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ROLE OF NANO-EMULSION IN PHARMACEUTICAL SCIENCES-A REVIEW

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ABSTRACT

Nanoemulsions are submicron sized emulsions that are under investigation as drug carriers for improving the delivery of therapeutic agents. These are the thermodynamically stable isotropic system in which two immiscible liquids are mixed to form a single phase by means of appropriate surfactant and cosurfactant. Nanoemulsion droplet sizes fall typically in the range of 20- 200nm and shows narrow size distribution. In this review attention is focused to give the brief regarding formulation aspect, method of preparation characterization techniques, evaluation parameters and various application of the nanoemulsions, several techniques are to be used for preparation of nanoemulsions like microfluidization, high pressure homogenization, low energy emulsification and solvent evaporation method and parameter that are to be used for its characterization like droplet size analysis ,viscosity determination, drug content, refractive index, pH, zeta potential, Transmission electron microscopy, thermal stability, release and *in vitro* skin permeation study. These are applicable in drug targeting. 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 and unusual elastic behaviour. Nanoemulsions have widespread applications in different fields such as pharmaceutics, food technology.

KEYWORDS

Nanoemulsion, Self emulsification, Co-surfactant and High-pressure Homogenization.

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INTRODUCTION

The term "Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. It is defined as oil- in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm, terms sub- micron emulsion (SME) and mini-emulsion are used as

synonyms. Since, the preparation of the first nanoemulsion in 1940s, it can be of three types such as oil-in-water (O/W), water-in-oil (W/O), and bi- continuous. The transformation between these three types can be achieved by varying the components of the emulsions. Each type of the nanoemulsions serves as a template for preparing polymer latex particles, nanoporous polymeric solids etc. Apart from this, the nanoemulsions with pharmaceutically accepted ingredients are utilized in the development of drug formulations for oral drug delivery. The Nanoemulsions are also referred as miniemulsions, ultrafine emulsions and submicron emulsions. Phase behaviour studies have shown that the size of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition. Studies on Nanoemulsion formation 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. The main application of Nanoemulsions is the preparation of nanoparticles using a polymerizable monomer as the disperse phase (the so-called miniemulsion polymerization method) where Nanoemulsion droplets act as nanoreactors. Another interesting application which is experiencing an active development is the use of Nanoemulsions as formulations, namely, for controlled drug delivery and targeting. The main application of nanoemulsions is the preparation of nanoparticles using a polymerizable monomer as the disperse phase where nanoemulsion droplets act as nanoreactors.

Classification of Nanoemulsions

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

O/W Nanoemulsion

Wherein oil droplets are dispersed in the continuous aqueous phase.

W/O Nanoemulsions

Wherein water droplets are dispersed in the continuous oil phase.

Bi-continuous Nanoemulsions

Wherein microdomains of oil and water are interdispersed within the system.

The surfactants used to stabilise such systems as shown in the Table No.1.

A combination of these, particularly ionic and non-ionic, can be very effective at increasing the extent of the Nanoemulsion region. Examples:

1. Non-ionic includes polyoxyethylene surfactants such as Brij 35 (C12E35) or sugar esters such as sorbitan monooleate (Span 80).
2. Zwitterionic surfactants include Phospholipids are a notable example and exhibit excellent biocompatibility.
3. Cationic surfactants include Lecithin preparations from a variety of sources including soybean and egg are available commercially and contain diacylphosphatidylcholine as its major constituent. Quaternary ammonium alkyl salts form one of the best known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide and cosurfactant to be added and the percent of oil phase that can be incorporated shall be determined with the help of pseudo-ternary phase diagram.

Ultrasonicator[1] can finally be used so to achieve the 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 (crosslinked polyacrylic acid polymers) are the most widely used gelling agent.

Multiple emulsions

The Multiple emulsions are novel carrier system which are complex and poly dispersed in nature where both w/o and o/w emulsion exists simultaneously in a single system. Lipophilic and hydrophilic surfactants are used for stabilizing these two emulsions respectively. The droplets of the dispersed phase contain even smaller dispersed

droplets themselves, therefore also called as "emulsions of emulsions". Each dispersed globule in the double emulsion forms a vesicular structure with single or multiple aqueous compartments separated from the aqueous phase by a layer of oil phase compartments. In multiple emulsions system solute has to transverse from inner miscible phase to outer miscible phase through the middle immiscible organic phase, so it also called as liquid membrane system[2].

Factors to Be Considered During Preparation of Nanoemulsion

Important conditions

1. Surfactants must be carefully chosen so that an ultra low interfacial tension (< 10-3 mN/m) can be attained at the oil / water interface which is a prime requirement to produce nanoemulsions.
2. Concentration of surfactant must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultra low interfacial tension.
3. The interface must be flexible or fluid enough to promote the formation of nanoemulsions.

