Nanoemulsion - A Review

Hitaishi Panchal[1], Reema Agarwal[2], Shraddha Patel[3]
[1, 2, 3] Shree Swaminarayan Sanskar Pharmacy College At &
Post: Zundal, Gandhinagar- 382421.

Abstract- *Nanoemulsions are a colloidal particulate system in the submicron size range which act as drug carriers for improving the delivery of therapeutic agents. Nanoemulsion globule size falls typically in the range of 20-200 nm and shows a narrow size distribution. Nanoemulsions plays a major role in pharmaceutical and cosmaceutical fields. These are the thermodynamically stable isotropic system in which two immiscible liquids are mixed to form a single phase by means of an emulsifying agent, i.e., surfactant and co-surfactant.This review provides brief information about the method of preparation and evaluation of nanoemulsion as drug carriers for improving the delivery of therapeutic agents. There are several techniques are to be used for preparation of nanoemulsions like high pressure homogenization, micro fluidization, spontaneous emulsification, low energy emulsification, Hydrogel method and solvent evaporation method. Parameter that are to be used for its characterization like droplet size analysis, viscosity determination, dilution test, drug content, refractive index, dye test, polydispersity, pH, zeta potential Study.*

Keywords- Nanoemulsion,Globules, Emulsification, Surfactant, Droplet, Pharmaceutical.

I. INTRODUCTION

Nanoemulsions are a colloidal particulate system in the submicron size range acting as carriers of drug molecules. Nanoemulsions are colloidal dispersions com-posed of an oil phase, aqueous phase, and surfactant and co-surfactants at appropriate ratios. Unlike coarse emulsions micronized with external energy nanoemulsions are based on low interfacial tension. This is achieved by adding co-surfactants, which leads to spontaneous formation of a thermodynamically stable nanoemulsion. The droplet size in the dispersed phase is small, usually below 140 nm in diameter, which makes the nanoemulsions transparent liquids. Their size varies from 10 to 1,000 nm. These carriers are solid spheres and their surface is amorphous and lipophilic with a negative charge. An emulsion is abiphasic system in which one phase is intimately dispersed in the other phase in the form of minute droplets ranging indiameter from 0.1 to 100 lm. It is a thermodynamically unstable system, which can be stabilized by the presence of an emulsifying agent (Emulgent or emulsifier). The dispersed phase is also known as internal phase or the discontinuous phase while the outer phase is called dispersion medium, external phase or continuous phase. The emulsifying agent is also known as intermediate or inter phase. The term 'nanoemulsion' also refers to a miniemulsion which is fine oil/water or water/oil dispersion stabilized by an interfacial film of surfactant molecule having droplet size range 20–600 nm. Because of small size, nanoemulsions are transparent.[1]

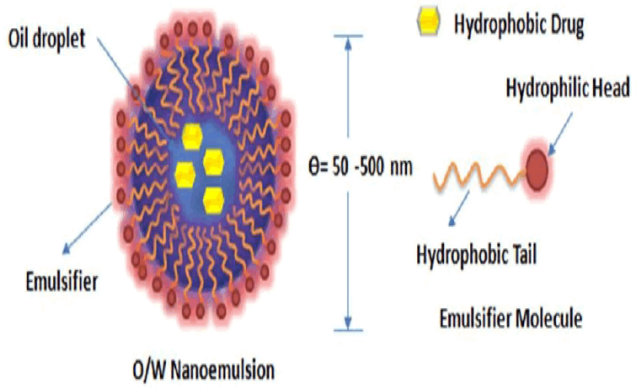

Figure 1: Nanoemulsion droplet.

Figure 1: Nano emulsion Droplet

II. CLASSIFICATION OF NANOEMULSIONS

The Nanoemulsions are most likely to be formed depending on the composition.

1) **O/W Nanoemulsion:** Wherein oil droplets are dispersed in the continuous aqueous phase.
2) **W/O Nanoemulsions:** Wherein water droplets are dispersed in the continuous oil phase.
3) **Bi-continuous Nanoemulsions:** Wherein microdomains of oil and water are interdispersed within the system.[2]

III. ADVANTAGES OF NANOEMULSIONS OVER OTHER DOSAGE FORMS

1. Eliminates variability in absorption
2. Increases the rate of absorption.
3. Provides aqueous dosage form for water insoluble drugs.
4. Increases bioavailability.
5. Helps in solubilizing lipophilic drug.

