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DIFFERENT TECHNIQUES FOR PREPARATION OF NANOEMULSION WITH CHARACTERISATION AND VARIOUS APPLICATION OF IT - A REVIEW

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ABSTRACT

Nanoemulsion are the most of the rapidly developing field of nanotechnology with several applications in drug delivery, medical and research as that of other medical sciences and develop new therapeutics. 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. Nanoemulsions/ Sub-micron emulsions (SMEs)/ Mini-emulsions are thermodynamically stable transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant and co surfactant molecules having a droplet size of less than 100 nm. The present review gives highlight on the different techniques for preparation and characterization of Nanoemulsions as particle size, zeta potential, stability study and the importance of Nanoemulsions in pharmaceutical applications.

KEYWORDS: Nanoemulsion, nanotechnology, surfactant and co surfactant, techniques for preparation, pharmaceutical applications.

INTRODUCTION

Nanotechnology it is the preparation of the nanosized structures cotaining the drugs.[1] The defination of nanotechnology it is study and structures in the size range of 1-100nm. In the nanotechnology other important drug delivery system it will be developed that is Nanoparticles, SLNs, Nanosuspension, NLCs, Nanoemulsion, Nanocrystals, LDCs etc.[4,5,6] This review focused on the Nanoemulsion for drug delivery and targeting application. NEs

are a group of dispersed particles used for pharmaceutical and biomedical aids and vehicles that show great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies. NEs can be 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. 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.[7] The NEs are also referred as miniemulsions, ultrafine emulsions and submicron emulsions. Phase behavior studies have shown that the size of the droplets is governed by the surfactant phase structure at the inversion point induced by either temperature or composition. Studies on NE 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.[8] Nanoemulsions are part colloidal dispersions of two immiscible liquids.[9] Nanoemulsion possesses stability of outstanding application like it waiver the destabilization process of emulsion i.e., creaming, flocculation, coalescence and sedimentation.[10] 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.

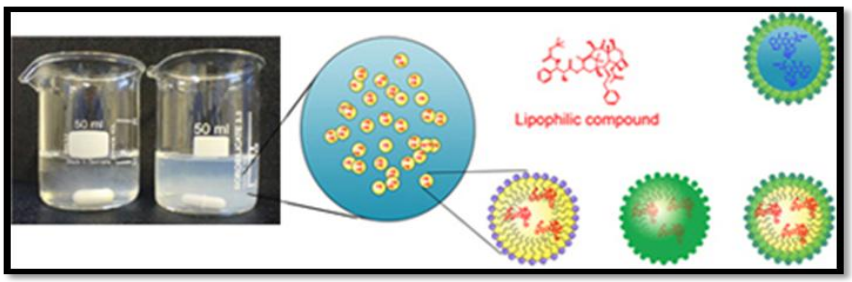

Image of a nanoemulsion (left) and a macro-emulsion right)

Table 1. Formulation ingredients of Nanoemulsion.