The two major types of multiple emulsions are the water-oil-water (w/o/w) and oil-water-oil (o/w/o) double emulsions. The most common multiple emulsions are of W/O/W type, although some specific applications O/W/O emulsions can also be prepared. Multiple emulsions may find many potential applications in various fields such as chemistry, pharmaceutics, cosmetics, and food. These emulsions have been investigated as controlled-release drug delivery systems (DDS), as 'emulsion liquid membranes' for simultaneous liquid extraction and stripping of metals, organic acids and antibiotics, as microcapsules for the protection and controlled release of functional food ingredients, for the formulation of reduced-calorie food emulsions , etc. Other applications include the use of multiple emulsions as intermediate products to the preparation of inorganic particles, lipid nanoparticles, polymeric microspheres, biodegradable microspheres, gel microbeads, and vesicles such as polymerosomes[3].

Preparation Methods of Nano Emulsions

The drug is be dissolved in the lipophilic part of the nanoemulsion i.e. oil and the water phases can be 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 shall be determined with the help of pseudo-ternary phase diagram. Ultrasonicator[1] can finally be used so to achieve the 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 (crosslinked polyacrylic acid polymers) are the most widely used gelling agent as shown in the Table No.2.

Phase Diagram

Pseudo-ternary phase diagrams of oil, water, and co-surfactant/surfactants mixtures are constructed at fixed cosurfactant/surfactant weight ratios. Phase diagrams are 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. Several methods have been suggested for the preparation of nanoemulsion. Here some methods are discussed which are freely used for the nanoemulsion preparation[4].

Phase Inversion Method

In this method, fine dispersion is obtained by chemical energy resulting of phase transitions occur through emulsification method. The adequate phase transitions are produced by changing the composition at constant temperature or by changing the temperature at constant composition, phase inversion temperature (PIT) method was introduced by Shinoda *et al*. based on principle of the changes of solubility of polyoxyethylene-type surfactant with

temperature. This surfactant becomes lipophilic as increase in temperature because of dehydration of polymer chain. At low temperature, the surfactant monolayer has a great positive spontaneous curvature forming oil swollen micellar solution phase[2].

Method

Sonication method is best way to prepare nanoemulsions. In sonication method the droplet size of conventional emulsion or microemulsions are reduced with the help of sonication mechanism. This method is not applicable for large batches, but only small batches of nanoemulsions can be prepared by this method[5].

Ultrasonic System

In ultrasonic emulsification, the energy input is provided through so called sonotrodes (sonicator probe) containing piezoelectric quartz crystals that can be expand and contract in response to alternating electrical voltage. As the tip of sonicator probe contacts the liquid, it generates mechanical vibration and therefore cavitations occurs, which is the main phenomenon responsible for ultrasonically induced effects. Cavitation is the formation and collapse of vapour cavities in a flowing liquid. Such a vapour cavity forms when the local pressure is reduced to that of at the temperature of the flowing liquid because of local velocity changes. The collapse of these cavities causes powerful shock waves to radiate throughout the solution in proximity to the radiating face of the tip, thereby breaking the dispersed droplets. Within the ultrasound range, the power available varies inversely with the frequency and only powerful ultrasound (0-200kHz) is able to produce physical and chemical changes such as emulsification. Ultrasound can be used directly to produce emulsion, but since breaking an interface requires a large amount of energy, it is better to prepare coarse emulsion before applying acoustic power. Due to small product throughput the ultrasound emulsification process mainly applied in laboratories where emulsion droplet size as low as 0.2 micrometer can be obtained[6].

Microfluidizer

It is possible to produce emulsion at much higher pressures up to approximately 700 Mpa, in the nozzle of microfludizer that is the heart of this device (the interaction chamber) two jets of crude emulsion from two opposite channels collide with one another. The process stream is delivered by a pneumatically powered pump that is capable of pressurizing the in-house compressed air (150-650 Mpa) up to about 150 Mpa. Forcing the flow stream by high pressure through microchannels toward an impingement area creates a tremendous shearing action, which can provide an exceptionally fine emulsion[7].

Jet Disperser

Forcing the flow stream by high pressure through microchannels towards an impregnated area creates a tremendous shearing action, which can provide an exceptionally fine emulsion. In general, initial forces in turbulent flow along with cavitations are predominantly responsible for droplet disruption in microfludizer. Disruption in laminar elongation flow is also possible, especially when emulsion has high viscosity. In the jet disperser two or more jets of crude emulsion each from opposing bores collide with one another but at a different design than microfludizer, the diameter of the bores in jet dispersers are typically 0.3-0.5mm. Finally an "orifice plate" is the simplest construction form for a homogenizing nozzle. The diameter of orifice bore is of same order of magnitude as the jet dispersers and inlet head diameter of orifice plate is typically 10-60nm, in jet dispersers and orifice plates[8]. Droplets are disrupted predominantly due to laminar elongation flow ahead of the bores. Unlike radial diffusers, the nozzle is microfludizer; jet dispersers and orifice plate contain no moving parts, so they can be used at high pressures up to 300-400 Mpa.

