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A Novel Ophthalmic Pharmaceutical Nano emulsion: Methods of Preparations, Characterizations, and Applications

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ABSTRACT

Ocular delivery drug is the major challenge faced by formulation scientists and pharmacologists because of the poor ophthalmic bioavailability. One of the most promising technologies is the Nanoemulsion drug delivery system. Nanoemulsions have the potential advantages in pharmaceutical industries because of the transparency at high droplet volume fraction, safe, patient compliant formulation which is being applied to enhance the solubility and higher rate of bioavailability of lipophilic drugs, ease of manufacturing and permeation over conventional formulations that convert them to important drug delivery systems. As the drug is lipophilic in nature it can be easily soluble into the oil phase that is used in the formulation of the nanoemulsions and also reduced particle size to the nanometer range favors the formulation to achieve more surface area there by solubility of the lipophilic drug can be achieved. Nanoemulsions are submicron sized emulsion that is under extensive investigation as drug carriers for improving the delivery of therapeutic agents. These 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 5 - 200 nm and shows a narrow size distribution. Reduction in droplet size to nanoscale leads to change in physical properties such as optical transparency & unusual elastic behavior. Nanoemulsions have future widespread applications in different fields such as pharmaceutics, food technology, diagnostics drug therapies and biotechnologies. This review mainly discussed about the importance of ophthalmic nanoemulsions over other dosage forms, advantage and disadvantage various methods of preparation, characterization of nanoemulsions and applications.

Keywords: Opthalmic, Nano Emulsion,

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INTRODUCTION

The ophthalmic eye is a complex organ with an unique anatomy and physiology. The structure of eye can be divided into two main parts: anterior segment and posterior segment. Tissues such as cornea, conjunctiva, aqueous humor, iris, ciliary body and lens makeup the anterior portion. Back of the eye or posterior segment of the eye include sclera, choroid, retinal pigment epithelium, neural retina, optic nerve and vitreous humor. The anterior and posterior segment of eye is affected by various vision threatening diseases. Diseases affecting anterior segment include, glaucoma, allergic conjunctivitis, anterior uveitis and cataract. While, age-related macular degeneration (AMD) and diabetic retinopathy are the most prevalent diseases affecting posterior segment of the eye figure (1) [(1)].

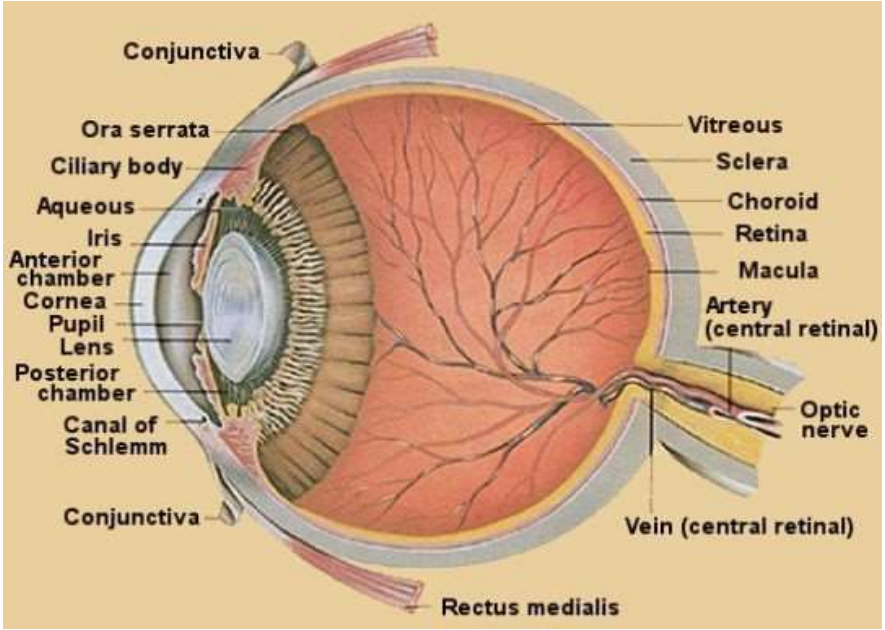

Figure 1: Anatomy of Eye

Topical instillation is the most widely preferred noninvasive route of drug administration to treat diseases affecting the anterior segment. Conventional dosage forms such as eye drops account for 90% of the marketed ophthalmic formulations. The reason may be attributed to ease of administration and patient compliance[(2,3).]

Nonetheless, the ocular bioavailability is very low with topical drop administration. Numerous anatomical and physiological constraints such as tear turnover, nasolachrymal drainage, reflex blinking, and ocular static and dynamic barriers pose a challenge and impede deeper ocular drug permeation[(3)]. Hence, less than 5% of topically applied dose reaches to deeper ocular tissues [(4)].