6. Helps in taste masking.
7. Various routes like topical, oral and intravenous can be used to deliver the product.
8. Rapid and efficient penetration of the drug molecule
9. Use of Nanoemulsion as delivery systems improves the efficacy of a drug, al-lowing the total dose to be reduced and thus minimizing side effects.
10. Provides protection from hydrolysis and oxidation as drug in oil phase in o/wemulsion
11. Less amount of energy required
12. Liquid dosage form increases patient compliance
13. Nanoemulsions are thermodynamically stable systems and the stability allows self-emulsification of the system whose properties are not dependent on the process followed.
14. Nanoemulsionscarry both lipophilic and hydrophilic compounds. [3]

IV. DISADVANTAGES OF NANOEMULSION BASED SYSTEMS

1. Limited solubilizing capacity for high melting substances.
2. Use of a large concentration of surfactant and co-surfactants necessary for stabilizing the Nano droplets.
3. The surfactant must be nontoxic for pharmaceutical applications.
4. Nanoemulsion stability is influenced by environmental parameters such as temperature and pH.These parameters change upon Nanoemulsion delivery to patients. [4]

V. COMPONENTS OF NANOEMULSION [5, 6]

Main three components of Nanoemulsions are

1. Oil
2. Surfactant/Co surfactant
3. Aqueous phase

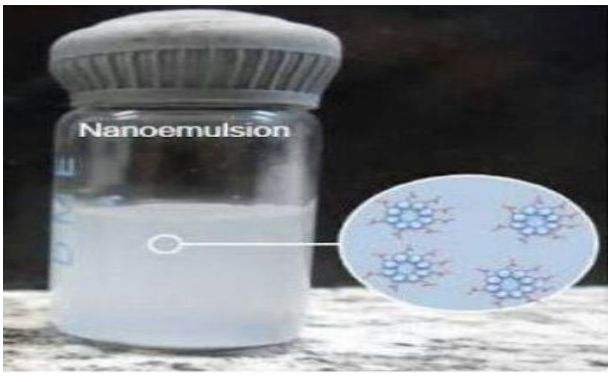

Figure 2: Component of Nano emulsion

Table 1: Component of Nanoemulsion

Sr .no	Component	Examples
1	Oils	Castor oil, Corn oil, Coconut oil, Evening primrose oil, linseed oil, Mineral oil, olive oil, peanut oil.
2	Emulgent	Natural lecithins from plant or animal Source, phospholipids, castor oil Derivatives, polysorbates, sterylamine.
3	Surfactant	Polysorbate20, Polysorbate80, Polyoxy 60, castor oil, Sorbitanmonooleate, PEG300, Caprylic glyceride.
4	Co- Surfactant	Ethanol, glycerine, PEG300, PEG400, Polyene glycol, Poloxamer.
5	Antioxidants	Ascorbic acid and tocopherol.
6	Tonicity modifiers	Glycerol, Sorbitol and xylitol.
7	Additives	Lower alcohol (ethanol), propylene glycol, 1, 3-butylanes glycol, sugars such as butylenes glycol, sugars such as glucose, sucrose, fructose, and maltose.

VI. METHOD OF PREPARATION OF NANOEMULSION [7]

There are two primary methods to prepare a nanoemulsion

A. Persuasion
B. Brute force

A. By Persuasion:

(1)Phase Transition from Near-Optimum State via Change in Single Variable: This method involves change in one formulation variable such as salinity or temperature for a system near optimal (HLD (hydrophilic lipophilic deviation), such as applying a higher temperature to a micro emulsion.

(2)Phase Transition from Near-Optimum State via Change in Multiple Variables: This method involves change in more than one formulation variable, such as applying higher temperature and inclusion of additional salt in a micro emulsion.

(3) Catastrophic Inversion: This method involves causing a low internal phaseemulsion to invert such that the internal phase becomes the external phase.

(4) Phase Transition Stabilized by Liquid Crystal Formation: This method involves stabilization of Nano droplets by liquid crystal formation from a state near HLD=0.