Preparation of multiple emulsions

Multiple emulsions are usually formed by a two-step emulsification process using conventional rotor-stator or high pressure valve homogenizers[9]. The primary W/O or O/W emulsion is prepared under high-shear conditions to obtain small inner droplets, while the secondary emulsification step is carried out

with less shear to avoid rupture of the liquid membrane between the innermost and outermost phase. However, the second step often results in highly polydisperse outer drops (if homogenizing conditions are too mild) or in small encapsulation efficiency (if homogenization is too intensive). Multiple emulsions can alternatively be produced by forcing a primary emulsion through a microporous membrane [10-13] or micro- fabricated channel arrays[1, 14, 15] into a continuous phase liquid. This results in much less shear than in conventional emulsification processes so that the droplets are intact and both a high entrapment efficiency and monodispersity can be achieved different Surfactant and Co-surfactant used in Nanoemulsions as shown in the Table No.3.

Formulation of Multiple Emulsions

Florence and Whitehil[16] described three different types of multiple emulsions, which they termed A, B, and C. Type A multiple emulsions were those in which only one large internal drop was contained in the secondary emulsion droplet. In type B emulsions, there were several small internal droplets contained in the secondary emulsion droplet, and type C emulsions were those with a large number of internal droplets present. Only the type C systems have applications in drug delivery and drug targeting. The objectives will be to produce a multiple emulsion system that has a high yield of multiple droplets containing the drug entrapped in the innermost phase, and for such a system to have good stability *in vitro* and the desired release characteristics *in vivo*. The following factors are identified as being of importance[17] and will be discussed in turn with reference to the w/o/w system:

Emulsification equipment

The primary emulsion can be prepared using a laboratory mixer or homogenizer to provide a good dispersion of droplets within the appropriate continuous phase.

Nature of the oil phase

The oil phase to be employed in a pharmaceutical emulsion must be nontoxic. The various oils of vegetable origin (soybean, sesame, peanut, safflower, etc.) are acceptable if purified correctly. Refined hydrocarbons such as light liquid paraffin squalane, as well as esters of fatty acids (ethyl oleate and isopropyl myristate) have also been used in double emulsions.

Volumes of the two dispersed phases

The quantity of water dispersed in the initial w/o emulsion [expressed as a phase volume ratio, (w/o/w)] can have an influence on both the yield and stability of the final emulsion system.

Nature and quantity of the emulsifying agents

Two different emulsifiers (lipohilic and hydrophilic) are required to form a stable emulsion. In general, for a w/o/w emulsion the optimal HLB value will be in the range 2-7 for the primary surfactant and in the range 6-16 for the secondary surfactant[18].

Characterization and Evaluation of Nanoemulsion

Different characterization parameters for nanoemulsion include transmission electron microscopy, nanoemulsion droplet size analysis, viscosity determination, refractive index, *in vitro* skin[3], permeation studies, skin irritation test, *in vivo* efficacy study, thermodynamic stability studies, and surface characteristics. The surface charge of the nanoemulsion droplets has a marked effect on the stability of the emulsion system and the droplet *in vivo* disposition and nanoemulsion droplets were in the size range of 25-40 nm with some particle aggregates in the size range of 100-150 nm.

Nanoemulsion Droplet Size Analysis

Droplet size distribution is one of the important physicochemical characteristics of a nano-emulsion, was measured by a diffusion method using a light-scattering particle size analyzer Coulter LS-230. It measures the size distribution using the diffusion of laser light by particles. Polarization intensity differential scattering (PIDS) is the assembly consists of an incandescent light source and polarizing filters, a PIDS sample cell and an additional seven photodiode detectors.

It is used to measure the droplets size distribution, like 0.5 ml emulsion was introduced in the measure compartment (125 ml of water). The results were presented as the volume distribution. Many other techniques that have been developed to measure droplet size of nanoemulsions, two are of interest in

this article in which laser light scattering (LLS) and energy filtering transmission electron microscopy (EFTEM). The small droplet size gives them inherent stability against creaming, sedimentation, flocculation and coalescence.

Polydispersity Index

The average diameters and polydispersity index of samples were measured by photon correlation spectroscopy. The measurements were performed at 25^0C using a He-Ne laser.

Viscosity Determination

The viscosity of the formulations was determined as such without dilution using a Brookfield DV III ultra V6.0 RV cone and plate rheometer using spindle[19, 20].

