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RESEARCH ARTICLE

NANOEMULSION SYSTEMS: POTENTIAL APPROACH FOR DRUG DELIVERY

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ABSTRACT

Nanoemulsions are transparent or translucent dispersions,having the droplet size less than 100 nm with ultra low interfacial tension,large o/w interfacial areas and long term physical stability Recently,much attention has been paid to the application of nanoemulsion as drug delivery systems, since nanoemulsions are thermodynamically stable and are formed spontaneously by simple mixing of various components.An attempt to combine the two phases require energy input to establish water – oil contacts that would replace the water-water and oil-oil contacts.The interfacial tension between bulk oil and water can be as high as 30-50 dynes/cm.To overcome this, surfactants can be used because they contain water loving (hydrophilic) and oil loving (lipophilic) moietie and they tend to adsorb at the water -oil interface.If enough surfactant molecules are present, they align and create an interface between the water and the oil by decresing the interfacial tension.

Key words: Nanoemulsions,drug delivery

INTRODUCTION

An emulsion is a dispersed system containing at least two immiscible liquid phases. In order to ensure stability, an emulsion must contain a suitable emulsifying agent aside form the dispersed phase and dispersion medium[1,2].there are two basic forms of emulsion.The first is the oil-in-water(O/W) emulsion in which oil droplet are dispersed and encapsulated within the water column[3].The second is the water-in-oil (W/O) emulsion in which droplet of water is dispersed and encapsulated within the oil. An emulsion is formed when a small amount of an appropriate surfactant is mechanically agitated with the il in water.this results in a two phase dispersion where one phase exists as droplets coated by surfactant that is dispersed throughut the continuous,other phase.[4] These emulsion are milky or turbid in appearance due to the fact that the droplet size range from 0.1 to 1 micron in diameter.As a general rule, the type of surfactant used in the system determine which phase is continuous. If the surfactant is hydrophilic, then oil will be emulsified in droplets throughout a continuous water phase.the opposite is true fr more lipophilic surfactants. Water will be emulsified in droplets that are dispersed throughout a continuous oil phase in this case[5].

Emulsions with droplet size in the nanometric scale(20-200nm) [6,7].nanemulsion are part of a broad class of multiphase colloidal dispersions also known as mesphases,microemulsion,miceller phases.may appear to be similar to nanoemulsion in composition and nanoscale structure,such

ISSN 2319-1074

phases are quite different[9].this are made from surfactant approved from human consumption and common food substances that are "Genrally Recognized as Safe" by the FDA[8].

The term nanoemulsion is preferred because in addition to give an idea of the nanoscale size range of the droplets it is concise and it avoids misinterpretation with the term microemulsion (which are thermodynamically stable systems).Due to their characterstic size, nanoemulsions appear transparent or translucent to the naked eye and small droplet size makes them stable against sedimentation or creming.There is a fundamental difference between microemulsion and nanoemulsion.microemulsion are equilibrium systems,while nanoemulsions are non-equilibrium system with a spontaneous tendency to seprate into the constituent phases. Nanoemulsion has been widely used in pharmaceutics, cosmetics and it also play important role as a reaction template for synthesis of polymer dispersions and nanoparticles,because of the controllable droplet size, relatively long term stability and powerful solubilization ability and so on[10].

From the last two decades, formulation scientist has been playing attention to nanoemulsion systems using various surfactants to resolve many issues for instance solubility and their drug delivery applications.Currently,research on nanoemulsion system is aiming towards the specificity of drugs action and target,to facilitate the bioavailability of drugs through biological membranes,or to protect a drug against enzyme inactivation. It is an effort to summarize the recent development in the area of nanoemulsion and self nanoemulsifying drug delivery system(SNEDDS)which are examined in relation to their use in different route of administrations.

