to attack by water and air.

11. Liquid dosage form increases patient compliance.

12. Less amount of energy requirement.

13. Microemulsion lower the skin irritation: alcohol-free microemulsions have been reported with much lower irritation potential.

14. Long self life as compared to other colloidal drug delivery system.

15. High drug loading.

16. Improve therapeutic efficacy of drugs and allow reduction in the volume of the drug delivery vehicle, thus minimizing toxic side effects\(^{[47]}\).

17. Easy to administer in child and adults who have difficulty swallowing solid dosage forms.

**WHY MICROEMULSIONS ARE CHOSEN FOR NOSE TO BRAIN DRUG DELIVERY**

Literature survey revealed that intranasal administration of microemulsion offers a practical, noninvasive, alternative route of administration for drug delivery to the brain\(^{[13, 14]}\). Intranasal administration allows transport of drugs to the brain circumventing BBB, thus providing better option to target drugs to the brain\(^{[15-19]}\). Microemulsion lower the skin irritation: alcohol-free microemulsions have been reported with much lower irritation potential.

**CHALLENGES IN NOSE TO BRAIN DRUG DELIVERY VIA MICROEMULSION**

1. The main problem in a microemulsion application is a high concentration and a narrow range of physiologically acceptable surfactants and co-surfactants\(^{[48, 49]}\).

2. Large surfactant concentration (10-40%) determines their stability\(^{[50]}\).
3. Selection of components: if the systems are to be used topically, selection of components involves a consideration of their toxicity, irritation and sensitivity.[51]
4. Nasal congestion due to cold or allergies may interfere with absorption of drug through nasal mucosa.
5. Delivery is expected to decrease with increasing molecular weight of drug.
6. Some therapeutic agents may be susceptible to partial degradation in the nasal mucosa or may cause irritation to the mucosa.
7. Concentration achievable in different regions of the brain and spinal cord varies with each agent.
8. Fluidity of interfacial film should be low to promote the formulation of microemulsion[52].

FORMULATION OF MICROEMULSIONS

Microemulsions are isotropic systems, which are difficult to formulate, than ordinary emulsions because formulation is a highly specific process involving spontaneous interactions among the constituent molecules. Generally, the microemulsion formulation requires following components:

a) **Oil Phase:** Toluene, Cyclohexane, mineral oil or vegetable oils, silicone oils or esters of fatty acids etc. have been widely investigated as oil components[53].

b) **Aqueous phase:** Aqueous phase may contain hydrophilic active ingredients and preservatives. Some workers have utilized buffer solutions as aqueous phase.

c) **Primary surfactant:** The surfactants are generally ionic, non ionic or amphoteric. The surfactants chosen are generally for the non ionic group because of their good cutaneous tolerance. Only for specific cases, amphoteric surfactants are being investigated[48, 49].

d) **Secondary surfactant (cosurfactant):** In most cases, single-chain surfactants alone are unable to reduce the o/w interfacial tension sufficiently to enable a microemulsion to form[45,54]. The presence of co-surfactants allows the interfacial film sufficient flexibility to take up different curvatures required to form microemulsion over a wide range of composition[53-57]. Co surfactants originally used were short chain fatty alcohols (pentanol, hexanol, benzyl alcohol). These are most often polyols, esters of polyols, derivatives of glycerol and organic acids. Their main purpose is to make inter facial film fluid by wedging themselves between the surfactant molecules.
PREPARATION OF MICROEMULSION

1. Phase Titration Method:

Microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. Microemulsions are formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. The understanding of their phase equilibria and demarcation of the phase boundaries are essential aspects of the study. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component Fig. (3).

![Pseudoternary phase diagram of oil, water and surfactant showing microemulsion region.](image)

The region can be separated into w/o or o/w microemulsion by simply considering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the metastable systems are not included. The methodology has been comprehensively discussed by Shafiq-un-Nabi et al.\textsuperscript{[58]}.
2. Phase Inversion Method:

Phase inversion of microemulsions occurs upon addition of excess of the dispersed phase or in response to temperature. During phase inversion drastic physical changes occur including changes in particle size that can affect drug release both in vivo and in vitro. These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, this can be achieved by changing the temperature of the system, forcing a transition from an o/w microemulsion at low temperatures to a w/o microemulsion at higher temperatures (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. This method is referred to as phase inversion temperature (PIT) method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone.

