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NANOEMULSION BASED DRUG DELIVERY-A REVIEW

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ABSTRACT

Nanoemulsions are submicron sized emulsion that is under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Nanoemulsions have the potential in pharmaceutical industries because of the transparency at high droplet volume fraction, higher rate of bioavailability or diffusion and increased shelf life of the pharmaceuticals. Nanoemulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water, surfactant and co-surfactant. These are oil-in-water (o/w) type of emulsions with the average droplet size ranging from 5nm to 100 nm. Reduction in droplet size to nanoscale leads to change in physical properties such as optical transparency & unusual elastic behavior. Thus the aim of this review is focused on nanoemulison advantage and disadvantage, various methods of preparation, characterization techniques and the various applications of sub micron size emulsion in different areas such as various route of administration, in chemotherapy, in cosmetic, etc.

KEYWORDS: Nanoemulsion Submicron size droplet Self emulsifying agent Drug delivery system.

INTRODUCTION

Nanoemulsions consist of fine oil-in-water dispersions, having droplets covering the size range of 100–600 nm. Nanoemulsions, usually spherical, are a group of dispersed particles used for pharmaceuticals biomedical aids and vehicles that shows great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies.[1] The emulsifier used is generally a surfactant, but proteins and lipids have also been effective in the preparation of nanoemulsions.[2–12] Nanoemulsion is one of the growing technologies especially in food and pharmaceutical industries as a novel delivery system for drugs and lipophilic materials such as flavours, colours, fatty acids etc.[13-15] The terms sub-micron emulsion (SME),[16] miniemulsion[17] and ultra fine emulsion[18] are used as synonyms. Nanoemulsion is a heterogeneous mixture of lipid and aqueous phase and stability are achieved by using a suitable material known as emulsifying agents. Nowadays this dosage form is frequently used for the delivery of various biopharmaceuticals as vaccines, DNA encoded drugs[19], and antibiotics.[20] A lot of techniques are available for enhancing absorption of poorly water soluble drugs, like use of lipid-based systems. Thus enhancement of aqueous solubility in such case is a valuable goal to successfully formulate them into bioavailable dosage forms. A range of novel strategies are currently being developed for efficient delivery of poorly water-soluble drugs, such as the formulation of amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes. Among all, the most accepted approach is the lipid-based formulation approach.[21-22] Nanoemulsion possesses stability of outstanding application like it waiver the destabilization process of emulsion i.e., creaming, flocculation, coalescence and sedimentation.[23] Mainly GRAS (Generally regarded as safe) Nanoemulsion, formulated with oil, surfactant and co-surfactant are nontoxic, nonirritant and approved for human consumption that are "generally recognized as safe" by the FDA[24] (Table 1)

Table 1: Formulation ingredients of Nanoemulsion.[9]

Component	Examples
Oils	Castor oil, Corn oil, Coconut oil, Evening primrose oil, linseed oil, Mineral oil, olive oil, peanut oil
Emulgent	Natural lecithins from plant or animal source, phospholipids, castor oil Derivatives, polysorbates, sterylamine
Surfactant	Polysorbate20, Polysorbate80, Polyoxy 60, castor oil, Sorbitan monooleate, PEG300, Caprylic glyceride
Co- Surfactant	Ethanol, glycerine, PEG300, PEG400, Polyene glycol, Poloxamer
Tonicity modifiers	Glycerol, Sorbitol and xylitol
Additives	Lower alcohol (ethanol), propylene glycol, 1, 3-butylenes glycol, sugars such as butylenes glycol, sugars such as glucose, sucrose, fructose, and maltose
Antioxidants	Ascorbic acid and tocopherol

Applications of Nanoemulsions in Drug Delivery

Nanoemulsions and Intranasal Drug Delivery

Intranasal drug delivery system has now been recognized as a reliable route for the administration of drugs next to parenteral and oral routes. Nasal mucosa has emerged as a therapeutically viable channel for the administration of systemic drugs and also appears to be a favourable way to overcome the obstacles for the direct entry of drugs to the target site.[25] This route is also painless, non-invasive and well tolerated. The nasal cavity is one of the most efficient sites because of its reduced enzymatic activity, high availability of immunoactive sites and its moderately permeable epithelium.[26] 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 systemic circulation and barrier between the blood and brain.[27] The olfactory region of the nasal mucosa provides a direct connection between the nose and brain, and by the use of nanoemulions loaded with drugs, conditions such as Alzheimer's disease, migraine, depression, schizophrenia, Parkinson's diseases, meningitis, etc. can be treated.[28,29] Preparation of nanoemulsions containing risperidone for its delivery to the brain via nose has been reported.[29] It is inferred that this emulsion is more effective through the nasal rather than intravenous route. Another application of intranasal drug delivery system in therapeutics is their use in development of vaccines. Immunity is achieved by the administration of mucosal antigen. Currently, the first intranasal vaccine has been marketed.[30] Among the possible delivery systems, the use of nano based carriers hold a great promise to protect the biomolecules, promote nanocarrier interaction with mucosae and to direct antigen to the lymphoid tissues. Therefore the use of nanoemulsions in intranasal drug delivery system is set to bring about significant results in targeting drugs to the brain in treatment of diseases related to the central nervous system.[31]