Refractive Index

The refractive index, n, of a medium is defined as the ration f the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium.

n=c/vp

It was determined using an Abbes type refractrometer (Nirmal International) at 25 ± 0.5^0C.

pH

The apparent pH of the formulation was measured by pH meter[19, 20].

Transmission Electron Microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using transmission electron microscopy. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of nanoemulsion droplets. Observations was performed as, a drop of the nanoemulsion was directly deposited on the holey film grid and observed after drying.

Drug Content

Drug content was determined by reverse phase HPLC method using C18 column[21].

Zeta Potential

Zeta potential is a technique which is used to measure the surface charge properties and further the long term physical stability of nanoemulsions, the instrument which is used to measure the surface charge is known as Zeta PALS. The measurements were carried out with diluted nanoemulsion formulations[22] and its values were determined from the electrophoretic mobility of the oil droplets. The minimum zeta potential of ±20mv is desirable[3].

Percentage Transmittance

Percentage transmittance of the prepared nanoemulsion formulations was determined spectrophotometrically using UV-VIS Spectrophotometer[23].

***In vitro* Skin Permeation Studies**

In vitro skin permeation studies were performed by using Keshary Chien-diffusion cell. It was performed on abdominal skins and was obtained from male rats weighing 250±10 gm with a recirculating water bath and 12 diffusion cells. The skins were placed between the donor and the receiver chambers of vertical diffusion cells. The receiver chambers were filled with freshly water containing 20% ethanol. The receiver chambers were set at 37^0C and the solution in the receiver chambers was stirred continuously at 300 rpm. The formulations were placed in the donor chamber. At 2, 4, 6, 8 h, 0.5 ml of the solution in the receiver chamber was removed for GC analysis and replaced immediately with an equal volume of fresh solution[24]. Each sample was performed three times. The cumulative corrections were made to obtain the total amounts of drugs permeated at each time interval. The cumulative amounts of drug permeated through rat skins were plotted as a function of time. The permeation rates of drug at a steady-state through rat skins were calculated from the slope of linear portion of the cumulative amount permeated through the rat skins per unit area versus time plot[16].

Thermodynamic Stability Studies

During the thermodynamic stability of drug loaded Nanoemulsions following stress tests as given below.

Heating Cooling Cycle

Nanoemulsion formulations were subjected to six cycles between refrigerator temperature (4°C) and 45°C. Stable formulations were then subjected to centrifugation test.

Centrifugation

Nanoemulsion formulations were centrifuged at 3500 rpm and those that did not show any phase separation were taken for the freeze thaw stress test.

Freeze Thaw Cycle

In this the formulation were subjected to three freeze thaw cycles between 21°C and +25°C kept under standard laboratory conditions. These studies were performed for the period of 3 months[4].

Nanoemulsions in pharmaceutical science

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 and unusual elastic behavior. Nanoemulsions have widespread applications in different fields such as pharmaceutics, food technology. Nanoemulsion offers a promising vehicle for increasing the aqueous solubility of poorly water-soluble drugs. Nanoemulsions have many advantages; for instance, enhance drug solubility, perfect thermodynamic stability, ease of manufacturing and permeation over conventional formulations that convert them to important drug delivery systems[25].

The design and development of nanoemulsions aimed at controlling or improving required bioavailability levels of therapeutic agents. The use of nanotechnology in pharmaceuticals and medicine has grown over the last few years. The pharmaceuticals developed on the basis of nanotechnology are termed as "Nanopharmaceuticals". The various nano pharmaceuticals currently being used or in the process of development are Nanoemulsions (NE) (submicron sized emulsions), nanosuspensions (submicron sized suspensions), nanospheres (drug nanoparticle in polymer matrix), nanotubes (sequence of nanoscale C60 atoms arranged in a long chain cylindrical structure), nanoshells (concentric sphere nanoparticles consisting of a dielectric core and a metal shell), nanocapsules (encapsulated drug nanoparticles), lipid nanoparticles (lipid monolayer enclosing a solid lipid core) and dendrimers (nanoscale three- dimentional macromolecules of polymer). NEs are group of dispersed particles for pharmaceutical and biomedical aids and vehicles that show great promise for the future of cosmetics. NEs can be defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000nm. Usually, the average droplet size is between 100 and 500nm. The particles can exist as water-in-oil and oil-in-water forms, where the core of the particle is either water or oil, respectively. NEs are made from surfactants approved for human consumption and common food substance that are "Generally Recognised as Safe" (GRAS) by the FDA. These emulsions are easily produced in large quantities by mixing a water-immiscible oil phase into an aqueous phase with a high-stress, a mechanical extrusion process[4].