Additionally, a transition in the spontaneous radius of curvature can be obtained by changing the water volume fraction. By successively adding water into oil, initially water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a w/o microemulsion to an o/w microemulsion at the inversion locus. Short-chain surfactants form flexible monolayer at the o/w interface resulting in a bicontinuous microemulsion at the inversion point.

CHARACTERIZATION OF MICROEMULSION

The characterization of these systems is highly challenging due to their small droplet size with fluctuating boundaries and complex structure. The droplet size, viscosity, density, turbidity, refractive index, phase separation and pH measurements shall be performed to characterize the microemulsion. The droplet size distribution of microemulsion vesicles can be determined by either light scattering technique or electron microscopy. This technique has been advocated as the best method for predicting microemulsion stability.

1. Dynamic light-scattering measurements\[^{3,59-61}\]: The DLS measurements are taken at 90° in a dynamic light-scattering spectrophotometer which uses a neon laser of
wavelength 632 nm. The data processing is done in the built-in computer with the instrument.

2. **Polydispersity:** This property is characterized by Abbe refractometer.

3. **Phase analysis:** To determine the type if microemulsion that has formed the phase system (o/w or w/o) of the microemulsion is determined by measuring the electrical conductivity using a conductometer.

4. **Viscosity measurement:** The viscosity of microemulsions of several compositions can be measured at different shear rates at different temperatures using Brookfield type rotary viscometer. The sample room of the instrument must be maintained at 37 ± 0.2°C by a thermobath, and the samples for the measurement are to be immersed in it before testing.

5. **In Vitro Drug Permeation Studies: Determination of permeability coefficient and flux:**

   Excised human cadaver skin from the abdomen can be obtained from dead who have undergone postmortem not more than 5 days ago in the hospital. The skin is stored at 4°C and the epidermis separated. The skin is first immersed in purified water at 60°C for 2 min and the epidermis then peeled off. Dried skin samples can be kept at -20°C for later use. Alternatively the full thickness dorsal skin of male hairless mice may be used. The skin shall be excised, washed with normal saline and used. The passive permeability of lipophilic drug through the skin is investigated using Franz diffusion cells with known effective diffusional area. The hydrated skin samples are used. The receiver compartment may contain a complexing agent like cyclodextrin in the receiver phase, which shall increase the solubility and allows the maintenance of sink conditions in the experiments. Samples are withdrawn at regular interval and analyzed for amount of drug released.

6. **In Vivo Studies:**

   **A. Bioavailability studies: Skin bioavailability of topical applied microemulsion on rats:** Male Sprague–Dawley rats (400–500 g), need to be anesthetized (15 mg/kg pentobarbital sodium i.p.) and placed on their back. The hair on abdominal skin shall be trimmed off and then bathed gently with distilled water. Anesthesia should be maintained with 0.1-ml pentobarbital (15 mg/ml) along the experiment.
Microemulsions must be applied on the skin surface (1.8 cm²) and glued to the skin by a silicon rubber. After 10, 30 and 60 min of in vivo study, the rats shall be killed by aspiration of ethyl ether. The drug exposed skin areas shall be swabbed three to four times with three layers of gauze pads, then bathed for 30 s with running water, wiped carefully, tape-stripped (X10 strips) and harvested from the animals.

B. Determination of residual drug remaining in the skin on tropical administration: The skin in the above permeation studies can be used to determine the amount of drug in the skin. The skin cleaned with gauze soaked in 0.05% solution of sodium lauryl sulfate and shall bath with distilled water. The permeation area shall be cut and weighed and drug content can be determined in the clear solution obtained after extracting with a suitable solvent and centrifuging.

C. Pharmacological Studies: Therapeutic effectiveness can be evaluated for the specific pharmacological action that the drug purports to show as per stated guidelines.

D. Estimation of Skin Irritancy: As the formulation is intended for dermal application skin irritancy should be tested. The dorsal area of the trunk is shaved with clippers 24 hours before the experiment. The skin shall be scarred with a lancet. 0.5 ml of product is applied and then covered with gauze and a polyethylene film and fixed with hypoallergenic adhesive bandage. The test be removed after 24 hours and the exposed skin is graded for formation of edema and erythema. Scoring is repeated 72 hours later. Based on the scoring the formulation shall be graded as ‘non-irritant’, ‘irritant’ and ‘highly irritant’.