Nanoemulsions and Transdermal Delivery

Drug delivery through the skin to the systemic circulation is convenient for a number of clinical conditions due to which there has been a considerable interest in this area.[32,33] It offers the advantage of steady state controlled drug delivery over extended period of time, with self administration also being possible, which may not be the case with parenteral route. The drug input can be eliminated at any time by the patient just by removing the transdermal patch. Their transparent nature and fluidity, confers on nanoemulsions a pleasant skin feel. An extra advantage is the total absence of gastrointestinal side effects like irritation and bowel ulcers which are invariably associated with oral delivery. Transdermal drug products have been developed for a number of diseases and disorders including cardiovascular conditions, Parkinsons' and Alzheimer diseases, anxiety, depression, etc. However, the fundamental disadvantage which limits the use of this mode of administration is the barrier imposed by the skin for effective penetration of the bioactives. The three routes by which drugs can primarily penetrate the skin are through the hair follicles, sweat ducts or directly across stratum corneum, which restricts their absorption to a large extent and limits their bioavailability. For improved drug pharmacokinetics and targeting, the primary skin barriers need to be overcome. Also the locally applied drug redistribution through cutaneous blood and lymph vessel system needs to be controlled. Nano sized emulsions are able to easily penetrate the pores of the skin and reach the systemic circulation thus getting channelized for effective delivery.[34] Caffeine has been used for treatment of different types of cancer by oral delivery. Water-in-oil nanoemulsion formulations of caffeine have been developed for transdermal drug delivery. Comparison of in vitro skin permeation profile between these and aqueous caffeine solutions showed significant increase in permeability parameters for the nanoemulsion loaded drugs.[35] Use of nanoemulsions in transdermal drug delivery represents an important area of research in drug delivery, which enhances the therapeutic efficacy and also the bioavailability of the drugs without any adverse effects. It is also regarded as a promising technique with many advantages including, high storage stability, low preparation cost, thermodynamic stability, absence of organic solvents, and good production feasibility. They have also made the plasma concentration profiles and bioavailability of drugs reproducible. These systems are being used currently to provide dermal and surface effects, and for deeper skin penetration.[34] Many studies have shown that nanoemulsion formulations possess improved

transdermal and dermal delivery properties in vitro[35-43], as well as in vivo.[44-46] Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as emulsions[47,48] and gels.[49,50]

Nanoemulsions and Parenteral Drug Delivery

This is one of the most common and effective routes of drug administration usually adopted for actives with low bioavailability and narrow therapeutic index. Their capacity to dissolve large quantities of hydrophobics, together with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation make nanoemulsions ideal vehicles for the purpose of parenteral transport. Further, the frequency and dosage of injections can be reduced throughout the drug therapy period as these emulsions guarantee the release of drugs in a sustained and controlled mode over long periods of time. Additionally, the lack of flocculation, sedimentation and creaming, combined with a large surface area and free energy, offer obvious advantages over emulsions of larger particle size, for this route of administration.[34] Their very large interfacial area positively influences the drug transport and their delivery, along with targeting them to specific sites. Major clinical and preclinical trials have hence been carried out with parenteral nanoemulsion based carriers. The advances in these novel drug delivery systems have been reviewed by Patel and Patel.[51] Nanoemulsions loaded with thalidomide have been synthesized where a dose as low as 25 mg leads to plasma concentrations which can be therapeutic.[52] However, a significant decrease in the drug content of the nanoemulsion was observed at 0.01% drug formulation after two months storage which could be overcome by the addition of polysorbates 80. Chlorambucil, a lipophilic anticancer agent has been used against breast and ovarian cancer. Its pharmacokinetics and anticancer activity has been studied by loading it in parenteral emulsions prepared by high energy ultrasonication method. Treatment of colon adenocarcinoma in the mouse with this nanoemulsion leads to higher tumor suppression rate compared to plain drug solution treatment concluding that the drug loaded emulsion could be an effective carrier for its delivery in cancer treatment.[53] Carbamazepine, a widely used anticonvulsant drug had no parenteral treatment available for patients due to its poor water solubility. Kelmann *et al.*[54] have developed a nanoemulsion for its intravenous delivery, which showed favorable in vitro release kinetics.

Nanoemulsions and Drug Targeting

Another interesting application, which is experiencing an active development, is the use of nanoemulsion formulations, for controlled drug delivery and targeting.[55] Because of their submicron size, they can easily be targeted to the tumor area. Although nanoemulsions are chiefly seen as vehicles for administering aqueous insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizes, neutron capture therapy agents, or diagnostic agents. The development of magnetic nanoemulsions is an innovative approach for cancer therapy. These can deliver photo sensitizers like Foscan to deep tissue layers across the skin thereby inducing hyperthermia for subsequent free radical generation. This methodology can be used for the treatment of cancer in the form of photodynamic therapy.[56]

Nanoemulsions and Vaccine Delivery

A vaccine carrier system using nanoemulsions is currently being researched. This medication delivery system uses nanotechnology to vaccinate against human immunodeficiency virus (HIV). There is recent evidence that HIV can infect the mucosal immune system. Therefore, developing mucosal immunity through the use of nanoemulsions may become very important in the future fight against HIV.[57] The oil-based emulsion is administered in the nose, as opposed to traditional vaccine routes. Research is demonstrating that genital mucosa immunity may be attained with vaccines that are administered into the nasal mucosa.[58]

Nanoemulsions and Pulmonary Drug Delivery

Until now, the submicron emulsion system has not yet been fully exploited for pulmonary drug delivery and very little has been published in this area.[59] Emulsion systems have been introduced as alternative gene transfer vectors to liposomes.[60] Other emulsion studies for gene delivery (non-pulmonary route) have shown that binding of the emulsion/DNA complex was stronger than liposomal carriers.[61] This stable emulsion system delivered genes more efficiently than liposomes.[62] Bivas-Benita *et al.*[63] reported that cationic submicron emulsions are promising carriers for DNA vaccines to the lung since they are able to transfect pulmonary epithelial cells, which possibly induce cross priming of antigen presenting cells and directly activate dendritic cells, resulting in stimulation of antigen specific T-cells. Therefore the nebulization of submicron emulsions will be a new and upcoming research area. However, extensive studies are required for the successful formulation of inhalable submicron emulsions due to possible adverse effects of surfactants and oils on lung alveoli function (adverse interactions with lung surfactant).