B. By Brute Force: This method may involve the use of a high speed mixer, a high pressure homogenizer, a high frequency ultra-sonic device, a small pore membrane, etc. Formation of O/W and W/O nanoemulsion by dispersion or high-energy emulsification methods is apparently fairly common, while nanoemulsion formation by condensation or "low-energy" emulsification methods, take advantage of the physicochemical properties of these systems based on the

phase transition that takes place during the emulsification process.

It can be carried out by operating in particular areas of the phase diagram with a very low interfacial tension, which are areas of liquid crystals and microemulsions; at the end of the emulsification process, nanoemulsion formed. Properties of nanoemulsions, such as small droplet size, relative high kinetic stability and optical transparency seem to depend not only on composition variables but also on preparation variables such as emulsifying path, degree of mixing energy input and emulsification time.

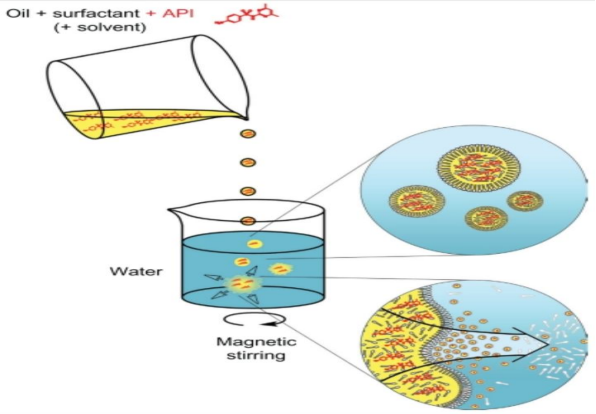

Figure 3: By Brute Force Method

VII. TECHNIQUES OF PREPARATION OF NANOEMULSION

Nanoemulsions have very small particle size range; they can be most effectively produced using high-pressure equipment.

- The most commonly used methods for producing nanoemulsions are 'High-pressure homogenization' and 'Micro fluidization' used at both laboratory and industrial scale.
- Other methods like 'Ultrasonification' and 'In-situ emulsification' are also suitable for preparation of nanoemulsion.

1. **High-Pressure Homogenization:** The preparation of nanoemulsions requires high- pressure homogenization. This technique include use of high-pressure homogenizer/piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1nm). The dispersion of two liquids is achieved by forcing their mixture through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear which results in extremely fine particles of emulsion. The particles which are formed exhibit a liquid, lipophilic core separated from the surrounding aqueous phase by a monomolecular layer of phospholipids. This technique has great efficiency.The only disadvantage being high energy consumption and increase in temperature of emulsion during processing.

In, high pressure homogenization shows the formation of nanoemulsion. [5,6,7]

- **Effect of Homogenization Pressure:** Effect of this pressure optimized the process parameter ranging from 100 to 150 bars. The higher is the size the lower is the particle size obtained e.g., RMRP 22.
- **Effect of Homogenization cycles:** The higher the homogenization cycles the smaller is the particle size obtained. Here, 3, 4 or 10 cycles are carried out. The number of cycles is analyzed by polydispersity index of drug after each cycle.

Advantages

- Ease of scale-up and little batch-to-batch variation.
- Narrow size distribution of the Nanoparticulate drug.
- Flexibility in handling the drug quality.
- Effectively used for thermolabile substances.

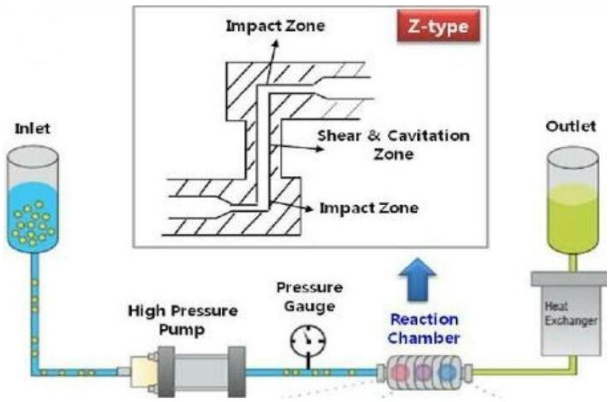

Figure 4: High Pressure Homogenization

2. **Micro-fluidization:** It is a mixing technique, which makes use of a device called micro- fluidizer. This device uses a high-pressure positive displacement pump (500 to 20000psi), which forces the product through the interaction chamber, it consists of small channels called 'micro-channels'. The product flows through the micro-channels on to an impingement area resulting in very fine particles of micrometer range. The two phases (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is into a microfluidizer where it is further processed to obtain a stable

nanoemulsion. The coarse emulsion is passed through the interaction chamber microfluidizer repeatedly until desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion.