This poor ocular bioavailability implies the necessity of frequent instillations in order to achieve the therapeutic effect of solution that is frequently associated with undesirable side effects caused by

systemic drug absorption. It is clear that the increase of both the retention of the drug in the precorneal area and the penetration of the drug through the cornea would be a great benefit in ophthalmic therapy((Bochot et al., 1998) Figure 2.

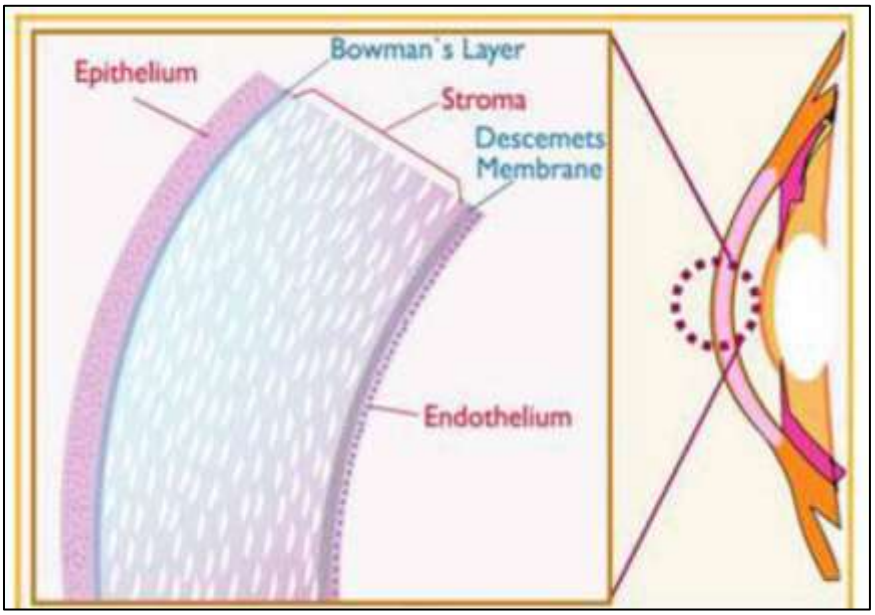

Figure 2: Structure of cornea

To overcome the ocular drug delivery barriers and improve ocular bioavailability, Nanoemlsion a novel ophthalmic drug delivery system have been developed to deliver drug to diseased ocular tissues for the treatment of ocular diseases.

Topical nanoemulsionis considered as the easiest method for ophthalmic drug delivery and has gained patient compliance over the period of time[(5)]. Once a drug is topically applied, due to the natural tear drainage and blinking action of the eye there is a 10 fold reduction in drug concentration in eye within 4-20 minutes[(6)]. Thus the residence time of the drug in precorneal space and penetration to ocular tissue is reduced to 5-6 minutes and 1% to 3% respectively. The best advantage of using ophthalmic nanoemulsion is that their surface can be modified as per requirement. Thus, surface modifications in nanoemulsioncan significantly increase the bioavailability, corneal penetration and conjunctival uptake of drug loaded nanoemulsion[(7)].

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. A Nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. The dispersed phase typically comprises small particles or droplets, with a size range of 5 nm-200 nm, and has very low oil/water interfacial tension. Because the droplet size is less than 25% of the wavelength of visible

light, Nanoemulsions are transparent. The Nanoemulsion is formed readily and sometimes spontaneously, generally without high-energy input. In many cases a cosurfactant or cosolvent is used in addition to the surfactant, the oil phase and the water phase[8].

The main difference between emulsions and Nanoemulsions lies in the size and shape of the particles dispersed in the continuous phase: these are at least an order of magnitude smaller in the case of Nanoemulsions (5-200 nm) than those of conventional emulsions (1-20 μm). Also, whereas emulsions consist of roughly spherical droplets of one phase dispersed into the other, nanoemulsions constantly evolve between various structures ranging from droplet-like swollen micelles to bicontinuous structures, making the usual "oil in water" and "water in oil" distinction sometimes irrelevant(9). Nanoemulsions are formed when and only when the interfacial tension at the oil/water interface is brought to a very low level and the interfacial layer is kept highly flexible and fluid. These two conditions are usually met by a careful and precise choice of the components and of their respective proportions, and by the use of a "co-surfactant" which brings flexibility to the oil/water interface. These conditions lead to a thermodynamically optimised structure, which is stable as opposed to conventional emulsions and does not require high input of energy (i.e. through agitation) to be formed(10) figure 3.