Prophylactic in Bio-Terrorism Attack

Based on their antimicrobial activity, research has begun on use of nanoemulsion as a prophylactic medication, a human protective treatment, to treat people exposed to bioattack pathogens such as anthrax and ebola. A broad spectrum nanoemulsion was tested on surfaces by the USA army in Dec 1999 for decontamination of Anthrax spore surrogates. It was tested again by Rest Ops in March 2001 as a chemical decontamination agent. All tests were successful. The technology has been tested on gangrene and *Clostridium botulism* spores and can even be used on contaminated wounds to salvage limbs. The

nanoemulsion technology can be formulated into a foam, liquid, cream, or spray to decontaminate a variety of materials as has been done by Nano Bio Corporation.[64]

Limitation of nanoemulsion

Although this formulation provide great advantages as a delivery system for the consumers but sometimes the reduced size of droplets are responsible for the limited use of nanoemulsion formulation. Some limitations of nanoemulsion are as follows.[65]

- The manufacturing of nanoemulsion formulation is an expensive process because size reduction of droplets is very difficult as it required a special kind of instruments and process methods. For example, homogenizer (instrument required for the nanoemulsion formulation) arrangement is an expensive process. Again microfludization and ultrasonication (manufacturing process) require high amount of financial support.
- Stability of nanoemulsion is quite unacceptable and creates a big problem during the storage of formulation for the longer time period. Ostwald ripening is the main factor associated with unacceptability of nanoemulsion formulations. This is due to the high rate of curvature of small droplet show greater solubility as compared to large drop with a low radius of curvature.
- Less availability of surfactant and cosurfactant required for the manufacturing of nanoemulsion is another factor which marks as a limitation to nanoemulsion manufacturing.

Advantages of Nanoemulsions over other dosage forms

1. Eliminates variability in absorption
2. Increases the rate of absorption.
3. Helps in solublizing lipophilic drug.
4. Provides aqueous dosage form for water insoluble drugs.
5. Increases bioavailability.
6. Various routes like topical, oral and intravenous can be used to deliver the product.
7. Rapid and efficient penetration of the drug molecule.
8. Helps in taste masking.
9. Provides protection from hydrolysis and oxidation as drug in oil phase in o/w emulsion.
10. Less amount of energy required.
11. Liquid dosage form increases patient compliance.
12. Nanoemulsions are thermodynamically stable systems and the stability allows self emulsification of the system whose properties are not dependent on the process followed.
13. Nanoemulsions carry both lipophilic and hydrophilic compounds.
14. Use of Nanoemulsion as delivery systems improves the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.[66]

Disadvantages of Nanoemulsion Based Systems

1. Use of a large concentration of surfactant and cosurfactants necessary for stabilizing the Nanodroplets.
2. Limited solubilizing capacity for high melting substances.
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.[67]

Components of Nanoemulsion

Main three components of Nanoemulsions are

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

Nanoemulsions are colloidal dispersions com-posed of an oil phase, aqueous phase, 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. They are used to deliver drugs to the patients via several routes, but the topical application of nanoemulsions has gained much interest. The three main factors determining the transdermal permeation of drugs are: mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. Thus they improve the transdermal delivery of drugs over the conventional topical preparations such as emulsions and gels. Mobility of drugs in nanoemulsions is more facile as compared to the nanoemulsion with gel which will increase its viscosity and further decrease the permeation into the skin. The superior transdermal flux from nanoemulsions hasbeen shown to be mainly due to their high solubilization potential for lipophilic and hydrophilic drugs. This creates an increased thermodynamic activity towards the skin. They may affect the permeability of drug into the skin. In this case, the components of nanoemulsions serve as permeation enhancers. Several compounds used in nanoemulsions have been mentioned to improve the transdermal permeation by altering the structure of the stratum cornea. For example, short chain alkanols are widely used as permeation enhancers. It is known that oleic acid, a fatty acid with one double bond in the chain structure, perturbs the lipid barrier in the stratum corneum by forming separate domains which interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways in the stratum corneum.[68-70]

Factors affecting the Formulation of Nanoemulsion

1. The surfactant is the most important part of the Nanoemulsion. They should not form lyotropic liquid crystalline "micro-emulsions" phases. Systems containing short chain alkanes, alcohols, water, and surfactants form the phases which are generally used with the co surfactant.
2. Appropriate composition is required to avoid Oswald ripening andthe dispersed phase should be highly insoluble in the dispersion medium.
3. The presence of excess surfactants enables new surface area of nanoscale to be rapidly coated during emulsification there by inhibiting induced coalescence.[71]

Methods of preparation of nanoemulsions

The drug is to be dissolved in the lipophilic part of the nanoemulsion i.e. oil and the water phases and is combined with surfactant and a cosurfactant is then added at slow rate with gradual stirring until the system is transparent. The amount of surfactant and cosurfactant to be added and the percent of oil phase that can be incorporated are determined with the help of pseudoternary phase diagrams. Ultrasonicators and high pressure homogenizerscan then be used so as to achieve desired size range for dispersed globules. It is then being allowed to equilibrate. Gel may be prepared by adding a gelling agent to the above nanoemulsion. Carbomers (cross linked polyacrylic acid polymers) are the most widely used gelling agent.