The NEs are also referred as microemulsions, ultrafine emulsions and submicron emulsions. Phase behavior studies have shown that the size of the droplets is governed by the surfactant phase structure (biocontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition. Studies on NE formation by the phase inversion temperature (PIT) method have shown a relationship between minimum droplet size and complete solubilization of the oil in a microemulsion biocontinuous phase independently of whether the initial phase equilibrium is single or multiphase. Nanoemulsions are submicron sized emulsions that are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. They are by far the most advanced nanoparticle systems for the cosmetics. It helps to give skin care formulations good skins feel, an increasingly important characteristic for formulators[3].

NEs posses various advantages such, NEs have a much higher surface area and free energy than macroemulsions that make them an effective transport system.

1. NEs do not show the problems of inherent creaming, flocculation, coalescence, and sedimentation, which are commonly associated with macroemulsions.

2. NEs can be formulated in variety of formulations such as foams, creams, liquids and sprays.
3. NEs are non-toxic and non-irritant hence can be easily applied to skin and mucous membranes.
4. NEs are formulated with surfactants, which are approved for human consumption (GRAS), they can be taken by enteric route.
5. NEs do not damage healthy human and animal cells, hence are suitable for human and veterinary therapeutic purposes[26].

Nanoemulsions as a Vehicle

From *in vitro* and *in vivo* data, it was concluded that the developed nanoemulsion s have great potential for transdermal drug delivery of aceclofenac[27]. The nanoemulsion of the system containing ketoprofen evidenced a high degree of stability. Ketoprofen-loaded nanoemulsions enhanced the *in vitro* permeation rate through mouse skins as compared to the control[28]. The study was developed to evaluate the potential of nanoemulsions for increasing the solubility and the *in vitro* transdermal delivery of carvedilol. The prepared nanoemulsions were subjected to physical stability tests. Transdermal permeation of carvedilol through rat abdominal skin was determined with the Keshary-Chien diffusion cell. Significant increase ($P < 0.05$) in the steady state flux (Jss) and permeability coefficient (Kp) was observed in nanoemulsion formulations as compared to control or drug-loaded neat components. The irritation studies suggested that the optimized nano-emulsion was a non-irritant transdermal deliver system[29].

Self-Nanoemulsifying Drug Delivery Systems

Self-nanoemulsifying drug delivery systems for oral delivery of protein drugs: Formulation development, *in vitro* transport study and *in vivo* oral absorption study[30]. The research project was done to develop a self-nanoemulsifying drug delivery system (SNEDDS) for non-invasive delivery of protein drugs. An experimental design was adopted to develop SNEDDS. Fluorescent- labeled beta-lactamase (FITC-BLM), a model protein, was loaded into SNEDDS through the solid dispersion technique. The experimental design provided 720 compositions of different oil, surfactant, and co-surfactant at various ratios, of which 33 SNEDDS prototypes were obtained. A SNEDDS was developed to load FITC- BLM into the oil phase that can spontaneously form O/W NE upon the addition of water. Fluorescently labeled BLM (FITC-BLM), a model protein, formulated into 16 SNEDDS preparations through a solid dispersion technique were studied for transport across monolayer. All the SNEDDS NEs resulted in higher transport rate than the free solution. The transport rate by SNEDDS depends on the SNEDDS composition. The SNEDDS significantly increased the transport of FITC-BLM across MDCK monolayer *in vitro*. SNEDDS may be a potential effective delivery system for non- invasive protein drug delivery. The oral absorption of BLM in rats when delivered by such a SNEDDS was investigated and showed significantly enhance in the oral bioavailability of BLM. So the SNEDDS has a great potential for oral protein delivery[3].

Nanoemulsions as a Mucosal Vaccine

Nanoemulsions are being used to deliver either recombinant proteins or inactivated organisms to a mucosal surface to produce an immune response. The first applications, an influenza vaccine and an HIV vaccine, can proceed to clinical trials. The nanoemulsion causes proteins applied to the mucosal surface to be adjuvant and it facilitates uptake by antigen-presenting cells. Additional research is ongoing to complete the proof of concept in animal trials for other vaccines including Hepatitis B and anthrax[20] Mice and guinea pigs intranasally immunized by the application of recombinant HIV gp120 antigen mixed in nanoemulsion demonstrated robust serum anti-gp120 IgG, as well as bronchial, vaginal, and serum anti-gp120 IgA in mice.

Nanoemulsion as Non-Toxic Disinfectant Cleaner

A breakthrough nontoxic disinfectant cleaner for use in commercial markets that include healthcare, hospitality, travel, food processing, and military applications has been developed by Enviro Systems, Inc. that kills tuberculosis and a wide spectrum of viruses, bacteria and fungi in 5-10 min without any of the hazards posed by other categories of disinfectants. The product needs no warning labels.