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

STRUCTURE OF NANOEMULSION

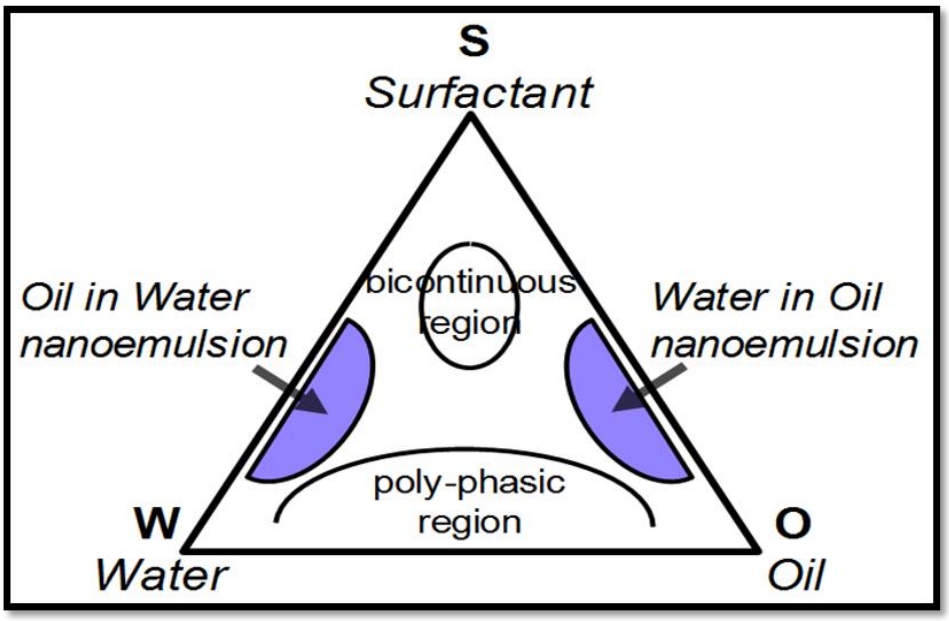

Figure No 1: Structure of Nanoemulsion.

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, Nano porous polymeric solids etc. Apart from this, the nanoemulsions with pharmaceutically accepted ingredients are utilized in the development of drug formulations for oral drug delivery. Phase behavior studies have shown that the size of the droplets is governed by the surfactant phase structure (bicontinuous micro emulsion 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.[11,12,13,14]

ADVANTAGES OF NANOEMULSION[15]

1. Increase the rate of absorption.
2. Eliminates variability in absorption.
3. Helps in solublizing lipophilic drug.
4. Provides aqueous dosage form for water insoluble drugs.
5. Increases bioavailability.
6. Various routes like tropical, oral and intravenous can be used to deliver the product.
7. Rapid and efficient penetration of the drug moiety.

8. Helpful in taste masking.

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

10. Liquid dosage form increases patient compliance.

11. Less amount of energy requirement.

12. Nanoemulsions are thermodynamically stable system and the stability allows self emulsification of the system whose properties are not dependent on the process followed.

13. Same Nanoemulsions can carry both lipophilic and hydrophilic drugs.

14. The use of Nanoemulsion as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

LIMITATION OF NANOEMULSION[16]

1. Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.

2. Limited solubilizing capacity for high-melting substances.

3. The surfactant must be nontoxic for using pharmaceutical applications.

4. Nanoemulsion stability is influenced by environmental parameters such as temperature and pH. These parameters change upon Nanoemulsion delivery to patients.

COMPONENTS OF NANOEMULSION[17]

Main three components of Nanoemulsions are as follows:

1. Oil

2. Surfactant/Cosurfactant

3. Aqueous phase

DIFFERENT TECHNIQUES FOR PREPARATION OF NANOEMULSION

1. High pressure homogenization (HPH)

1. Hot homogenization

2. Cold homogenization

2. Ultrasonication or high speed homogenization

3. Microemulsion

4. Phase inversion

5. Solvent Evaporation Technique

6. Double emulsion technique

7. Solvent emulsification-diffusion method

8. Spontaneous Emulsification

9. Microfluidization

10. Hydrogel Method

1. High pressure homogenization (HPH)[17,23]

High pressure homogenization technique used for the formulation of NLCs. High pressure homogenizers push a liquid with high pressure (100–2000 bar) through a narrow gap. The fluid accelerates on a small distance to high velocity (over 1000 Km/h). Very high Shear stress and cavitation forces disrupt the particles down to the submicron range. Generally 5-10% lipid content is used but up to 40% lipid content has also been investigated.

High pressure homogenization is of two types-hot homogenization and cold homogenization. In this two, a formulation step gives the drug incorporation into the bulk lipid by dissolving or dispersing the drug in the lipid melt or liquid lipid.

1. Hot homogenization[24,28]

In this method, homogenization occurs at temperatures upper than melting point of lipid. Drug loaded lipid melt is dispersed in hot aqueous surfactants phase (isothermal) by mixing device (Ultra-Turrax) and leads to the formation of pre-emulsions. Because of the reduced viscosity at high temperatures, particle size becomes lesser mainly. This technique is illustrated in Figure 2 Hot homogenization has three basic problems. The first is temperature-dependent degradation of the drug, the second is the drug penetrates into the aqueous phase during homogenization and the third is complexity of the crystallization step of the nano-emulsion leading to several modifications and/or super cooled melts.