FORMULATION OF NANOEMULSION

Screening of Excipients

The solubility of the drug in various oils, surfactants and cosurfactants is determined by dissolving an excess amount of the drug in small quantities of the selected oils, surfactants and cosurfactants and mixed using a vortexmixer. A combination of oils can also be used for the determination of solubility. The mixtures are allowed to equilibrate at ambient temperature in an isothermal shaker. Samples are removed from the shaker and centrifuged. The supernatant is filtered through a 0.45 µm membrane filter. The concentration of the drug is determined in each oil, surfactant, cosurfactant and combination of oils by HPLC or UV Spectrophotometer at their respective λmax.

Construction of Pseudo Ternary phase diagram

Pseudoternary phase diagrams of oil, water, and Smix are constructed at fixed cosurfactant and surfactant weight ratios. Phase diagrams were obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring; the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the nanoemulsion region of existence which is given in the Figure 1. Several methods have been suggested for the preparation of nanoemulsion. Here some methods discussed which are freely used for the nanoemulsion preparation.[71]

1. High Energy Approaches

The nanoemulsions are formed by high-energy methods which are based on selected composition, i.e. surfactant and functional compound, and on the quantity of energy supplied. The mechanical processes generating nanoemulsions are divided into three major groups based on the used devices.

A) High Pressure Homogenizer

High pressure homogenizer is the most commonly used device to produce fine emulsions. In this method, the oil-water-surfactant mixture is subjected to very high pressure and is pumped through a resistive valve. A very high shear stress causes the formation of very fine emulsion droplets. The combination of two theories, turbulence and cavitation, explain the droplet size reduction during homogenization process. The high velocity gives the liquid high energy in the homogenizer valve generates intense turbulent eddies of the same size as the mean diameter droplet (MDD). Droplets ate thus torn apart by these eddie currents resulting in a reduction in droplet size. Simultaneously, due to considerable pressure drop across the valve, cavitation occurs and generates further eddies disruption droplets. Decreasing the gap size increases the pressure drop, which causes a greater degree of cavitation. Emulsion droplet diameters as small as 100 nm can be produced using this method if there is sufficient surfactant present to completely cover the oil-water interface formed and the adsorption kinetics is high enough to prevent droplet coalescence.[72]

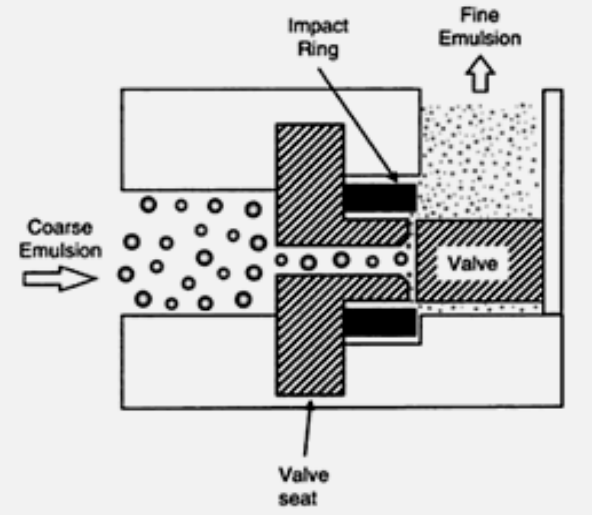

Figure 1. Schematic diagram of high pressure valve homogenizer.

B) Ultrasonication

One of the first applications of ultrasound was to make emulsions and the first patent on this technology is more

than fifty years ago. Since then, different types of ultrasonic devices have been developed for emulsion applications. Cavitation is the main phenomenon of this method in which formation and collapse of vapor cavities, in flowing liquid, takes place. Two mechanisms are proposed for ultrasonic emulsification. First, the application of an acoustic field produces interfacial waves resulting in the dispersion of the oil phase in the continuous phase in the form of droplets. Secondly, the application of ultrasound causes acoustic cavitation causing the formation and subsequent collapse of microbubbles by the pressure fluctuations of the simple sound wave, which creates extreme levels of highly localized turbulence. Therefore, the turbulent micro-implosions break up primary droplets into sub-micron size. Since the emitted sound field is typically inhomogeneous in most ultrasonic devices, it is necessary to recirculate the emulsions through the region of high power so that all droplets experience the higher shear rate. The nanoemulsions produced by ultrasonication show wider and bimodal size distributions and greater dependence on coarse emulsion preparation methods than do those prepared using microfluidization.[73,74] It is worth noting that all these researchers use small lab scale ultrasonic experimental setups. Commercial ultrasonic devices for nanoemulsion applications are not directly available since there are some design issues to be solved.