7. Stability Studies:

The physical stability of the microemulsion must be determined under different storage conditions (4, 25 and 40 °C) during 12 months. Fresh preparations as well as those that have been kept under various stress conditions for extended period of time are subjected to droplet size distribution analysis. Effect of surfactant and their concentration on size of droplet is also studied.

APPLICATION OF MICROEMULSION IN BRAIN TARGETING

The treatment of CNS disorders are challenging because of a variety of formidable obstacles for effective and persistent delivery of drugs. Even though the drugs used for the treatment of CNS disorders are potent, their clinical failure is often not due
to lack of drug efficacy but mainly due to shortcomings in the drug delivery approach. Hence, scientists are exploring the novel approaches so that delivery of the drugs can be enhanced and/or restricted to the brain and CNS. Many advanced and effective approaches to the CNS delivery of drugs have emerged in recent years. Intranasal administration confers a simple, economic, convenient and noninvasive route for rapid drug delivery to the brain \cite{62-64}. It allows a direct transport of drugs to the brain through brain barriers \cite{16-19}.

1. Treatment of Epilepsy and schizophrenia:

Vyas et al. prepared mucoadhesive microemulsion for an antiepileptic drug clonazepam \cite{15}. The aim was to provide rapid delivery to the rat brain. Brain/blood ratio at all sampling points up to 8h following intranasal administration of clonazepam mucoadhesive microemulsion compared to i.v. was found to be 2-fold higher indicating larger extent of distribution of the drug in the brain. Kwatikar et al \cite{65} prepared microemulsion containing valproic acid showed a fractional diffusion efficiency and better brain bioavailability efficiency. Hence microemulsions are the promising approach for delivery of valproic acid to the brain for treatment of epilepsy.

Florence et al \cite{20} has prepared Clobazam microemulsion and mucoadhesive microemulsion. Formulations were assessed for the average onset of seizures in pentylene tetrazole treated mice. This study demonstrated high brain targeting efficiency of prepared Clobazam mucoadhesive microemulsion and delayed onset of seizures induced by pentylene tetrazole in mice after intranasal administration of developed formulation. However, clinical evaluation of the developed formulation may result into a product suitable for the treatment of acute seizures due to status epileptics and patients suffering from drug tolerance and hepatic impairment on chronic use in the treatment of epileptics, schizophrenia and anxiety.

Shende et al \cite{66} prepred microemulsion of lomotrigone from nose to brain delivery. Intranasal administration allows transport of the drug to the brain circumventing BBB, thus providing the better option to target drug to the brain with quick onset of action in case of emergency in epilepsy. Lorazepam (LZM) is a poorly water-soluble drug which can be used as tranquilizers, muscle relaxant, sleep inducer, sedative and antiepileptic agent\cite{21}. However, cosolvent
based parenteral formulations suffer from several disadvantages such as pain and tissue damage at the site of injection and precipitation of the drug on dilution in several cases \[22\]. Furthermore, parenteral administration of the organic cosolvents can also cause hemolysis \[23\]. Amit et al\[24\] has prepared Lorazepam microemulsions and investigate that microemulsion have very low hemolytic potential and exhibit good physical and chemical stability and can be considered as a viable alternative to the currently marketed Lorazepam formulations.

2. Treatment of Migraine:

Migraine treatment has evolved into the scientific arena, but opinions differ on whether migraine is primarily a vascular or a neurological dysfunction \[25, 26\]. Sumatriptan is rapidly but incompletely absorbed following oral administration and undergoes first-pass metabolism, resulting in a low absolute bioavailability of 14% in humans. \[27\]. Moreover, the transport of Sumatriptan across the blood-brain barrier (BBB) is very poor\[13\]. Studies have demonstrated that intranasal administration offers a practical, noninvasive, alternative route of administration for drug delivery to the brain \[13, 14\]. Vyas et al\[18\] Prepared mucoadhesive microemulsion of Sumatriptan which shows rapid and larger extent of selective Sumatriptan nose-to-brain transport compared with Suspension and Microparticles of the same in rats. Enhanced rate and extent of transport of Sumatriptan following intranasal administration of microemulsion may help in decreasing the dose and frequency of dosing and possibly maximize the therapeutic index.