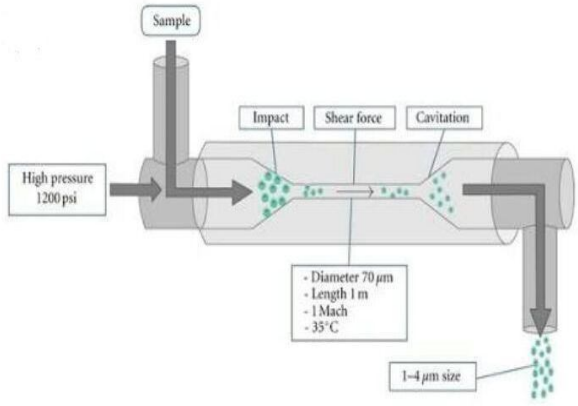

Figure 5: Micro Fluidization

3. Spontaneous Emulsification: It involves three main steps.

- Preparation of homogeneous organic solution composed of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant.
- The organic phase was injected in the aqueous phase in magnetic stirrer magnetic stirring is done then the o/w emulsion was formed.
- The water-miscible solvent was removed by evaporation under reduced pressure.[11]

4. Low Energy Emulsification: o/w nanoemulsion preparation is done by this method. Take advantage of the physicochemical properties of these systems based on the phase transition that takes place during the emulsification process. [12, 13]

5.Solvent Evaporation Technique: This technique involves preparing a solution of drug followed by its emulsification in another liquid that is non-solvent for the drug. Evaporation of the solvent leads to precipitation of the drug. Crystal growth and particle aggregation can be controlled by creating high shear forces using a high-speed stirrer. [14]

6. Hydrogel Method: It is similar to solvent evaporation method. The only difference between the two methods is that the drug solvent is miscible with the drug anti-solvent. Higher shear force prevent crystal growth and Ostwald ripening. Other method used for Nanoemulsion preparation is the phase inversion temperature technique. [14]

VIII. EVALUATION OF NANOEMULSION

1. Droplet size

Droplet size analysis of nanoemulsion is measured by a diffusion method using a light-scattering, particle size analyzer counter, LS 230. It is also measured by correlation spectroscopy that analyses the fluctuation in scattering of light due to Brownian motion. Droplet size analysis of nano emulsion can also be performed by transmission electron microscopy (TEM).

2. Viscosity determination

The viscosity of nanoemulsion is measured by using Brookfield-type rotary viscometer at different shear rates at different temperatures.

3. Dilution test

Dilution of a nano emulsion either with oil or with water can reveal this type. The test is based on the fact that mor eof the continuous phase can be added into a nanoemulsion without causing the problem of its stability. Thus, an o/w nanoemulsion can be diluted with water and a w/o nanoemulsioncan be diluted with oil.

4. Drug content

Preweighed nanoemulsion is extracted by dissolving in a suitable solvent, extract is analyzed by spectrophotometer or HPLC against standard solution of drug.

5. Polydispersity

It indicates the uniformity of droplet size in nanoemulsion. The higher the value of polydispersity, lower will be uniformity of droplet size of nanoemulsion. It can be defined as the ratio of standard deviation to mean droplet size. It is measured by a spectrophotometer.

6. Dye test

If a water-soluble dye is added in an o/w nanoemulsion then a noemulsion takes up the colour uniformly. Conversely, if the emulsion is w/o type and the dye being soluble in water, the emulsion takes up the colour only in the dispersed phase and the emulsion is not uniformly coloured. This can be revealed immediately by microscopic examination of the emulsion.

7. Refractive index

Refractive index of nanoemulsion is measured by Abbesrefract meter.

8. pH

The pH of nanoemulsion can be measured by pH meter.

9. Heating cooling cycle

Between refrigerators temperature 4°C and 45°C of six cycles with storage at each temperature of not less than 48 h was studied. Those formulations, which were stable at these temperatures, were subjected to centrifugation.