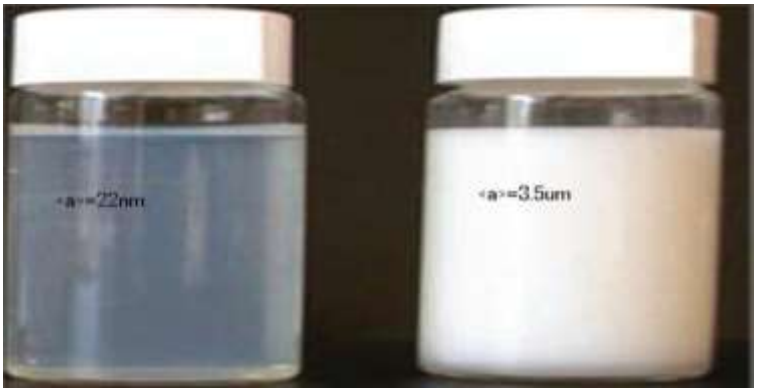

Figure 3: left(nanoemulsion=22nm), right(emulsion=3.5um)

TYPES OF NANOEMULSION

Three types of Nanoemulsions are most likely to be formed depending on the composition:

1. Oil in water Nanoemulsions wherein oil droplets are dispersed in the continuous aqueous phase
2. Water in oil Nanoemulsions wherein water droplets are dispersed in the continuous oil phase.
3. Bi-continuous Nanoemulsions wherein microdomains of oil and water are interspersed within the system.

In all three types of Nano emulsions, the interface is stabilized by an appropriate combination of surfactants and/or co-surfactants.

Advantages of Nanoemulsion over other dosage forms[(11)]

1. It improves water solubility and bioavailability of lipophilic drugs.
2. It increases the rate of absorption.
3. Fine oil droplets empty rapidly from the stomach thus providing wide distribution of drug throughout the intestinal tract by minimizing the irritation.
4. Has higher solubilization capacity and is thermodynamically stable over emulsions and suspensions.
5. It is non-toxic; non-irritant so can be applied in the skin and the mucous membranes.
6. Rapid and efficient penetration of the drug moiety.
7. Nanoemulsions are formulated with surfactants that are approved for human consumption so can be used in enteric route.
8. It does not damage healthy human and animal cells so can be used for human and veterinary therapeutic purpose.
9. Various routes like topical, oral and intravenous can be used to deliver the product.
10. Liquid dosage forms increases patient compliance. Can be formulated in various formulations like spray, foam, liquid and creams.
11. Does not show problems of coalescence, creaming, flocculation and sedimentation such as that of macroemulsions.

Disadvantages of Nanoemulsion

1. Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
2. Limited solubilising 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. These parameters change upon Nanoemulsion delivery to patients.

Compositions of ophthalmic Nanoemulsion [(12)]

The main components of ophthalmic nan emulsions are as follows:

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

4. Miscellaneous additives like preservatives, tonicity modifiers and viscosity modifiers can be added to impart the system with enhanced ocular compatibility.

Oil

Prior to the formulation design of the lipid emulsions data are needed concerning the drug solubility in the oil vehicle. Additionally, prerequisite information is needed on compatibility of the oil vehicle with other formulation additives and the established ocular tissues-oil vehicle matching before the dosage form can be prepared.

Tocopherol (0.001–0.002% w/w) should be included in a typical lipid emulsion formulation for ocular use. The final oil phase concentration in ocular lipid emulsions is now widely accepted at or even below 5% (w/w) taking into account that the lipid emulsion must be kept in a low viscosity range, of between 2and 3 cp, which is considered an adequate viscosity for ocular preparations (Lee and Robinson, 1986). Sometimes, a mixture of oils rather than single oil is employed since drug solubilization in the oil phase is a prerequisite to exploit the lipid emulsion advantages. Jumaa and Muller reported the effect of mixing castor oil with medium chain triglycerides on the viscosity of castor oil (Jumaa and Muller,1998;Jumaaet al., 1999). The oil combination, at the ratio of 1:1 (w/w) led to a decrease in the viscosity of castor oil and simultaneously to a decrease in the interfacial tension of the oil phase. This was related to the free fatty acids contained in castor oil, which can act as a coemulsifier resulting in lower interfacial tension and, simultaneously in a more stable formulation in comparison with the other oil phases. Oils: include Captex 355, Captex 200, Captex 8000, Witepsol, Isopropyl Myristate.

Surfactant/Co-surfactant (Emulsifier)

Traditionally, lecithin or phospholipids have been the emulsifiers of choice to produce ocular lipid emulsions. However, emulsifier of this kind is not suitable to produce submicron sized emulsion droplets or to withstand the heat

during steam sterilization. Therefore, additional emulsifiers preferably dissolved in the aqueous phase are usually included in the lipid emulsion composition. A typical example of the aqueous soluble emulsifiers is non-ionic surfactants

(e.g. Tween 20) after taking into consideration their non-irritant nature when compared to ionic surfactants. The non-ionic block copolymer of Polyoxyethylene-Polyoxypropylene, Pluronics F68 (Poloxamer 188), is included to stabilizethe lipid emulsion through strong steric repulsion. However, amphoteric surfactants, Miranol MHT (Lauroamphodiacetate and Sodium tridecethsulfate) and Miranol C2M (Cocoamphodiacetate) were also used in an earlier ophthalmic lipid emulsion.