2. Cold homogenization[29, 30]

Like the hot homogenization method, the drug is dissolved in the lipid melt, and then rapidly cooled by liquid nitrogen or dry ice. Milling leads to formation of nanoparticles in the range of 50-100 nm which are dispersible in a cold surfactant phase that form a pre-suspension. PHP is done at ambient temperature that leads to break the nanoparticles to NLCs. Cold homogenization technique has been expanded to resolve the problems of the hot homogenization technique[1,2] Schematic diagram of this method is given in Figure 2.

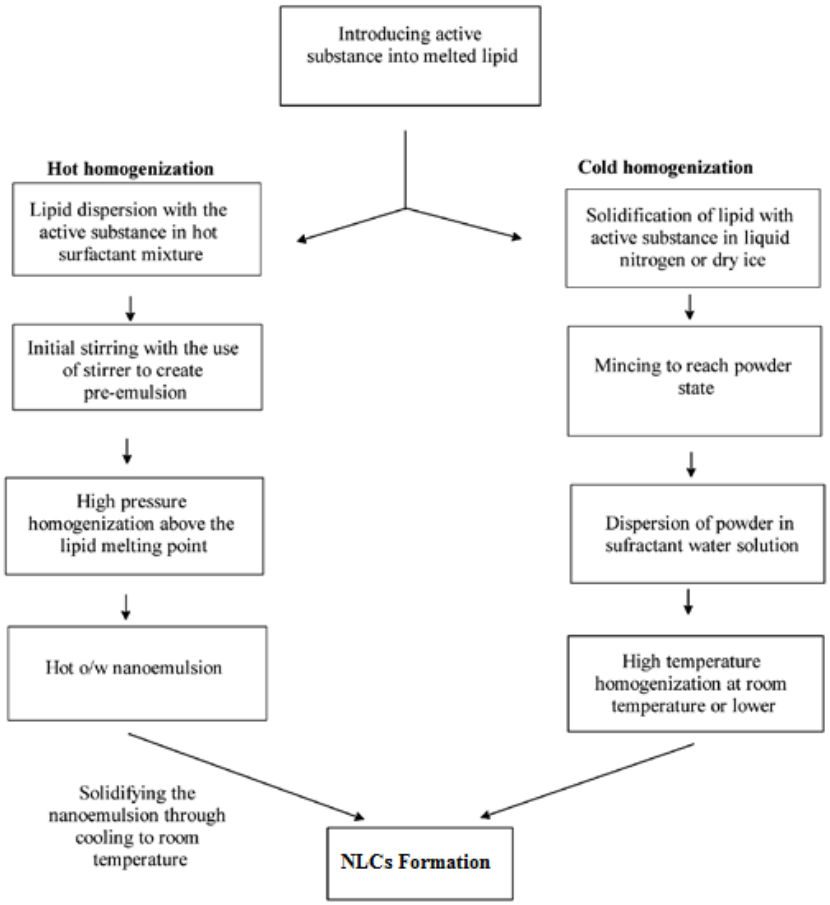

Figure 2: Hot homogenization and cold homogenization method.

2. Ultrasonication or high speed homogenization[31,33]

NLCs were also developed by high speed stirring or sonication. A most advantages are that, equipment whatever use here is very common in every lab. The problem of this method is broader particle size distribution ranging into micrometer range. This lead physical instability likes particle growth upon storage. Potential metal contamination due to ultrasonication is also a big problem in this method. So for making a stable formulation, studies have been performed by various research groups that high speed stirring and ultrasonication are used combined and performed at high temperature. Schematic diagram of this method is given in Figure 4.

Figure 3: Ultasonication.