10. Centrifugation

Those formulations that passed were centrifuged at3500 rpm for 30 min by using centrifuge. The formulations that did not show any phase separated were taken to further tests.

11. Freeze thaw cycle

Between –21°C and +25°C three freeze thaw cycles with storage at each temperature for not less than48 h was done for the formulations, which passed these thermodynamic stress tests, were further taken for the dispersibility tests.

12. Dispersibility tests

Dispersibility tests were done using a dissolutionapparatus 2. 1mL of each formulation was added to 500 ml of water at 37}0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm provided gentle agitation. *In vitro* performance of the formulation was visually assessed using the following grading system:

Grade A: Clear or bluish appearance rapidly forms within 1 min.
Grade B: Slightly less clear emulsion having a bluish white forms rapidly.
Grade C: Fine milky emulsion that formed within 2min.
Grade D:Dull, greyish white emulsion having slightlyoily appearance that is slow to emulsify (longer than2 min).
Grade E: Poor or minimal emulsification with large oil globules present on the surface.The formulations that passed the thermodynamic stability and also dispersibility tests in Grade A and B were selected for further studies. The selected formulations were prepared by dissolving 10 mg (single dose) of amphotericin B in oil (10%, 15%, 20%, 25% etc.). Respective mix ratio was added to the oil, mixed using magnetic stirrer and aqueousphase was added. The resulting mixture gave Nanoemulsion.

13. Viscosity determination

The viscosity of the formulations (0.5 g) was determined as such without dilution using Brookfield DV-II ultra-+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA)using spindle # CPE 40 at 25}0.5oC. The software used for the calculations was Rheocalc V2.6.

14. Electro-conductivity study

For the conductivity measurements, the tested nanoemulsions were prepared with a 0.01 Non-aqueous solution of NaCl instead of distilled water. The test was measured by an electroconductometer(Conductivity meter 305, Systronic).

15. Refractive index and percent transmittance

The refractive index of the system was measured byan Abbe refract meter (Bausch and Lomb optical company, NY) by placing 1 drop of nanoemulsionon the slide. The percent transmittance of the system was measured at 650 nm using a UV spectrophotometer keeping distilled water as blank.[14,15]

16. Drug content

The drug content was calculated by UV visible spectrophotometer. The formulation was diluted to required concentration using methanol as solvent and the absorbance was measured at 416nm against a solvent blank. The drug content was calculated as:
Drug content = Analyzed content x 100Theoretical content

17. Transmission electron microscopy (TEM)

TEM analysis should be done for the formulation nanoemulsion to determine the globule size of oil present in the nanoemulsion. This can be done by TOPCON 002B operating at 200 kV capable of point to point resolution. To perform the TEM observations, the nanoemulsion formulation wasdiluted with water (1/100). A drop of the diluted nano emulsion was then directly deposited on the holey film grid and observed after drying.

18. In vitrodrug release

Invitrodrug release for the nanoemulsion formulation should be done in order to measure and detect the formulation

that release the maximum amount drug (amphotericin B) release from the nanoemulsion formulation. This test was performed in 500 mL of Phosphate buffer pH 7.4using USP Dissolution apparatus Type II at 75 rpm and 37}0.5oC. 2 mL of nanoemulsion formulation containing single dose 10mg of amphoteric in B was placed in a dialysis bag (Himedia dialysis membrane150). Samples (5mL) were withdrawn at regular time

Intervals (0, 0.5, 1, 1.5, 2, 4, 6, 8 h) and an aliquot amount of phosphate buffer was replaced. The release of drug from nanoemulsion formulation was compared with the conventional tablet formulation (ZOCOR TM) and the suspension of pure drug. The samples were analyzed for the drug content using UV-Visible spectrophotometer at 416nm.[17]