Mechanisms of Emulsion Formation

In order to disperse one liquid in another in the form of an emulsion,an amount of work (W) in ergs/ cm must be done upon the system.W is equal to the interfacial tension (γ) in ergs/cm2 multipied by the ΔS (cm2) which is the increase in surface area of dispersed phase due to formation of emulsion droplets.

$W = \gamma \Delta S$……..Equation 1

To reduce the amount of energy required for emulsification and yet obtain small droplets, the interfacial tension between water and oil must be lowered to a marked degree.This can be achieved using appropriate emulsifying agent or and homogenization. To have thermodynamic stability,W must be very small.This implies that the surface area and interfacial tension must be small.However,to have a small specific surface area, the droplets must be large.This could lead to aggregation or coalescence and faster breakdown of emulsion.Therefore,there must be a balance of the thermodynamic and physical stability,i.e. keeping W as possible and keeping the z-average small enough[10].

Emulsion droplets are deformed and disrupted by viscous or inertial forces.Viscous forces generate tangential and normal stresses at the drop surface.Inertial forces generate pressure differences.Laminar or turbulent flow can cause shear or elongation.In laminar flow, viscous forces are predominant.The flow can cause shear or elongation.In turbulent flow,inertial forces predominate; however,viscous force may also be involved.During cavitation,small vapor bubbles are formed which subsequently collapse extremely fast,causing heavy shock waves in liquid.These may disrupt droplets.The liquid is intensely agitated and flow is turbulent.Hence,cavitation is comparable to disruption by turbulence.

The occurrence of any of these mechanisms depend on the type of apparatus,viscosity of liquid and constructional details.The scale of the apparatus may considerably affect the operation.A larger machine operated at appropriate speed gives more turbulence.Emulsion droplets may also

ISSN 2319-1074

be disrupted due to interfacial instability caused by surface tension gradients.This is dependent on the surfactant and can occur in any equipment[11].Emulsion can be prepared by high energy emulsification methods which includes high pressure homogenization and ultrasound generators or by low energy emulsification methods such as spontaneous emulsification or solvent diffusion method such as spontaneous emulsification or solvent diffusion method and phase inversion temperature (PIT)method.

ADVANTAGES AND DISADVANTAGES [12]

Advantages

- Increase the rate absorption.
- Eliminates variability in absorption.
- Helps solublize lipophilic drug.
- Provides aqueous dosage form for water insoluble drugs.
- Increases bioavailability.
- Various routes like topical, oral and intravenous can be used to deliver the product.
- Rapid and efficient penetration of the drug moiety.
- Helpful in taste masking.
- Provides protection from hydrolysis and oxidation as drug in oil phase in O/W Nanoemulsion is not exposed to attack by water and air.
- Liquid dosage form increases patient compliance.
- Less amount of energy requirement.
- Nanoemulsions are thermodynamically stable system and the stability allows self-emulsion of the system.

Disadvantages

- Use of a large concentration of surfactant and co-surfactant necessary for stabilizing the nanodroplets.
- Limited solubilizing capacity for high-melting substances.
- The surfactant must be nontoxic for using pharmaceutical applications.
- Nanoemulsion stability is influenced by environmental parameters such as temperature pH.

Component of nanoemulsion[12]

Main three components of nanoemulsions are as follows:

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

Table 1 :List of oils used in nanoemulsions

Name	Chemical Name	Make

ISSN 2319-1074

Captex 355	Glyceryl Tricaorylate/Caprate	Abitec
Captex 200	Propylene Dicaprylate/Dicaprate	Abitec
Captex 8000	Glyceryl tricaprylate[Tricaprylin]	Abitec
Witepsol	90:10%w/w c12 Glyceride tri: trimesters	Sasol pharmaceutical excipients
Myritol 318	C8/c10 triglycerides	Russia
Isopropyl myristate	Myristic acid Isopropyl ester	Fluka