It does not irritate eyes and can be absorbed through the skin, inhaled, or swallowed without harmful effects. The disinfectant formulation is made up of nanospheres of oil droplets #106 mm that are suspended in water to create a NE requiring only miniscule amounts of the active ingredient, PCMX (parachlorometaxylenol). The nanospheres carry surface charges that efficiently penetrate the surface charges on microorganisms' membranes-much like breaking through an electric fence. Rather than "drowning" cells, the formulation allows PCMX to target and penetrate cell walls. As a result, PCMX is effective at concentration levels 1-2 orders of magnitude lower than those of other disinfectants; hence, there are no toxic effects on people, animals, or the environment[5].

Enhancement of drug penetration through the skin

Both the desired and the undesired effect of a drug are dependent on the concentration of the drug at the site of action, which in turns depends upon the dosage form. In transdermal delivery, the goal of dosage design is to maximize the flux through the skin into the systemic circulation. There are two pathways such as tansappendageal and intercellular pathway by which drug can cross the skin and reach the systemic circulation. In the transappendageal route the drug substances penetrate via the sweat glands and the hair follicle. But this route is not an appropriate pathway of penetration for most molecules because it has smaller surface area (less than 0.1%). Also it lack suitable animal model for drug testing. Skin contains an uppermost layer epidermis, which has basal layer, spiny layer, stratum granulosum and upper most layer stratum corneum. The stratum corneum consists of corneocytes which is 200-300 nm thick, 30-50 um in diameter and hexagonal or polygonal in shape. It consists of alternating cell and lipid layers and is only 6- 10 um thick which equivalent to about[14-18] cellular layers. The majority of molecules that cross the epidermis must partition into the stratum corneum before diffusing across the viable epidermis. Therefore, the major pathway for a compound is highly dependent upon its partition coefficient.

Hydrophilic compounds may preferably partition into the intracellular domain, while lipophilic ones may cross the stratum corneum through the Intercellular route. This series of partitioning into and diffusing across multiple hydrophilic and hydrophobic domains is unfavorable for most drugs. Increase the drug penetration into the skin. Moreover, the most of the pharmaceutical substances are lipophilic in nature. The clinical efficacy of such drug is being impeded by their low aqueous solubility resulting in poor penetration and absorption when they are designed for transdermal administration. There has been a continue interest during recent years for modifying drug penetration into and through the skin. It can be possible by use of physical or chmical means of penetration enhancement.

Physical means of penetration enhancement include use of iontophoresis, Sonophoresis and microneedle. Chemical means of penetration enhancement include use of chemical penetration enhancers[19]. The physical means are relatively complicated to use and will affect patients compliance. Most of the topical vehicles contain chemical enhancers and non-friendly solvents to achieve improved permeability[20]. But these vehicles usually results in various degree of irritancy and permanent damage to skin in case of chronic treatments. Therefore it is desirable to develop topical vehicles that do not use chemical enhancers to facilitate drug penetration into and through the skin[21]. One of the most promising techniques for enhancement of transdermal permeation of drug is to develop microemulsion or nanoemulsion[22]. Microemulsions are quaternary systems composed of an oil phase, a water phase, and surfactant in combination with cosurfactant[23]. These spontaneously formed systems pose specific physicochemical properties such as transparency, optical isotropy, low viscosity and thermodynamic stability. In stable microemulsion, droplet diameter is usually within the range of 10-100 nm (100-1000A0), and therefore this system is also termed as nanoemulsion (NE)[24]. Due to unique

physicochemical properties, NE offer advantages over traditional topical and transdermal drug delivery formulation. Many studies have shown that NE formulation possess improved transdermal drug delivery properties both *in vitro*[30-34] as well as *in vivo*[9, 10, 35]. One of the unique characteristics of the NE technology is the relatively high percentage of total particle volume occupied by the internal hydrophobic oil core of the droplets. This provides high solubilization of lipophilic compound as compared to other lipoidal vehicle such as liposomes[11]. Both increase in solute concentration and the tendency of the drug to favor partitioning into stratum corneum make NE a useful vehicle to enhance transdermal drug permeability.