Also, a combination of non-ionic and cationic emulsifiers is generally preferred to obtain a stable cationic emulsion system. Non-ionic surfactants like polyoxyethylene-polyoxypropylene block copolymers (Pluronic F68™, Pluronic L-62™ and PluronicL62D™), polysorbates (polysorbate 80), polyoxyethylene fatty acid esters (Emulphor™), Hydrogenated castor oil derivatives (Cremaphor® EL and Cremaphor® RH 40), Tyloxapol, Polyethylene glycol succinate etc are preferred due to their physiological acceptance and a very low irritation potential to the eye.These are generally used in a concentration of 0.5 to 3% w/v.[13].

Other, surfactants include Capryol 90, Gelucire 44/14, 50/13, Cremophor RH 40, Imwitor 191, 308(14), 380, 742, 780 K, 928, 988, Labrafil M 1944 CS, M 2125 CS, Lauroglycol 90, PEG MW > 4000, PlurolOleique CC 497, Poloxamer 124 and 188, Softigen 701, 767, Tagat TO and Tween 80.

Cationic lipids (Co-surfactant) orient themselves at the interface of the submicron oil-in-water emulsions with the emulsifiers. Thus they act as interfacial stabilizers in addition to their role of imparting a positive charge to the submicron oil globules. The strength of charge conferred on the globules depends on the chemical nature (chain length etc) of the lipid. The most common cationic lipids used comprise of C12-C18 alkylamines and alkanolamines like Stearylamine and Oleylamine. Additionally cationic cholesteryl esters like Cholesterylbetainate and long chain quaternary ammonium compounds like Cetrimide and benzalkonium chloride can also be used. Cationic lipids are used in a concentration ranging from 0.05 % w/v to 2 % w/v for conferring a suitable positive zeta potential like 10 to 40 mV.[15].

Preservatives

Additives other than antioxidants such a preservatives like benzalkonium chloride, chlorocresol, parabens etc. are regularly included in ophthalmic lipid emulsions to prevent microbial spoilage of multi-dose ophthalmic lipid emulsions. The presence of components of natural origin like lecithin or oils with high calorific potential render the lipid emulsion a good medium to promotemicrobial growth when it is packed in multi dose containers. Sznitowska et al. studied the physicochemical compatibility between the lecithin-stabilized lipid emulsion and 12 antimicrobial agents over 2 years of storage at room temperature. Preliminary physicochemical screening results indicate that addition of chlorocresol, phenol, benzyl alcohol, thiomersal, chlorhexidine gluconate and bronopol should be avoided due to the occurrence of an unfavorable pH change followed by the coalescence of the lecithin stabilized droplets of the lipid emulsion. Despite a good physicochemical compatibility, neither parabens nor benzalkonium chloride showed satisfying antibacterial efficacy in the lipid emulsion against the tested microorganisms and consequently did not pass the test.

Therefore, higher concentrations of antimicrobial agents or their combination may be required for efficient preservation of the lecithin-stabilized lipid emulsions probably because of unfavorable phase partitioning of the added antimicrobials within the different internal structures of the lipid emulsions.

Methyl paraben, Benzalkonium chloride and cetrimide in a concentration of 0.01 to 0.1 %w/v can be used to prevent microbial contamination in the aqueous phase [(16)].

Osmotic agents/Tonicity modifiers

Glycerine in a concentration ranging from 2.5 to 5% w/v and mannitol (0.15-0.3% w/v) are the two commonly used osmotic agents used due to their ocular tolerability [(17)].

Viscosity modifiers

Non-ionic polymer like HPMC of low viscosity grades in a concentration of 0.05 to 1% w/v can be used in order to modulate the viscosity of the aqueous phase in order to enhance the stability of the cationic emulsion. These also aid in imparting a muco adhesive property to the aqueous phase[(18)].

Ocular metabolism of lipid emulsions after instillation

Nanoemulsions for ophthalmic use aim to enhance drug bioavailability either by providing prolonged delivery to the eye or by facilitating transcorneal/ transconjunctival penetration. Drugs incorporated in o/w type lipid emulsions are lipophilic in nature and depending on the extent of lipophilicity, either the corneal or the conjunctival/ sclera route of penetration may be favored. For the more lipophilic drugs the corneal route was shown to be the predominant pathway for delivering drugs to the iris, whereas the less lipophilic drugs underwent the conjunctival/ scleral penetration for delivery into the ciliary body. Thus, transcorneal permeation has traditionally been the mechanism by which topically applied ophthalmic drugs are believed to gain access to the internal ocular structures. Relatively little attention has been given to alternate routes through which drugs may enter the eye[(19)].

METHODS OF PREPARATION OF NANOEMULSION[(20)]

Nanoemulsion can be prepared by both:

1. High energy methods.
2. Low energy methods.

Both high and low energy methods can produce stable nanoemulsions. High-pressure homogenizer and Microfluidization can be used for the preparation of nanoemulsion by high energy emulsification methods.