3. Microemulsion[34,37]

This method is based on the dilution of microemulsions. As micro-emulsions are two-phase systems composed of an inner and outer phase (e.g. o/w microemulsions). They are made by stirring an optically transparent mixture at 65-70°C, which typically composed of a low melting fatty acid (e.g. stearic acid), an emulsifier (e.g. polysorbate 20), co-emulsifiers (e.g. butanol) and water. The hot microemulsion is dispersed in cold water (2-3°C) under stirring. NLCs dispersion can be used as granulation fluid for transferring in to solid product (tablets, pellets) by granulation process, but in case of low particle content too much of water needs to be removed. High-temperature gradients facilitate rapid lipid crystallization and prevent aggregation.

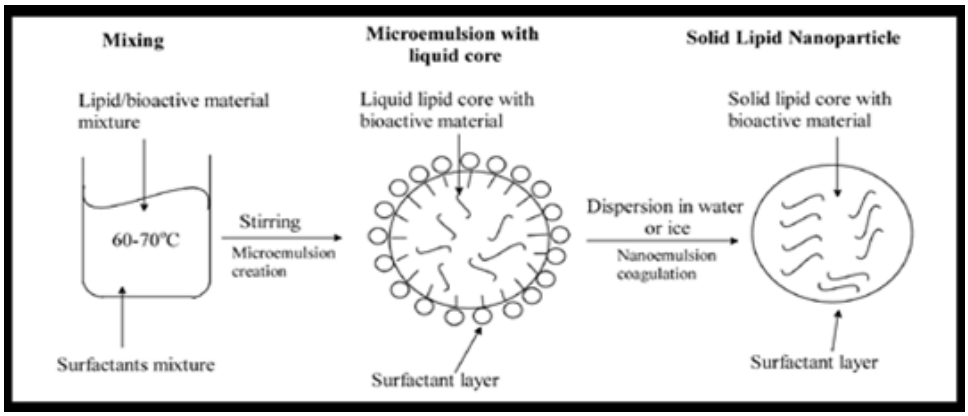

Figure 4: Microemulsion Method.

4. Phase inversion method[38]

In this method, fine dispersion is obtained by chemical energy resulting of phase transitions produced by emulsification pathway. The phase transition is produced by varying the composition of the emulsion and keeping temperature constant or vice versa. The phase inversion temperature was first done by Shinoda *et al*. it was concluded that increase in

temperature results in the chemical changes of polyoxyethelene surfactants by degradation of the polymer chain with the temperature.

5. Solvent Evaporation Technique[39, 40]

This is a method analogous to the production of NLCs solvent evaporation in o/w emulsions via precipitation. In the solvent emulsification-evaporation the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under condition of reduced pressure. The lipid precipitates upon evaporation of the solvent thus forming nanoparticles.

Firstly, an organic phase has produced containing the lipid material dissolved in a water-immiscible organic solvent and then the drug is dissolved or dispersed in that solution. This organic phase is emulsified in an o/w surfactant containing aqueous phase by mechanical stirring. Subsequent quick removal of solvent by evaporation from the obtained o/w emulsion under mechanical stirring or reduced pressure nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The solvent evaporation step must be quickly in order to avoid particle aggregation.

This method is suitable for the incorporation of highly thermolabile drugs due to avoidance of heat during the preparation but presence of solvent residues in the final dispersion may create problems due to regulatory concern. Limited solubility of lipids in organic materials generally leads to dilute dispersions and need to concentrate by means of another process such as ultra-filtration, evaporation or lyophilization. On the other hand small particle size around 100 nm with narrow size distribution can be achieved by this method. This procedure has schematically depicted in Fig no.5.