Nanoemulsion in Cancer Therapy and Targeted Drug Delivery

Targeted Drug Delivery the effects of the formulation and particle composition of gadolinium (Gd)-containing lipid nanoemulsion (Gd-nanoLE) on the biodistribution of Gd after its intravenous (IV) injection in D1-179 melanoma-bearing hamsters were evaluated for its application in cancer neutron-capture therapy. Biodistribution data revealed that Brij 700 and HCO-60 prolonged the retention of Gd in the blood and enhanced its accumulation in tumors. Upon dermal application, the drug was predominantly localized in deeper skin layers, with minimal systemic escape. This has amounted to an absolute bioavailability of 70.62%. Inhibition of P-glycoprotein efflux by D-tocopheryl polyethyleneglycol 1000 succinate and labrasol would have contributed to the enhanced peroral bioavailability of PCL. This investigation provides direct evidence on the localization of high-molecular-weight, lipophilic drug, PCL, in dermis. Further, the nanoemulsion formulation has enhanced the peroral bioavailability significantly to more than 70%. The developed nanoemulsion formulation was safe and effective for both peroral and dermal delivery of PCL[18] Camptothecin is a topoisomerase-I inhibitor that acts against a broad spectrum of cancers. However, its clinical application is limited by its insolubility, instability, and toxicity. The aim of the present study was to develop acoustically active nanoemulsions for camptothecin encapsulation to circumvent these delivery problems[30].

The nanoemulsions were prepared using liquid perfluorocarbons and coconut oil as the cores of the inner phase. These nanoemulsions were stabilized by phospholipids and/or Pluronic F68 (PF68). The effects of the formulation and particle composition of gadolinium (Gd)-containing lipid nanoemulsion (Gd-nanoLE) on the biodistribution of Gd after its intravenous (IV) injection in D1-179 melanoma-bearing hamsters were evaluated for its application in cancer neutron-capture therapy. Biodistribution data revealed that Brij 700 and HCO-60 prolonged the retention of Gd in the blood and enhanced its accumulation in tumors. Upon dermal application, the drug was predominantly localized in deeper skin layers, with minimal systemic escape. This has amounted to an absolute bioavailability of 70.62%. Inhibition of P-glycoprotein efflux by D-tocopheryl polyethyleneglycol 1000 succinate and labrasol would have contributed to the enhanced peroral bioavailability of PCL. This investigation provides direct evidence on the localization of high-molecular-weight, lipophilic drug, PCL, in dermis. Further, the nanoemulsion formulation has enhanced the peroral bioavailability significantly to more than 70%. The developed nanoemulsion formulation was safe and effective for both peroral and dermal delivery of PCL[22]. Camptothecin is a topoisomerase-I inhibitor that acts against a broad spectrum of cancers. However, its clinical application is limited by its insolubility, instability, and toxicity. The aim of the present study was to develop acoustically active nanoemulsions for camptothecin encapsulation to circumvent these delivery problems. The nanoemulsions were prepared using liquid perfluorocarbons and coconut oil as the cores of the inner phase. These nanoemulsions were stabilized by phospholipids[2, 5, 8].

Antimicrobial Nanoemulsions

Antimicrobial nanoemulsions are oil-in-water droplets that range from 200-600 nm. They are composed of oil and water and are stabilized by surfactants and alcohol. The nanoemulsion has a

broad spectrum activity against bacteria (e.g., E. coli, Salmonella, S. aureus), enveloped viruses (e.g., HIV, Herpes simplex), fungi (e.g., Candida, Dermatophytes), and spores (e.g., Anthrax). The nanoemulsion particles are thermodynamically driven to fuse with lipid-containing organisms[30, 36].

Nanoemulsions in Cell Culture Technology

Cell cultures are used for in vitro assays or to produce biological compounds, such as antibodies or recombinant proteins. To optimize cell growth, the culture medium can be supplemented with a number of defined molecules or with blood serum. The advantages of using nanoemulsions in cell culture technology are better uptake of oil- soluble supplements in cell cultures; improve growth and vitality of cultured cells, and allowance of toxicity studies of oil-soluble drugs in cell cultures[33].

Medical applications

A water-soluble therapeutic component can be solubilized within the inner W1 phase of the emulsion globule, which showed prolonged release properties and lessen toxic effects[21]. The stability and release properties of double emulsion can be improved by varying the type and concentrations of surfactants. As suggested by Sausville[18], combining targeted delivery with prolonged release would present a tremendous benefit in cancer therapy. The use of double emulsions to accomplish this combined capability merits consideration[37].

Multiple emulsion system possesses adequate biocompatibility, complete biodegradability and versatility in terms of different oils and emulsifiers being used. Both hydrophilic and hydrophobic drugs can be entrapped and protected, drug targeting especially to reticuloendothelial system (RES), taste masking and for slow or controlled delivery of drugs. Beside these advantages with multiple emulsions, there are certain associated disadvantages like being difficult to formulate, bulky and susceptible to various routes of physical and chemical degradation. Multiple emulsions have not been commercially exploited because of their inherent thermodynamic instability. A number of attempts have been made in last two decades for improving stability by several investigators. These attempts are; polymerization gelling, additives in different phases, surfactant concentration modulation, interfacial complexation, pro-multiple emulsion approach and steric stabilization. Many authors have reviewed the different stabilization methods and different drug release mechanisms[25, 33, 34].