C) High Speed Devices

Nanoemulsions produced by these rotor/stator devices do not possess good dispersion, as compared to other high-energy approaches, in terms of droplet size. The energy provided gets dissipated generating heat.[75]

2. Low Energy Approaches

In low energy approaches, nanoemulsions are obtained as a result of phase transition produced during the emulsification process which is carried out, usually, at constant temperature and changing the composition or at constant composition and changing the temperature.

A) Phase Inversion Temperature (PIT)

The phase inversion temperature is most extensively used in industry to form nanoemulsions. It is based on the changes in solubility of polyoxyethylene- type non-ionic surfactants with temperature. These types of surfactants become lipophilic with increasing temperature because of dehydration of the polyoxyethylene chains. At low temperature, the surfactant monolayer has a large positive spontaneous curvature forming oil-swollen micellar solution phases which may coexist with an excess oil phase. At high temperature, the spontaneous curvature becomes negative and water swollen micelles coexist with excess water phase. At intermediate temperature, the HLB temperature, the spontaneous curvature becomes close to zero and a bicontinuous, D phase microemulsion containing comparable amounts of water and oil phases coexists with both excess water and oil phases.[76]

B) Spontaneous Emulsification

Spontaneous emulsification has been reported to occur upon pouring, into water, a solution consisting of a small concentration of oil in a water-miscible solvent without the presence of surfactant. Oils droplets are produced with their diameter being a function of the ration of excess oil to water-soluble solvent. This method can be used as an alternative to ultrasonic and high-shear methods. It has some limitations such as the low oil content that can be dispersed and the requirement that the solvent used to be soluble in water in all proportions.[77] The removal of solvent is difficult.

C) Membrane Emulsification

It is a low energy nanoemulsion process that requires less surfactant and produces emulsions with a narrow size distribution range. This method involves formation of a dispersed phase through a membrane into a continuous phase. Nevertheless, this method has an limitation the low flux of the dispersed phase through the membrane, this being an issue during scale up.[78]

D) Emulsion Inversion Point

This method consists in varting the composition of the system at a constant temperature. The structures are formed through a progressive dilution with water or oil in order to create kinetically stable nanoemulsions.[75]

3. Microfluidization

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 - 20,000 psi), which forces the product through the interaction chamber, consisting of small channels called "microchannels". The product flows through the micro-channels on to an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion.[79] The coarse emulsion is introduced into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until the 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. High pressure homogenization and microfluidization can be used for fabrication of nanoemulsions at laboratory and industrial scale, whereas ultrasonic emulsification is mainly used at laboratory scale.

4. Phase Inversion Temperature Technique

Studies on nanoemulsion formulation by the phase inversion temperature method have shown a relationship between minimum droplet size and complete solubilization of the oil in a microemulsion bicontinuous phase independently of whether the initial phase equilibrium is single or multiphase. Due to their small droplet size nanoemulsions possess stability against

sedimentation or creaming with Ostwald ripening forming the main mechanism of nanoemulsion breakdown.[80] Phase inversion in emulsions can be one of two types: transitional inversion induced by changing factors which affect the HLB of the system, e.g. temperature and/or electrolyte concentration, and catastrophic inversion, which can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures.[81] Phase inversion temperature (PIT) method employs temperature dependent solubility of nonionic surfactants, such as polyethoxylated surfactants, to modify their affinities for water and oil as a function of the temperature. It has been observed that polyethoxylated surfactants tend to become lipophilic on heating owing to dehydration of polyoxyethylene groups. This phenomenon forms a basis of nanoemulsion fabrication using the PIT method. In the PIT method, oil, water and nonionic surfactants are mixed together at room temperature. This mixture typically comprises o/w microemulsions coexisting with excess oil, and the surfactant monolayer exhibits positive curvature. When this macroemulsion is heated gradually, the polyethoxylated surfactant becomes lipophilic and at higher temperatures, the surfactant gets completely solubilized in the oily phase and the initial o/w emulsion undergoes phase inversion to w/o emulsion. The surfactant monolayer has negative curvature at this stage. This method involves heating of the components and it may be difficult to incorporate thermolabile drugs, such as tretinoin and peptides, without affecting their stability. Although it may be possible to reduce the PIT of the dispersion using a mixture of components (surfactants) with suitable characteristics, in order to minimize degradation of thermolabile drugs.

5. Solvent Displacement Method

The solvent displacement method for spontaneous fabrication of nanoemulsion has been adopted from the nano-precipitation method used for polymeric nanoparticles. In this method, oily phase is dissolved in water-miscible organic solvents, such as acetone, ethanol and ethyl methyl ketone. The organic phase is poured into an aqueous phase containing surfactant to yield spontaneous nanoemulsion by rapid diffusion of organic solvent. The organic solvent is removed from the nanoemulsion by a suitable means, such as vacuum evaporation. Spontaneous nanoemulsification has also been reported when solution of organic solvents containing a small percentage of oil is poured into aqueous phase without any surfactant. Solvent displacement methods can yield nanoemulsions at room temperature and require simple stirring for the fabrication. Hence, researchers in pharmaceutical sciences are employing this technique for fabricating nanoemulsions mainly for parenteral use. However, the major drawback of this method is the use of organic solvents, such as acetone, which require additional inputs for their removal from nanoemulsion. Furthermore, a high ratio of solvent to oil is required to obtain a nanoemulsion with a desirable droplet size. This may be a limiting factor in certain cases. In addition, the process of solvent removal may appear simple at laboratory scale but can pose several difficulties during scaleup.