IX. APPLICATIONS OF NANOEMULSIONS

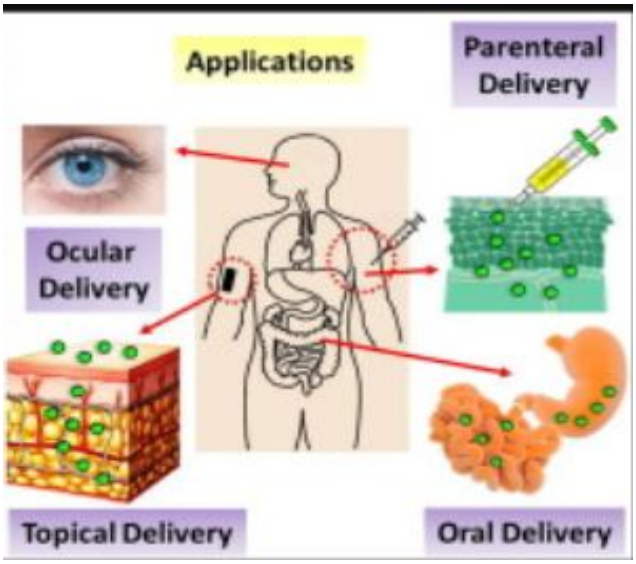

Figure 6: Application of Nano Emulsion

1. Parenteral Delivery

Nanoemulsion has advantages in intravenous administration, due to the strict requirement of this route of administration, particularly the necessity for the formulation droplet size lower than 1 micrometer. Parenteral (or Injectable) administration of nanoemulsion is employed for a variety of purposes, namely nutrition e.g. Fats, Carbohydrates, Vitamins etc. Nanoemulsion formulations have distinct advantages over macro emulsion systems when delivered parenterally because of the fine particle Nanoemulsion is cleared more slowly than the coarse particle emulsion and, therefore, have a longer residence time in the body. Both O/W and W/O Nanoemulsion can be used for parenteral delivery.

2. Oral Delivery

Nanoemulsion formulations offer many advantages over conventional oral formulation for oral administration including increased absorption, improved clinical potency and decreased drug toxicity. Thus, Nanoemulsion proves to be ideal in delivering of drugs such as steroids, hormones, diuretic and antibiotics. Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions.[18]

Primaquine when incorporated into oral lipid nanoemulsion showed effective antimalarial activity against Plasmodium bergheii infection in mice at a 25% lower dose level as com-pared to conventional oral dose. Lipid nanoemulsion of primaquine improved oral bioavailability by the liver with drug concentration higher by at least by 45% as compared with the pure drug.

3. Topical Delivery

Topical administration of drugs can have ad-vantages over other methods for several reasons, one of which is the avoidance of hepatic first pass metabolism of the drug and related toxicity effects. Another is the direct delivery and targetability of the drug to affected area of the skin or eyes. The nanoemulsion can achieve a high level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics. The nanoemulsion has broad spectrum activity against bacteria (e.g. E.coli, S. aureus) fungi (e.g. Candida, Dermatophytes).[19]

4. Ocular Delivery

For the treatment of eye diseases, drugs are essentially delivered topically Nanoemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to in-crease absorption and to attain prolong release profile. [20]

5.In Cosmetic

The aesthetic properties, i.e. low viscosity and transparent visual aspects of nanoemulsion-with droplet sizes below 200nm, its high surface area allowing effective transport of the active ingredient to the skin make them especially attractive for their application in cosmetics. Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation or coalescence that is observed with macro emulsion. The incorporation of potentially irritating surfactants can be avoided by using high energy equipment during

manufacturing. Nanogel technology to create miniemulsion from oil-in water concentrate suited to minimizing transepidermal water loss, enhanced skin protection and penetration of active ingredient. It would be useful for sun care products, moisturing and antiageing creams. It helps to give skin care formulations a good skin feels.[21]

6. Transdermal

Indomethacin a potent NSAID, the anti-inflammatory effects of true optimized nanoemulsion formulation were compared with marketed gel in carragenan induced paw edema in rats. The %inhibition value was significant for developed Nanoemulsion, so huge potential for transdermal application of indomethacin. Nanoemulsions for transdermal delivery of celecoxib, formulation which consisted of 2% celecoxib 10% oil phase (Sefsol 218 and Triacetin) 50% surfactant mixture (Tween 80 and Transcutol-P) and 40% water. The anti-inflammatory effect and percent inhibition value after 24h administration was found to be high for nanoemulsion formulation (81.2%) as compared to celecoxib gel (43.7%) and nanoemulsion gel (64.5%). The in vitro- in *vivo* studies revealed a significant increase in the anti-inflammatory effects of aceclofenacnanoemulsion (82.2%) as compared to nanoemulsion gel formulation (71.4%) and conventional gel (41.8%).