Advantages of nano-emulsion

1. Nanoemulsions have higher surface area and free energy that make them an effective transport system.
2. They do not show the problems of inherent creaming, flocculation, coalescence and sedimentation.
3. It can be formulated in variety of formulations such as foams, creams, liquids and sprays.
4. They are non-toxic; non-irritant hence can be easily applied to skin and mucous membranes.
5. It can be administered orally if the formulation contains surfactants which are biocompatible.
6. It do not damage healthy human and animal cells hence are suitable for human and veterinary therapeutic purposes.
7. It provides better uptake of oil-soluble supplements in cell cultures technology to improve growth of cultured cells and allows toxicity studies of oil-soluble drugs.
8. It may be applied as a substitute for liposomes and vesicles and it is possible to build lamellar liquid crystalline phases around the nanoemulsion droplets[28].
9. Due to their small size, nanoemulsions can penetrate through the “rough” skin surface and this enhances penetration of actives.
10. It constitutes the primary step in nanocapsules and nanospheres synthesis using nano precipitation and the interfacial poly-condensation[13, 23, 26, 37].

Advantages in the Transdermal Drug Delivery System

It avoids GIT side effect, inactivation of drug by GIT enzymes, interaction of drug with food and first-pass metabolism of drugs in GIT[38-40].

1. It provides controlled and sustained release of the medicament.
2. It improves the bioavailability of drug.

3. It provides uniform drug plasma concentration.
4. It improves the patient's compliance.
5. It can be administered to non- responsive, unconscious and nauseating patient.
6. It provides easy termination of drug in case of toxicity by removal of the formulation from the skin.

Disadvantages of Transdermal Drug Delivery Systems

Transdermal drug delivery system is unsuitable for a drug that causes irritation at the site of application.

1. It is suitable for potent drugs only.
2. It is limited for the drugs which are imposed by skin permeability[8, 14, 40]

Applications of Nanoemulsion

1. Use of nanoemulsions in cosmetics.
2. Antimicrobial Nanoemulsions.
3. Prophylactic in Bio-Terrorism Attack.
4. Nanoemulsions as Mucosal Vaccines.
5. Nanoemulsion as Non-Toxic Disinfectant Cleaner.
6. Nanoemulsions in Cell Culture Technology.
7. Nanoemulsion formulations for improved oral delivery of poorly soluble drug.
8. Self-nanoemulsifying drug delivery systems.
9. Nanoemulsions as a vehicle for transdermal delivery.
10. Nanoemulsion in the treatment of various other disease conditions like diclofenac cream, a potential treatment for osteoarthritis.
11. Solid self-nanoemulsifying delivery systems as a platform technology for formulation of poorly soluble drugs.
12. Nanoemulsion in cancer therapy and in targeted drug delivery[1, 18, 27].

Table No.1: Types of Surfactant

S.No	Types of Surfactant
1	Non-ionic
2	Zwitterionic
3	Cationic
4	Anionic surfactants

Table No.2: Oils used in nanoemulsions

S.No	Name	Chemical name
1	Captex 355	Glyceryl Tricaorylate/Caprate
2	Captex 200	Propylene Dicaprylate/Dicaprate Glycol
3	Captex 8000	Glyceryl Tricaprylate (Tricaprylin)
4	Witepsol	90:10 % w/w c12 Glyceride tri: diesters
5	Myritol 318	c8/c10 triglycerides
6	1sopropyel myristate	Myristic acid isopropyl ester

Table No.3: Surfactant and Co-surfactant used in Nano-emulsions

S.No	Surfactant	Co-surfactant
1	Capryol 90	TranscutolP
2	Gelucire 44/14, 50/13	Glycerin, Ethylene glycol
3	Cremophor RH 40	Propylene glycol
4	Imwitor 191, 308(1), 380, 742, 780 K, 928, 988	Ethanol
5	Labrafil M 1944 CS, M 2125 CS	Propanol
6	Lauroglycol 90	---
7	PEG MW > 4000	---
8	Plurol Oleique CC 497	---
9	Poloxamer 124 and 188	---
10	Softigen 701, 767	---
11	Tagat TO	---
12	Tween 80	---

CONCLUSION

Nanoemulsion formulations offer several advantages for the delivery of drugs, biologicals, or diagnostic agents and able to protect labile drug, control drug release, increase drug solubility, increase bioavailability and reduce patient variability. Traditionally, Nanoemulsions have been used in clinics for more than four decades as total parenteral nutrition fluids. 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, photosensitizers, neutron capture therapy agents, or diagnostic agents. Because of their submicron size, they can be easily targeted to the tumor area. Moreover, targeting moiety has opened new avenues for targeted delivery of drugs, genes, photosensitizers, and other molecules to the tumor area.

It is expected that further research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicles.

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