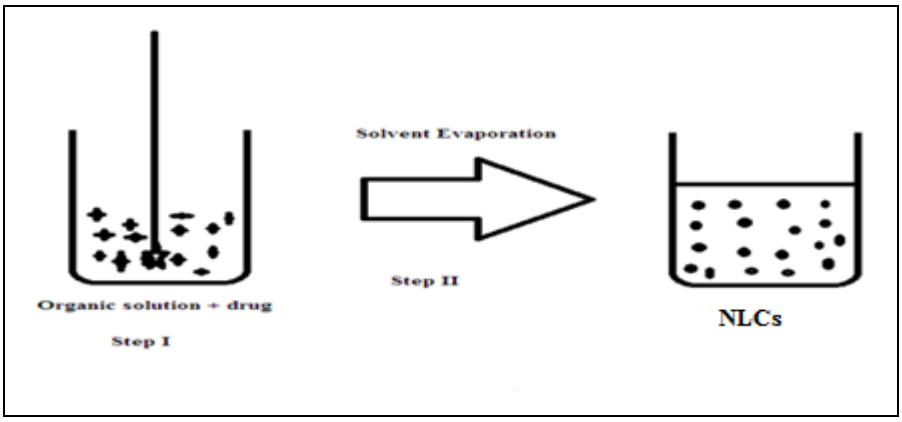

Figure 5: Solvent Evaporation Technique.

6. Double emulsion technique[41]

In double emulsion technique used for preparation NLCs. In this the drug (mainly hydrophilic drugs) was dissolved in aqueous solution and then was emulsified in melted lipid. This primary emulsion was stabilized by adding stabilizer (e.g. gelatin, poloxamer-407). Then this stabilized primary emulsion was dispersed in aqueous phase containing hydrophilic emulsifier (e.g. PVA). Thereafter, the double emulsion was stirred and was isolated by filtration. Double emulsion technique avoids the necessity to melt the lipid for the preparation of peptide-loaded lipid nanoparticles and the surface of the nanoparticles could be modified in order to sterically stabilize them by means of the incorporation of a lipid/-PEG derivative. Sterical stabilization significantly improved the resistance of these colloidal systems in the gastrointestinal fluids. This technique is mainly used to encapsulate hydrophilic drug (peptides).

7. Solvent emulsification-diffusion method[42]

NLCs can also be produced by solvent emulsification-diffusion technique. The mean particle size depends upon lipid concentration in the organic phase and the emulsifier used. Particles with average diameters of 30-100 nm can be obtained by this technique. Avoidance of heat during the preparation is the most important advantage of this technique. Here, the lipid matrix is dissolved in water-immiscible organic solvent followed by emulsification in an aqueous phase. The solvent is evaporated under reduced pressure resulting in nanoparticles dispersion formed by precipitation of the lipid in aqueous medium.

8. Spontaneous Emulsification[43]

It involves three main steps:

i. Preparation of homogeneous organic solution composed of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant.

ii. The organic phase was injected in the aqueous phase under magnetic stirring the o/w emulsion was formed.

iii. The water-miscible solvent was removed by evaporation under reduced pressure.

9. Microfluidization[44]

Microfluidization is a mixing technique, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 to 20000psi), which forces the product through the interaction chamber, which consists of small channels called

"microchannels". The product flows through the microchannels on to an impingement area resulting in very fine particles of sub- micron range.

The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is into a microfluidizer where it is further processed to obtain a stable nanoemulsion. The coarse emulsion is passed through the interaction chamber microfluidizer repeatedly until desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform nanoemulsion.

7. Hydrogel Method[45]

It is similar to solvent evaporation method. The only difference between the two methods is that the drug solvent is miscible with the drug anti-solvent. Higher shear force prevent crystal growth and Ostwald ripening.

CHARACTERIZATION OF NANOEMULSION

Characterization of Nanoemulsion[46]

The droplet size, viscosity, density, turbidity, refractive index, phase separation and pH measurements shall be performed to characterize the Nanoemulsion. The droplet size distribution of Nanoemulsion vesicles can be determined by either light scattering technique or electron microscopy. This technique has been advocated as the best method for predicting Nanoemulsion stability.

Dye Solubilization

A water soluble dye is solubilized within the aqueous phase of the W/O globule but is dispersible in the O/W globule. An oil soluble dye is solubilized within the oil phase of the O/W globule but is dispersible in the W/O globule.

Dilutability Test

O/W Nanoemulsions are dilutable with water whereas W/O are not and undergo phase inversion into O/W Nanoemulsion.