7. Nanoemulsions in Cancer Therapy

Nanoemulsions can beused as vehicle in cancer chemotherapy for prolonging the rate of drug release after intramuscular and intratumoral injection (W/O systems). It also enhances the transdermal drug delivery due to increase in the transport of anti-cancer drugs via lymphatic permeation through the skin and it is alsonon-irritant system. [21, 22]

8. Nanoemulsions in intranasal drug delivery

Intranasal drug delivery system has now been recognized as a reliable route for the administrationof drugs next to parenteral and oral routes. Nasal mucosa has emerged as a therapeuticallyviable channel for the administration of systemic drugs and also appears to be afavourable way to overcome the obstacles forthe direct entry of drugs to the target site.This route is also painless, non-invasive and well tolerated. The nasal cavity is one of the mostefficient sites because of its reduced enzymatic activity, high availability of immune active sitesand its moderately permeable epithelium. There are several problems associated with-targeting drugs to brain, especially the hydrophilic ones and those of high molecular weight.This is because of the impervious nature of the endothelium, which divides the systemiccirculation and barrier between the blood and brain. The olfactory region of the nasal-mucosa provides a direct connection between the nose and brain and by the use of nanoemulsions loaded with drugs, conditions such as Alzheimer's disease, migraine, depression, schizophrenia,Parkinson's diseases, meningitis, etc. can be treated. Preparation ofnanoemulsions containing risperidone for its delivery to the brain via nose has been reported. It is inferred that this emulsion is more effective through the nasal rather than intravenousroute. Another application of intranasal drug delivery system in therapeutics is their usein development of vaccines. Immunity is achieved by the administration of mucosal antigen.Currently, the first intranasal vaccine has been marketed. Among the possible delivery-systems, the use of nano based carriers hold a great promise to protect the bio-molecules, promotenano-carrier interaction with mucosa and to direct antigen to the lymphoid tissues.Therefore the use of nanoemulsions in intranasal drug delivery system is set to bring aboutsignificant results in targeting of the drugs to the brain in treatment of diseases related to the central nervous system. Developed intranasal nanoemulsion and gel formulationsfor rizatriptan benzoate for prolonged action. Various mucoadhesive agents were tried out to form thermo triggered mucoadhesive nanoemulsions.

9. Nanoemulsions in pulmonary drug delivery

The lung is the most important target for drug delivery due to noninvasive administration viainhalation aerosols, avoidance of first-pass metabolism, direct delivery to the site of action forthe treatment of respiratory diseases, and the availability of a huge surface area for local drugaction and systemic absorption of drug. Colloidal carriers (ie, nanocarrier systems) in pulmonarydrug delivery offer many advantages such as the potential to achieve relatively uniformdistribution of drug dose among the alveoli, achievement of improved solubility of the drug from its own aqueous solubility, a sustained drug release which consequently reducesdosingfrequency, improves patient compliance, decreases incidence of side effects, and the potential of drug internalization by cells.

10. Nanoemulsions in gene delivery vector

Emulsion systems have been emerged as alternative gene transfer vectors to liposomes.Other emulsion studies for gene delivery (non-pulmonary route) have shown that binding ofthe emulsion/DNA complex is stronger than liposomal carriers. This stable emulsion system delivered genes more efficiently than liposomes. Silva et alevaluated factors thatinfluence DNA compaction in cationic lipid nanoemulsions [cationic nanoemulsions containing stearylamine (a cationic lipid that presents a primary amine

group when in solution, is ableto compact genetic material by electrostatic interactions, and in dispersed systems such asNanoemulsions this lipid anchors on the oil/water interface conferring a positive charge tothem. The influence of the stearylamine incorporation phase (water or oil), time ofcomplexation, and different incubation temperatures were studied.Characterization was done by dynamic light scattering (DLS). The results demonstrate that thebest DNA compaction process occurs after 120 min of complexation, at low temperature (4 ±1 °C), and after incorporation of the cationic lipid into the aqueous phase. Although the zetapotential of lipoplexes was lower than the results found for basic nanoemulsions, the granulometrydid not change. Moreover, it was demonstrated that lipoplexes are suitable vehicles for gene deli-very.[22]

REFERENCES

[1] Nanoemulsion: an advanced mode of drug delivery systemManjitJaiswal, RupeshDudhe, P. K. Sharma

[2] Banker G S, Lieberman H A, Rieger M M. Pharmaceutical dosage forms, Disperse systems, Marcel Dekker, 2nd edition, Vol.3, **2002**, 339-40, 343-44.