6. Phase Inversion Composition Method (Self-Nanoemulsification Method)

This method has drawn a great deal of attention from scientists in various fields (including pharmaceutical sciences) as it generates nanoemulsions at room temperature without use of any organic solvent and heat. Kinetically stable nanoemulsions with small droplet size (~50 nm) can be generated by the stepwise addition of water into solution of surfactant in oil, with gentle stirring and at constant temperature. The spontaneous nanoemulsification has been related to the phase transitions during the emulsification process and involves lamellar liquid crystalline phases or D-type bicontinuous micro-emulsion during the process. Nanoemulsions obtained from the spontaneous nanoemulsification process are not thermodynamically stable, although they might have high kinetic energy and long term colloidal stability.[82]

Characterization of nanoemulsions

Characterization of nanoemulsions involves the physical and chemical tests related to oral liquid dosage forms which includes compatibility of the nanoemulsion components, isotropicity of the formulation, assay, uniformity of content, appearance, pH, viscosity, density, conductivity, surface tension, size and zeta potential of the dispersed phase etc. with respect to the effect of the composition on physical parameters.[83-93] Differential scanning calorimetry (DSC) provides information on the interactions of different components and polarization microscopy using crossed polarizers is employed to confirm isotropicity of the formulation.[88] The process of self-nanoemulsification can be evaluated by visual assessment. Its efficiency would be estimated by determining the rate of emulsification and droplet size distribution. Turbidity measurements are carried out to determine the rapid equilibrium reached by the dispersion and reproducibility of this process. The droplet size of the emulsion is a crucial factor in self-nanoemulsification performance because it determines the rate and extent of drug release as well as absorption. Photon correlation spectroscopy (PCS) and light scattering techniques like static light scattering (SLS), dynamic light scattering (DLS) are a useful method for determination of nanoemulsion droplet size.[100] Viscosity, conductivity and dielectric methods provide useful information at the macroscopic level. Viscosity measurements for example can indicate the presence of rod-like or worm-like reverse micelles and conductivity measurements provide the means of determining whether a nanoemulsion is oilcontinuous or water-continuous, as well as providing a means of monitoring phase inversion phenomena.[88] Dielectric measurements are a powerful means of probing both the structural and dynamic features of nanoemulsion system. Structural features of nanoemulsions have been studied using self-diffusion

nuclear magnetic resonance (SD NMR) and small angle x-ray scattering (SAXS). Freeze fracture electron microscopy has also been used to study nanoemulsion structure, however extremely rapid cooling of the sample is required in order to maintain the structure and minimize the possibility of artifacts.[90-92] Nanoemulsion droplet polarity is also a very important factor in characterizing emulsification efficiency. The HLB, chain length and degree of unsaturation of fatty acids, molecular weight of the hydrophilic portion and concentration of the emulsifier have an impact on the polarity of the oil droplets. Polarity represents the affinity of the drug compound for oil and/ or water and the type of forces formed. Rapid release of the drug into the aqueous phase is promoted by the polarity. The charge of the oil droplets of nanoemulsions is another property that should be assessed. Usually it is negative due to the presence of free fatty acids; however, incorporation of a cationic lipid, such as oleylamine at a concentration range of 1-3%, will yield cationic nanoemulsions.[93,94] The following sub-headings could be used to discuss briefly the parameters commonly employed in the assessment of nanoemulsions:

Morphology

The morphology of nanoemulsions can be determined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). SEM gives a three dimensional image of the globules.[94] The samples are examined at suitable accelerating voltage, usually 20 kV, at different magnifications. A good analysis of surface morphology of disperse phase in the formulation is obtained through SEM. Image analysis software may be employed to obtain an automatic analysis result of the shape and surface morphology.[95] In TEM, higher resolution images of the disperse phase are obtained. The sample is negatively stained with 1% aqueous solution of phosphotungstic acid or by dropping 2% uranyl acetate solution onto a 200 µm mesh size Pioloform™-coated copper grid or a microscopic carbon-coated grid using a micropipette and the sample examined under a transmission electron microscope at appropriate voltage. Qualitative measurements of sizes and size distribution of TEM micrographs can be performed using a digital image processing programme.[96] More sophisticated techniques, such as x-ray or neutron scattering, atomic force microscopy, or cryo-electron microscopy are typically required to explore the structure and behaviour of nanoemulsions.

Droplet size, polydispersity and zeta potential

Dynamic light scattering (DLS) otherwise called photon correlation spectroscopy (PCS) is used to analyze the fluctuations in the intensity of scattering by droplets/particles due to Brownian motion.[97] Nanoemulsion droplet size, polydispersity and zeta potential can be assessed by PCS using a particle size analyzer. This instrument also measures polydispersity index, which is a measure of the broadness of the size distribution derived from the cumulative analysis of dynamic light scattering. The polydispersity index indicates the quality or homogeneity of the dispersion.[98] PCS gives z-average particle diameter. Laser diffraction is another technique for measuring particle size. The fundamental particle size distribution derived by this technique is volume based and is expressed in terms of the volume of equivalent spheres (DN%) and weighted mean of the volume distribution (mass mean diameter). Since the laser diffraction system is used for this analysis, a rough equivalent of particle polydispersity could be given by two factors/values namely, uniformity (how symmetrical the distribution is around the median point) and span (the width of the distribution). The span value is defined by the expression:

Span = (D90%- D10%) / D50%

Where DN% (N=10%, 50%, 90%), means that the volume percentage of particles with diameters up to DN% equals to N%. The smaller the span value the narrower the particle size distribution.