Conductance Measurement

O/W Nanoemulsion where the external phase is water, are highly conducting whereas W/O are not, since water is the internal or dispersal phase. To determine the nature of the continuous phase and to detect phase inversion phenomena, the electrical conductivity

measurements are highly useful. A sharp increase in conductivity in certain W/O Nanoemulsion systems was observed at low volume fractions and such behaviour was interpreted as an indication of a "percolative behavior" or exchange of ions between droplets before the formation of bicontinuous structures. Dielectric measurements are a powerful means of probing both structural and dynamic features of Nanoemulsion systems.

Dynamic Light-Scattering measurements[47]

The DLS measurements are taken at 90° in a dynamic light scattering spectrophotometer which uses a neon laser of wavelength 632 nm. The data processing is done in the built-in computer with the instrument.

Polydispersity

The average diameters and polydispersity index of samples were measured by Photon Correlation Spectroscopy. The measurements were performed at 25oC using a He-Ne laser.

Phase analysis

To determine the type if Nanoemulsion that has formed the phase system (O/W or W/O) of the Nanoemulsions is determined by measuring the electrical conductivity using a conductometer.

Interfacial Tension[48]

The formation and the properties of Nanoemulsion can be studied by measuring the interfacial tension. Ultra low values of interfacial tension are correlated with phase behaviour, particularly the existence of surfactant phase or middle-phase Nanoemulsions in equilibrium with aqueous and oil phases. Spinning-drop apparatus can be used to measure the ultra low interfacial tension. Interfacial tensions are derived from the measurement of the shape of a drop of the low-density phase, rotating it in cylindrical capillary filled with high-density phase.

Viscosity measurement

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

pH: The apparent pH of the formulation was measured by pH meter.

Refractive Index[49]

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°C.

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.

***In Vitro* Skin Permeation Studies**[50]

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 37oC 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. Each sample was performed three times 7. 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.

Thermodynamic Stability Studies[51]

During the thermodynamic stability of drug loaded Nano-emulsions following stress tests as reported:

a. 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.

b. 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.

c. 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.

APPLICATIONS OF NLCs

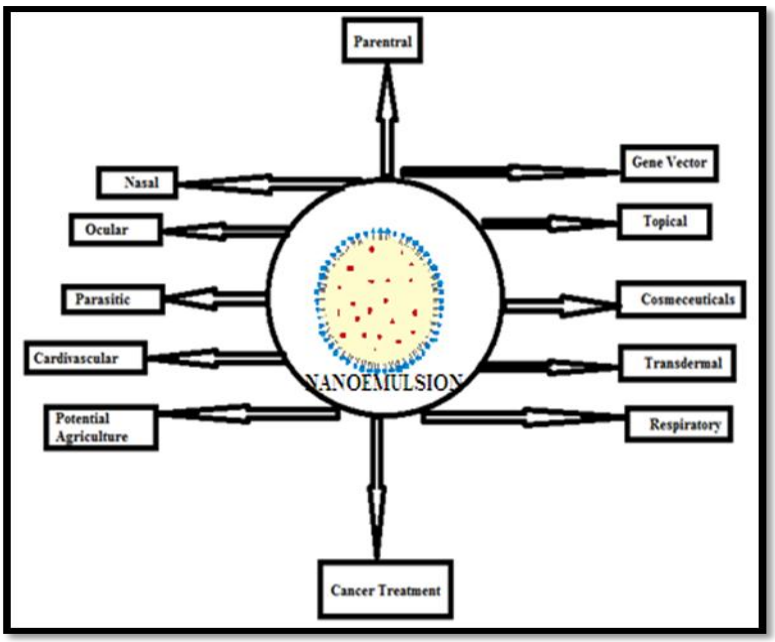

Figure 6: Application of Nanoemulsion in different area.

CONCLUSION

The aim has been to developed therapeutic nanotechnology under taking, particularly for targetted drug therapy The smart Nanoemulsion as the new generation offer much more flexibility in drug loading, modulation of release and improved performance in producing final dosage forms such as creams, tablets, capsules and injectables. The effort to develop alternative routes and to treat other diseases with Nanoemulsion should be continued to extend their applications. Permeation via the gastrointestinal tract and BBB may be a future trend. The combination of two therapeutically active agents to be included in a single nanosystem is another consideration for future development.

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