Phase inversion, Solvent displacement, and Self-emulsification methods are the low energy methods for the preparation of nanoemulsions.

High pressure homogenizer

This method is performed by applying a high pressure over the system having oil phase, aqueous phase and surfactant or co-surfactant. The pressure is applied with the help of homogenizer. Some problems associated with homogenizer are poor productivity, component deterioration due to generation of much heat. With this method only Oil in water (O/W) liquid nanoemulsion of less than 20% oil phase can be prepared figure 4.

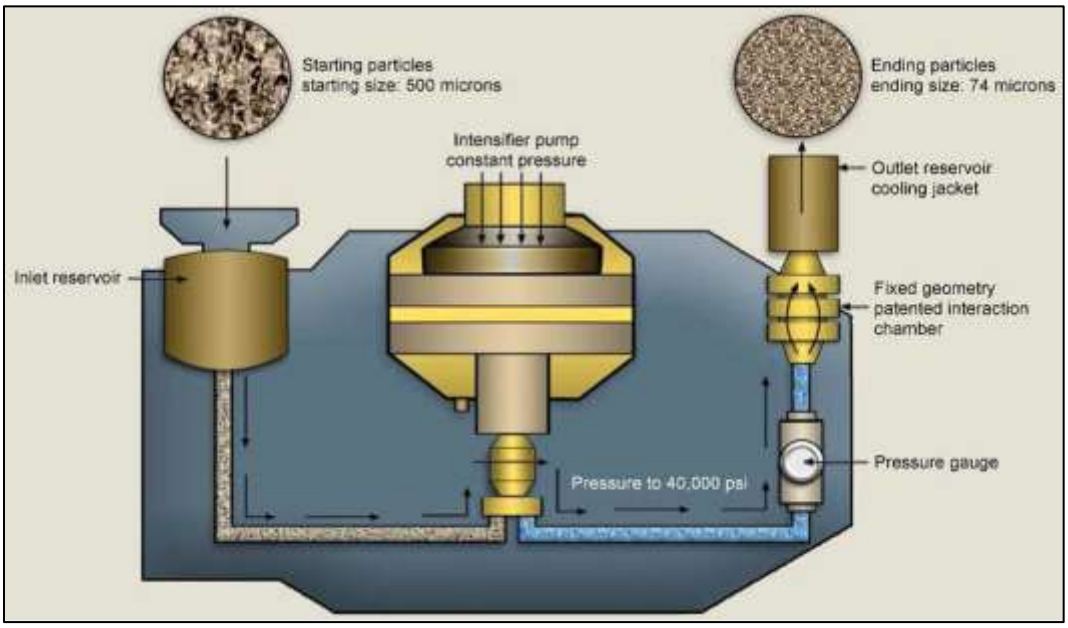

Figure 4:High pressure homogenization

Micro-fluidization

Micro-fluidization technology makes use of a device called ‘MICRO FLUIDIZER”. This device uses a high pressure positive displacement pump which forces the product through the interaction chamber, consisting of small channels called micro channels. The product flows through the micro channels on to an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous and oily phase) are combined together and processed in an inline homogenizer to yield a course emulsion. The course emulsion is into a micro fluidizer where it is further processed to obtain a stable nanoemulsion.

Phase Inversion 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 figure 5.

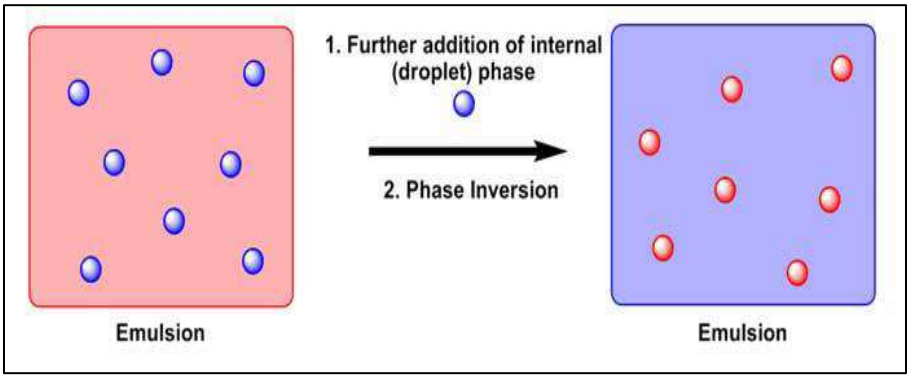

Figure 5: Phase Inversion

Solvent Displacement Method

This method is mainly used for polymeric nanoparticles. This method involves mixing of oily phase 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. Then the organic solvent is removed from the nanoemulsion by a suitable means, such as vacuum evaporation. The main advantage of this technique is that nanoemulsion can be formed at room temperature and require simple stirring for fabrication. This technique is mainly used for fabricating nanoemulsion for parenteral use. The major drawback of this method is use of organic solvents, such as acetone, which requires additional input for their removal from nanoemulsion. Additionally in this method high ratio of solvent to oil is required to obtain nanoemulsion with a desirable droplet size figure 6.