Shelke et al\[67\] has reported that Zolmitriptan microemulsion from nose to brain delivery provide the dual advantages of enhanced bioavailability with rapid onset of action in treatment of migraine. Tushar et al \[68\] has investigated zolmitriptan microemulsions (ZTME) for rapid drug delivery to the brain to treat acute attacks of migraine and to characterize microemulsions and evaluate biodistribution in rats. Studies of this investigation conclusively demonstrated rapid and larger extent of transport into the rat brain following intranasal administration of ZMME and can play a promising role in the treatment of acute attacks of migraine.

3. As an Antidepressant:
Tiwari et al. [28] has developed Eucalyptus oil microemulsion for intranasal delivery to the brain. This work demonstrated that the microemulsion of eucalyptus oil is cost effective and efficient formulation which provides the rapid onset in soothing stimulant and antidepressant action.

4. Treatment of angina Pectoris and neurological deficit:

Qizhi Zhang [29] has prepared the microemulsion to improve the solubility and enhance the brain uptake of nimodipine (NM), which was suitable for intranasal delivery. The uptake of NM in the olfactory bulb from the nasal route was three folds, compared with intravenous (i.v.) injection. The ratios of AUC in brain tissues and cerebrospinal fluid to that in plasma obtained after nasal administration were significantly higher than those after i.v. administration. These results suggest that the microemulsion system is a promising approach for intranasal delivery of NM for the treatment and prevention of neurodegenerative diseases.

Jing Yao [69] has prepared hyaluronic acid chitosan-based microemulsion (HAC-ME) containing nobiletin and determines its distribution in mice brain following i.v. administration. Based on AUC\(_{0-t}\), MRT and \(C_{max}\), HAC-ME delivered more nobiletin to the brain compared to nobiletin solution. These results indicate that HAC-ME may be presented as potential candidates for delivering more drugs into the brain.

5. Treatment of Amnesia:

Jogani and Misra, 2008 has studied microemulsion /mucoadhesive microemulsion of tacrine, assessed its pharmacokinetic and pharmacodynamic performances for brain targeting and for improvement in memory in scopolamine-induced amnesic mice [70]. The results demonstrated rapid and larger extent of transport of tacrine into the mice brain and faster regain of memory loss in scopolamine-induced amnesic mice after intranasal microemulsion administration.

OTHER APPLICATION OF PHARMACEUTICAL MICROEMULSION

1. Tumor Targeting:

Microemulsions successfully utilize to deliver the various antineoplastics/antitumor agents, viz. doxorubicin, idarubicin, tetrabenzamidine derivative [71]. Cyclosporine delivery has remained a challenge for the formulation scientists. It is an immunosuppressive agent and is widely used in recipients of organ transplants and in
various autoimmune diseases. The inherent insolubility of the cyclosporine provides the major role for the low and variable bioavailability and low formulation stability during storage. Due to poor bioavailability, higher doses may be needed which may result in undesirable adverse side effects such as nephrotoxicity and renal side effects. Sandoz Ltd. introduced an improved formulation later under the trademark Neoral® according to the composition disclosed in US Patent 5342625. The Neoral formulation was a microemulsion pre-concentrate which contained cyclosporine in an anhydrous oily vehicle, medium chain triglycerides, surfactants, glycerol and alcohol which exhibited better and more consistent bioavailability.\[72\]

Maranh et al.\[73\] suggested the utility of microemulsions as vehicles for the delivery of chemotherapeutic or diagnostic agents to neoplastic cells while avoiding normal cells. They claimed a method for treating neoplasms, wherein neoplasms cells have an increased number of LDL (low density, lipoprotein) receptors compared to normal cells. The microemulsion comprised of a nucleus of cholesterol esters and not more than 20% triglycerides surrounded by a core of phospholipids and free cholesterol and contained a chemotherapeutic drug. Microemulsions were similar in chemical composition to the lipid portion of low density lipoprotein (LDL), but did not contain the protein portion.

These artificial microemulsion particles incorporated plasma apolipoprotein E (apo E) on to their surface when they were injected in the blood stream or incubated with plasma. The apolipoprotein E served as a linking element between the particles of the microemulsion and the LDL receptors. The microemulsions could then be incorporated into cells via receptors for LDL and delivered the incorporated molecules.