Viscosity

This is carried out using a viscometer. The viscosity of nanoemulsions is a function of the surfactant, water and oil components and their concentrations. Increasing the water content lowers the viscosity, while decreasing the amount of surfactant and cosurfactant increases interfacial tension between water and oil resulting in increased viscosity. Viscosity is very important for stability and efficient drug release. Nanoemulsion carrier formulations are basically oil-in-water and so in addition to being less greasy than water-in-oil formulations, often possess lower apparent viscosities. They are therefore expected to exhibit faster release of active ingredients and wash out easily after application on the skin surface. Various equipment and methods are available for assessment of rheological properties of nanoemulsion carriers. Monitoring of viscosity change is a method of assessing stability of liquid and semi-solid preparations including nanoemulsion formulations.[88]

In vitro skin permeation

Franz diffusion cell is used to obtain the drug release profile of the nanoemulsion formulation in the case of formulations for transdermal application. The extent or depth of skin penetration by the released content can be visualized by confocal scanning laser microscopy. In vitro drug release can be determined by dispersing an amount of the preparation in the donor compartment of a Franz cell having a membrane as barrier and monitoring the appearance of the encapsulated drug in the receptor compartment, usually containing phosphate buffer saline (PBS, pH 7.4) and stirring on a magnetic stirrer at 100 rpm at 37 ± 1°C. Samples (1 ml) of the dispersion are withdrawn from the receptor medium and replaced with an equivalent amount of the medium at definite intervals. The withdrawn sample is then filtered using a 0.22-50 µm filter (e.g., Millipore, USA) and the drug released then analyzed using HPLC or UV-Vis spectroscopy at wavelength of peak absorption of the drug.[99] An alternative and popular method of ex-vivo release study

is performed using diffusion cell. The skin is cut from the ear or abdomen and underlying cartilage and fats carefully removed. Appropriate size of skin is cut and placed on the diffusion cell which had earlier been filled with receptor solution. Samples of the vesicular preparation are then applied on the dorsal surface of the skin and the instrument started. At intervals, up to 24 h, samples are withdrawn from the receptor medium and replaced with equal amounts of the medium and the withdrawn samples analyzed for the drug permeated using HPLC or UV spectroscopy.[100,101] Semipermeable membrane such as regenerated cellulose could be used in place of skin for in vitro release studies.[102,103] The flux J, of the drug across the skin or membrane is calculated from the formula:

$$J=Ddc/dx$$

Where D is the diffusion coefficient and is a function of the size, shape and flexibility of the diffusing molecule as well as the membrane resistance, c is the concentration of the diffusing species, x is the spatial coordinate.[103]

In vivo bioavailability/pharmacodynamic studies

In vivo release study otherwise referred to as dermato pharmacokinetics, is carried out by applying or administering the preparation to whole live animal. Blood samples are then withdrawn at intervals, centrifuged and the plasma (deproteinated) analyzed for the drug content using HPLC. Results obtained from in vitro and in vivo studies are extrapolated to reflect bioavailability of the drug formulation. Moreover, the pharmacodynamic properties of nanoemulsion formulations are also assessed depending on the pharmacological properties of the incorporated drug.[84, 90]

Marketed Product[104-106]

Some important patents related to NEs:

1. Patent name: Method of Preventing and Treating Microbial Infections. Assignee: NanoBio Corporation (US). US Patent number:6,506,803.
2. Patent name: NE based on phosphoric acid fatty acid esters and its uses in the cosmetics, dermatological, pharmaceutical, and/or ophthalmological fields. Assignee: L'Oreal (Paris, FR). US Patent number:6,274,150.
3. Patent name: NE based on ethylene oxide and propylene oxide block copolymers and its uses in the cosmetics, dermatological and/or ophthalmological fields. Assignee: L'Oreal (Paris, FR). US Patent number: 6,464,990.
4. NE of 5-aminolevulinic acid (6,559,183). Assignee: ASAT AG Applied Science and Technology (Zug, CH). PCT number: PCT/ EP99/08711.
5. NEs of poorly soluble pharmaceutical active ingredients and methods of making the same. Patent no.: WO/2007/103294.

Some commercially available NE formulations are shown in Table 2.

Drug therapeutic	Brand	Manufacturer	Indication
Propofol	Diprivan	Astra Zeneca	Anesthetic
Dexamethasone Palmitate	Limethason	Mitsubishi Pharmaceutical, Japan	Steroid
Alprostadil	Liple	Mitsubishi Pharmaceutical, Japan	Vasodilator platelet inhibitor
Flurbiprofen axetil	Ropion	Kaken Pharmaceuticals, Japan	Nonsteroidal analgesic
Vitamins A, D, E, K	Vitalipid	Fresenius Kabi, Europe	Parenteral nutrition