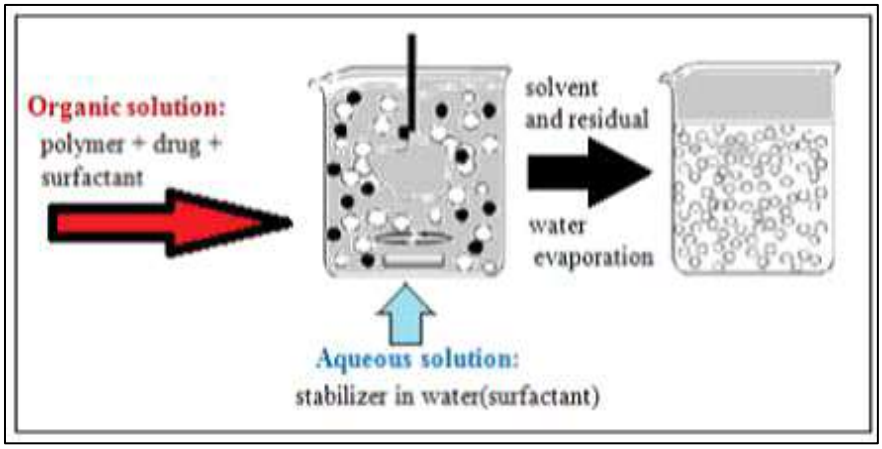

Figure 6: Solvent displacement method

Self-Nano emulsification Method

This method generates nanoemulsions at room temperature without any use of organic solvent and heat. Small droplet size of 50nm can be generated by step wise addition of water into solution of surfactant in oil, with gentle stirring and at constant figure 7 [(21)].

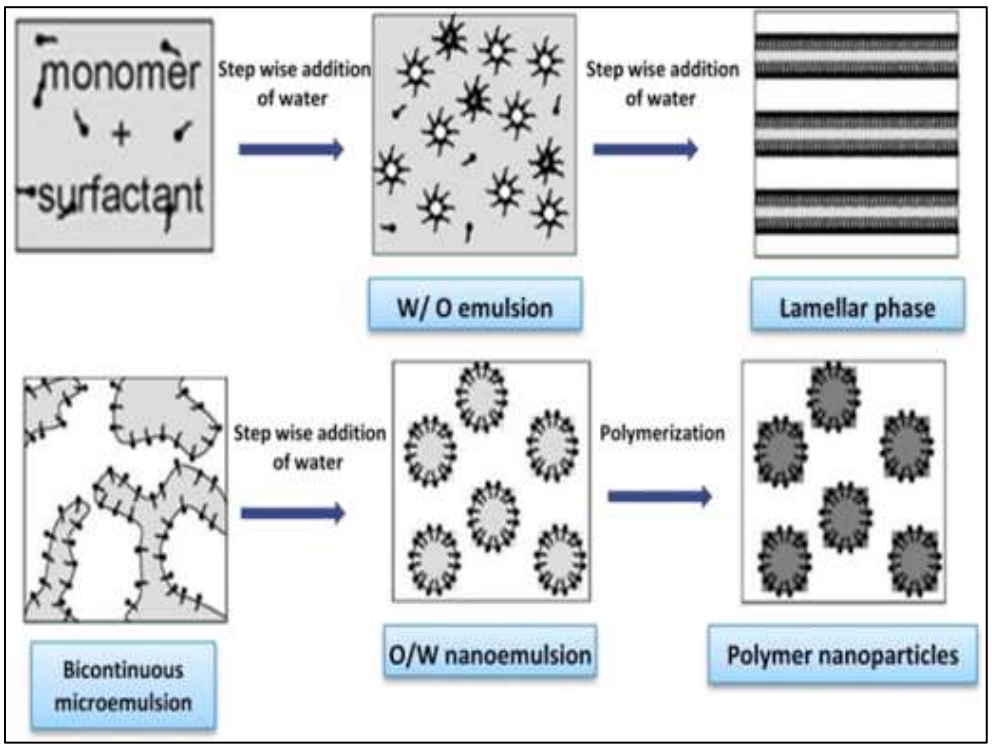

Figure 7: Self-nanoemulsification method.

Factors to be considered during preparation of nanoemulsion

Three 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 nanodroplets to be produced by an ultra-low interfacial tension.
3. The interface must be flexible or fluid enough to promote the formation of nanoemulsions.