Thus, higher concentration of anticancer drugs could be achieved in the neoplastic cells that have an increased expression of the receptors. In this way toxic effects of these drugs on the normal tissues and organs could be avoided. In human subjects, they observed no change in the plasma kinetics of the radioactively labeled microemulsion containing carmustine or cytosine-arabinoside thereby confirming that the incorporation of these drugs did not diminish the capacity of the microemulsion to incorporate apo E in the plasma and bind to the receptors. Shiokawa and coworkers reported a novel microemulsion formulation for tumor targeted drug carrier of lipophilic antitumor
antibiotic aclacinomycin A (ACM) \cite{74}. Their findings suggested that a folate-linked microemulsion is feasible for tumor targeted ACM delivery. The study showed that folate modification with a sufficiently long PEG chain on emulsions is an effective way of targeting emulsion to tumor cells.

Intravenous administration of cisplatin produces adverse effects like ototoxicity, renal failure, nephrotoxicity and chronic neurotoxicity\cite{75}. Hence, to overcome these inherent drawbacks associated with parenteral drug delivery of cisplatin an attempt is being made by Doizad \textit{et al} \cite{76} to provide cisplatin in the form of Solid-Lipid Nanoparticles using microemulsion technology to reduce the adverse effects and to enhance therapeutic efficacy of the drug.

Dongxing \textit{et al} \cite{77} has investigated the levels of raltitrexed (RTX) in blood and different brain tissues in rats and to find out whether there is any direct drug transport from nasal cavity to brain tissues following intranasal (i.n.) administration. AUC values in four brain regions by the nasal route were 54 to 121 fold compared with the i.v. route. Results showed that antineoplastic RTX could be directly transported into the brain tissue via the olfactory pathway in rats.

2. \textit{As a permeation enhancer}:

Microemulsions may affect the permeability of drug in the skin. In this case, the components of microemulsions serve as permeation enhancers. Several compounds used in microemulsions have been reported to improve the transdermal permeation by altering the structure of the stratum corneum. For example, short chain alkanols are widely used as permeation enhancers 3. The dispersed phase, lipophilic or hydrophilic (o/w or w/o type) can act as a potential reservoir of lipophilic or hydrophilic drugs that can be partitioned between the dispersed and the continuous phases. Coming in contact with a semi permeable membrane, such as skin or mucous membrane, the drug can be transported through the barrier\cite{71}. Microemulsions improve the transdermal delivery of several drugs over the conventional topical preparations such as emulsions\cite{78}.

3. \textit{Parenteral Delivery}:

Drugs with poor solubility are difficult to administer through parenteral route because of the extremely low amount of drug actually delivered to a targeted site. Microemulsion formulations are advantageous in Parenteral drug delivery because of the
fine particle microemulsion is cleared more slowly and, therefore, have a longer residence time in the body. An alternative approach was taken by Von Corsewant and Thoren\cite{48} in which C3-C4 alcohols were replaced with parenterally acceptable co-surfactants, polyethylene glycol (400) / polyethylene glycol (660) 12-hydroxystearate / ethanol, while maintaining a flexible surfactant film and spontaneous curvature near zero to obtain and almost balanced middle phase microemulsion.

4. Oral Drug Delivery:

Microemulsions formulations offer the several benefits over conventional oral formulation including increased absorption, improved clinical potency, and decreased drug toxicity\cite{79}. Literature survey revealed that microemulsion is ideal candidate for oral delivery of drugs such as steroids, hormones, diuretic and antibiotics. Because of low oral bioavailability, most protein drugs are only available as parenteral formulations. However, peptide drugs have an extremely short biological half life when administered parenterally, so require multiple dosing. A microemulsion formulation of cyclosporine, named Neoral® has been introduced to replace Sandimmune®, a crude oil-in-water emulsion of cyclosporine formulation. Neoral® is formulated with a finer dispersion, giving it a more rapid and predictable absorption and less inter and intra patient variability\cite{80}.

5. Ocular Drug Delivery: O/W microemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to increase absorption and to attain prolong release profile. The microemulsions containing pilocarpine were formulated using lecithin, propylene glycol and PEG 200 as co-surfactant and IPM as the oil phase. The formulations were of low viscosity with a refractive index lending to ophthalmologic applications\cite{44}.

6. Application in Biotechnology:

Many enzymes including lipases, esterases, dehydrogenases and oxidases often function in the cells in microenvironments that are hydrophobic in nature. In biological systems many enzymes operate at the interface between hydrophobic and hydrophilic domains and these usually interfaces are stabilized by polar lipids and other natural amphiphiles. Enzymatic catalysis in microemulsions has been used for a variety of
reactions, such as synthesis of esters, peptides and sugar acetals transesterification; various hydrolysis reactions and steroid transformation.

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