Future Industrial Perspectives

Nanoemulsion since its emergence has proved to be versatile and useful novel drug delivery system. Nano-emulsions are proposed for numerous applications in pharmacy as drug delivery systems because of their capacity of solubilizing non-polar active compounds. Future perspectives of nanoemulsion are very promising in different fields of therapeutics or application in development of cosmetics for hair or skin. One of the versatile applications of nanoemulsions is in the area of drug delivery where they act as efficient carriers for bioactive, facilitating administration by various routes. Their parenteral delivery has been adopted for supplying nutriational requirements, controlled drug release, vaccine delivery and for drug targeting to specific sites. The advantages and applications of oral drug delivery through these vehicles are numerous where the droplet size is related to their absorption in the gastrointestinal tract. Nanoemulsions have also been studied for their use in ocular delivery where pharmacological drugs are more sustained compared to their respective solutions. Pulmonary and transdermal routes are other successful ways of administering nanoemulsified delivery system. Although there have not been many reports of nanoemulsion applications in other fields, there is a great potential for nanoemulsion applications in other areas, such as in chemical and physical sciences, agriculture and engineering. In the production of nanoemulsions

there are some limitations, but pharmaceutical and food industries have to adjust their technologies to accommodate nanoemulsion production. Considering the versatile platforms nanoemulsions offer to formulation scientists in many fields, retooling of production facilities or outright change in technology of industries originally involved in production of parenteral and macro emulsions will lead to a lot economic windfall on the long run. This is because the effect of difficulty in preparation and the high energy input that may be involved in the production of nanoemulsion may just be felt on the short run. In as much as the cost of acquiring the technology for nanoemulsion production may be high, the production of nanoemulsions involves only a few steps, compensating the many steps involved in the production of some other products of lower versatility. Due to the renewed interest in herbal drug formulation, nanoemulsion may be the ideal delivery platform for these difficult-to-formulate phyto pharmaceuticals. Novel nanoemulsion dosage forms of herbal drugs will lead to higher remuneration for the pharmaceutical industries with the advent of new instruments for high pressure homogenization and the competition between various manufacturers, the cost of production of nanoemulsions will decrease. Fundamental research in investigation of the role of surfactants in nanoemulsion production process will lead to optimized emulsifier systems and more economic use of surfactants will emerge. Nanoemulsions can be manipulated for targeted delivery and this hold significant promise in the area of oncology for the treatment of tumors and drug delivery to the brain.

Table 3: Differences between emulsion, nanoemulsion and microemulsion.

Emulsion	Nanoemulsion	Microemulsion
Excellent kinetic stability.	Kinetically unstable.	They possess some kinetic stability
Thermodynamically unstable and will eventually phase separate.	Thermodynamically stable and no phase separation occur.	Thermodynamically stable
Emulsions appear cloudy.	Nanoemulsions are clear or translucent.	Microemulsion are clear
Methods involved in preparation of emulsion require a large input of energy.	Methods of preparation do not require energy input.	Methods of preparation do not require energy input.

Table 4: List of oils.

Name	Chemical name
Captex 355	GlycerylTricaorylate/Caprate
Captex 200	Propylene Dicaprylate/Dicaprate Glycol
Captex 8000	GlycerylTricaprylate (Tricaprylin)
Witepsol	90:10 % w/w c12 Glyceride tri: diesters
Myritol 318	C8/C10 triglycerides
Isopropyl Myristate	Myristic acid isopropyl ester

Table 5: List of surfactants.

Name	Chemical name
Tween 20	Polyoxyethylenesorbitanmonolaurate
Tween 80	Polyoxyethylene (20) sorbitanmonooleate
Labrasol	Caprylocaproyl macrogol-8 glycerides
Labrafil M 1944	Oleoyl macrogol-6 glycerides
Cremophor RH 40	Polyoxly 40 hydrogenated castor oil
PlurolOleique CC	Polyglyceryl-3 oleate

Table 6: List of cosurfactants.

Name	Chemical name
Transcutol P	Diethylene glycol monoethyl ether
Ethylene glycol	Ethane 1,2 diol
Propylene glycol	1,2 propanediol

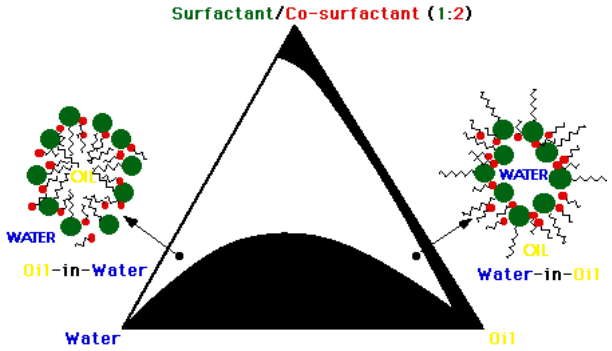

Fig. 2: Construction of pseudoternary phase diagram.

CONCLUSION

Nanoemulsion can act as a colloidal carrier for various lipophilic drug diagnostic agents etc The skin penetrative properties and low irritancy make it a suitable carrier for the transdermal delivery of the drugs. In the upcoming future further research work and development will be carried out for Clinical application of nanoemulsion. Nanoemulsion formulations offer several advantages for the delivery of drugs, biologicals, or diagnostic agents. Traditionally, NEs have been used in clinics for more than four decades as total parenteral nutrition fluids. Several o ther products for drug delivery applications such as Diprivan, Liple and Ropion have also reached the marketplace. Although NEs are chiefly seen as vehicles for administering aqueous insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers neutron capture therapy agents, or diagnostic agents. Because of their submicron size, they can be easily targeted to the tumor area. Recently, several research papers have been published for the improvement of drug delivery, but still there is a need to emphasis on its characterization part including in-vitro evaluation. Besides this, research papers shows higher percentage of surfactant (much higher than CMC level) used for the formation of Nanoemulsion, irrespective of different routes of administration, but there is a lack of toxicological evaluation of the prepared Nanoemulsion, which can be a broad research area in future.

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