Nanoemulsion, being non-equilibrium systems cannot be formed spontaneously. Consequently, energy input generally from mechanical devices or from the chemical potential of the components is required, Nanoemulsion formation by the so called dispersion or high energy emulsification method is generally achieved using high shear stirring, high pressure homogenizers and ultrasound generators. It has been shown that the apparatus supplying the available energy in the shortest time and having the most homogeneous flow produces the smaller sizes. High pressure homogenizers meet these requirements; therefore, they are the most widely used emulsifying machines to prepare nanoemulsion. Generally, the conventional high pressure homogenizers work in a range of pressures between 50 and 100 Mpa. Pressure as high as 350Mpa have been achieved in a recently

developed instrument. Ultrasonication emulsification is also very efficient in reducing droplet size but it is appropriate for small batches. On the preparation of polymerizable nanoemulsion has shown that the efficiency of dispersion process is strongly dependent on ultrasonication time at different amplitudes and that the more hydrophobic the monomer is the longer the sonication time required[22].

CHARACTERIZATION OF NANOEMULSION [23]

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 and these characteristics are measured by the various technique discuss below.

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.

Dilatability 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 behaviour’ 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. O/W Nanoemulsion where the external phase is water are highly conducting[24].

Dynamic light-scattering measurements

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.

Transmission Electron Microscopy (TEM)

It is a very simple method to determine the size, number, weight and structure (morphology characteristic). O/w nanoemulsion is stain with uranyl acetate and placed on a grid, coated with monolayer polymer, then water is evaporated and observation is done using TEM[25,26].

Polydispersity

Studied using Abbe refractometer.

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[27].

Interfacial Tension

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 behavior, 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.

Drug Entrapment Efficiency

Techniques like Ultracentrifugation and ultrafiltration are commonly used for determining the drug entrapment efficiency of cationic submicron emulsions. The emulsion system is subjected to centrifugation in Polyallomer 162,000×g) at 4°C for 2 hours. The bottom of the tubes is pricked with a syringe needle and the aqueous phase is collected. In case of Ultrafiltration, the emulsion is subjected to ultrafiltration in VIVASPIN 4 filters (VIVASCIENCE Ltd, Co., Germany) at 810 × g for 30 minutes. The amount of drug in the separated aqueous phase is calculated using HPLC. The concentration of the drug in the aqueous layer and the whole emulsion are determined using HPLC and the entrapment efficiency is calculated.

$$\text{Entrapment efficiency in percentage} = (Ct-Ca)/Ct \times 100$$

In-vitro Drug Permeation Studies

Release studies can be performed using vertical passive diffusion cells (HTD 96, HT Dialysis, USA), with a cellulose membrane. The cellulose (molecular weight <12 000) membrane was first

hydrated in the buffer solution at 20°C for 24 hours. The receptor solution will contain 0.20 mL of phosphate buffer pH 7.4 containing 1% SLS (Sodium lauryl sulphate) to, and it will be maintained at 37°C ± 0.5°C using a thermostatic shaker bath and stir at 200 rpm throughout the experiment. The donor compartment will contain 0.2 ml of nanoemulsion sample The release can be modulated (or) altered based on pharmacokinetic needs by selecting appropriate Formulation excipient at right composition. For example the formulation scientist can tailor the formulation for Sustained or immediate release by choosing high solubilizing oil or low solubilizing oil respectively. Also by reducing the oil content with respect to the aqueous content will give slightly enhanced flux with high solubilizing oil. The flux can be further increased by using high amount of surfactant in the nanoemulsion system irrespective of the solubilizing nature of the oil, since drug will be soluble in the surfactant solution. Another way of increasing the flux would be selection of low solubilizing oil with high amount of aqueous content for highly lipophilic compound. The sustained release can be achieved either by means of using high amount of medium/high solubilizing oils. There are several biological factors should be considered like thickness of the diffusion membrane, unionized state of the molecule at the absorption site because their degree of ionization depends upon the pH of the biological fluid. Only the unionized fraction of the drug, if sufficiently lipid soluble can permeate the membrane passively until the concentration of unionized drug on either side of the membrane becomes equal until equilibrium is attained. Also the amount of fluid available at the site, were dilution can takes place after ingestion of nanoemulsion will determine the effective formation of micro droplets. The existence of bile salts and few surfactants in biological system will also help in the effective formation of micro droplets along with the peristaltic movement present in the stomach.

Determination of permeability coefficient and flux

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

Sterility testing

The sterility testing is very relevant. The test is performed using Bactec L6 Apparatus. The testing is based on the quantitative measurement of radioactive carbon dioxide in bacterial culture vials inoculated with test samples. The test aids to detect the presence of both aerobic and anaerobic bacteria in the emulsion formulation[28].

In vivo irritation studies

The in vivo irritation profile ophthalmic nanoemulsion due to the presence of surfactants and cationic lipids can be studied using Draize test. The test comprises of instilling 2-3 drops of nanoemulsion in rabbit eyes and observing for inflammatory responses like reddening, chemosis, mucosal discharge etc at various time points of 0 hr, 6hrs, 12hrs, 24hrs, 48hrs and 72 hrs.