[3] Trotta M, Influence of phase transformation on indomethacin release from microemulsions. J Control Re-lease.**1999**; 60:399-405.

[4] Figueroa Alvarez, MJ and Blanco-Méndez, Transdermal delivery of me-thotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int J Pharm.**2001**; 215: 57-65.

[5] Bhatt P and Madhav S: A Detailed Review on Nanoemulsion Drug Deli-very System. International Journal of Pharmaceutical Sciences and Research. **2011**; 2(10):2482-2489.

[6] Liu P, Kurihara-Bergstrom T and Good WR. Cotransport of estradiol and ethanol through human skin in vitro: understanding the permeant/enhancer flux relationship.Pharm Res.**1991**; 8: 938-944.

[7] Quintero Lirio, Mckellar John Alexander, Clark David E: Nanoemulsion Assignee: Baker Hughes Incorporated (Houston, TX, US) **2010**.

[8] Lachman L, Lieberman HA, Kanig JL: The Theory and Practice of Industrial Pharmacy, Edition 3: 510-11

[9] O'Hagan DT Vaccine Adjuvants. Humana Press: 214.

[10] Bouchemal K, Briancon S, Fessi H, Perrier E: Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization .International Journal of Pharmaceutics **2004** ;280:243

[11] Ahuja A Ali J, Baboota S, Faisal M.S, Shakeell F and Shafiq S: Stability evaluation of Celecoxibnanoemulsion containing Tween 80. Thai J. Pharm. Sci. **2008**;4-9.

[12] Montserrat, Roca Foguet, (Leverkusen DE) United States Patent Application 20090324727 Assignee: Biofrontera Bioscience Gmbh (Leverkusen, De)

[13] Arunkumar N, Deecaraman M, Rani C: Nanosuspension Technology and its applications in drug delivery, IP **2007**; 124.124. 110.202

[14] Ali Javed, AhujaAlka, Baboota S, Shakeel F, Shafiq S: Design development and evaluation of novel nanoemulsion formulations for transdermal potential of Celecoxib. Acta Pharm.**2007**; 57 315–33210.2478/v10007-007-0025-5

[15] Bhatt P, Madhav S. A detailed review on nanoemulsion drug delivery system.International Journal of Pharmacy andPharmaceutical Sciences, **2011**, 2(10), 2482- 2489.

[16] Charles Lovelyn, Anthony A. Attama. Current state of nanoemulsion in drug delivery. Journal of Biomaterials and Nanobiotechnology, **2011**, 2, 626-639.

[17] Vinicius Raphael de Almeida, Borges, Alice S, Valeria Pereira de Sousa. Nanoemulsioncontainingdapsone for topical administration: a study of invitro release and epidermalpermeation. International Journal of Nanomedicine, **2013**, 3, 535-544.

[18] Aubrun O.S, Simonnet J.T, Alloret F.L: Nanoemulsions: a new for skincare products Ad. Collo. Inter Sci. **2004**; 108-109:145-149.

[19] Vyas T.K, Amiji M.M, Shahiwala A: Improved oral bioavailability and brain transport of saquinavir upon adminis-tration in novel nanoemulsion formula-tions. Int. J. Pharm. **2008**; 347:93-101.

[20] Tamilvanan S. Submicron emulsions as a carrier for topical (ocular and per-cutaneous)and nasal drug delivery. Indian J. Pharm. Educ. **2004**; 38(2):73-8.

[21] Heidi MM, Yun-Seok R, Xiao W. Na-nomedicine in pulmonary delivery. Int. J.Nanomed. **2009**; 4: 299–319.

[22] Junyaprasert B.V, Muller H.R, Souto B.E et al.: Q10 loaded NLC versus nanoemulsions; stability, rheology and in vitro skin permeation. Int. J Pharm. **2009**; 377:207-214.