Stability testing

The emulsion stability can be determined at various conditions like room temperature (25°C), stability chamber (40°C/75%RH), autoclaving(121°C,15 mins, 15psi), freeze-thawing and accelerated heating(100°C for 3-4 hours). Any traces of creaming, coalescence or phase separation indicate emulsion instability.

APPLICATIONS OF OPHTHALMIC NANOEMULSIONS[29]

Ophthalmic nanoemulsions are utilized for the treatment of eye diseases, drugs are essentially delivered topically. o/w nanoemulsions have been investigated for ocular administration, to dissolve poorly soluble drugs, to increase absorption and to attain prolong release profile.

Anionic o/w ophthalmic nanoemulsions with ciclosporin (cyclosporine A; Restas is, Allergan) were developed to increase tear production in patients whose tear production was presumed to be suppressed due to ocular inflammation (i.e. for patients with dry eye disease), or with difluprednate(Durezol, Alcon) for the treatment of inflammation and pain associated with eye surgery(30). Oil-in-water nanoemulsionswere demonstrated to be excellent vehicles forlipophilic drugs, such as ciclosporin or prostaglandin analogues like latanoprost or tafluprost, but also for delivering water unstable drugs[31,32].

Another important nanoemulsions extended, cationic o/w nanoemulsions one step further the benefits of the o/w nanoemulsions for drug delivery by improving their residence time over that observed with the anionic o/w nanoemulsions. These cationic o/w nanoemulsions take advantage of the negatively charged ocular surface to increase through electrostatic interactions their precorneal residence time, and thus the ocular drug bioavailability(33,34).

As a consequence, the first generation of cationic o/w nanoemulsions were developed to optimize penetration of drugs (among them ciclosporin) in ocular tissues(35-37). This first generation of

cationic o/w nanoemulsions used non compendial cationic surfactants and were not devoid of ocular toxicity side effects(38-40). Hence, the challenges for the development of cationic o/w nanoemulsions are in the choice of the most appropriate cationic agent used to bring the positive charge to the oil nanodroplets, and in the improvement of the ocular tolerance of these positively charged nanoemulsions. New topical ophthalmic drug delivery is cetalkonium chloride cationic oil-in-water nanoemulsions(CKC) was found tobe the best cationic agent to produce stable unpreserved cationic o/w nanoemulsions with unexpected beneficial biological activity for the ocular surface. CKC cationic o/w nanoemulsion was developed to improve the precorneal residence time and the spreading properties on negatively charged ocular surface cells of the nanoemulsions. This better spreading and improved residence time of the CKC cationic o/w nanoemulsion translated into twofold increase in ciclosporin ocular bioavailability over anionic ciclosporin nanoemulsions. This new type of vehicle was demonstrated to be perfectly safe and well toleratedby the ocular surface. CKC in the cationic o/w nanoemulsion exhibits neither detergent effect nor preservative role. Consequently, CKC cationic o/w nanoemulsion does not exhibitany of the observed ocular side effects related to benzelkinium chloride (BAK) in aqueous eye drops (tear film instability, ocular surface damage, mucus removal). In addition to the improved bioavailability of the loaded drug, these CKC cationic o/w nanoemulsions have ocular surface protective properties through the restoration of a healthy tear film and by favouring the corneal wound healing process through the promotion of re-epithelization and inflammation management.

Furthermore, El Qidra R.et al., 2008 [(41)] described the development of two different ophthalmic mucoadhesive chitosan based nanocarriers, nanoemulsion and nanoparticles adopting simple and convenient way for indomethacin (IM) ocular delivery. Unlike previously investigated colloidal systems, the interest of these formulations was their ability to interact and remain associated to the ocular mucosa thus prolonging the residence time in the cornea and slow gradual IM release during 24 h was achieved. Furthermore, CS nanoemulsion developed in this study can be proposed as promising carrier to enhance the therapeutic index of clinically challenging IM with potential application at both extra and intra ocular levels.

CONCLUSION

The study of basic and applied aspects of ophthalmic nanoemulsions is receiving increasing attention in recent years. Dispersion of high energy emulsification methods are traditionally used for nanoemulsions formation. Nanoemulsions are proposed for numerous applications in pharmacy

as drug delivery systems because of their capacity of solubilizing nonpolar active compounds. Thus the use of nanoemulsions as formulations for active delivery and targeting is also an active and interesting application of nanoemulsion. Overall ophthalmic nanoemulsion formulation may be considered as effective, safe, and patient compliance formulation for the delivery of pharmaceuticals and in cosmetics science after controlling the instability factors. Stability of formulation may be enhanced by controlling various factors such as type and concentration of surfactant and co-surfactant, type of oil phase, methods used, process variables and addition of additives over the interfaces of nanoemulsion formulation in ally, Nanoemulsions via ophthalmic route has proved significant advancement for future perspectives.

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