Table 2: List of Surfactant usd in nanoemulsion

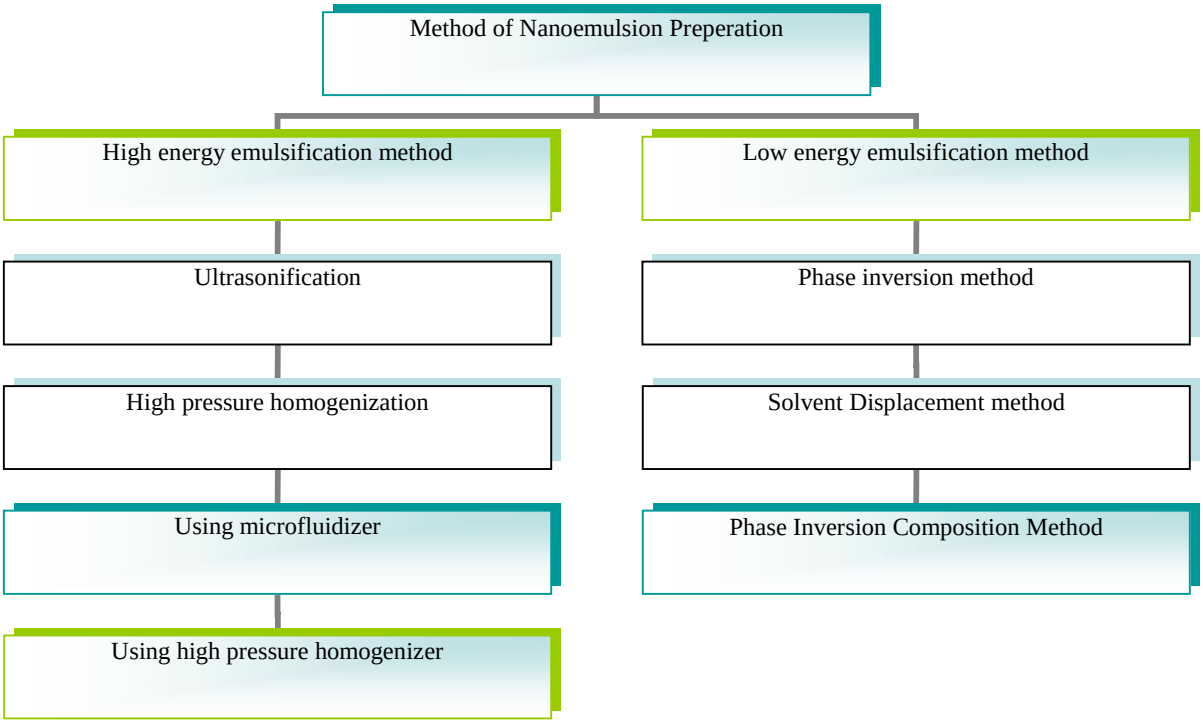
S.No.	**Surfactant,emulsifying agent**
1.	Caproyl 90
2.	Gelucire 44
3.	Cremophore RH 40
4.	Imwitor 191, 308
5.	Labrafil M 1944 C4
6.	Lauroglycol MW
7.	PEG MW>4000
8.	Plurol Olequie CC497
9.	Poloxamer 124 n 128
10.	Softigen
11.	Taget T0
12.	Tween 80

Table 3 :List of co surfactant used in nanoemulsions

S. No.	**Co-Surfactant**
1.	TranscutolP
2.	Glycerin, Ethylene Glycol
3.	Propylene Glycol
4.	Ethanol

ISSN 2319-1074

Preparation of Nanoemulsion[12]



Emulsification by Ultrasonication

Emulsions can be produced using a sonicator,a laboratory equipment that generates ultrasonic waves. This type of sonicator has an ultrasonic transducer which consists of piezoelectric crystal contained within a protective metal casing. application of a high electrical wave to the transducer causes the piezoelectric crystal to rapidly oscillate and generate an ultrasonic wave. At the beginning of emulsification, waves are produced at the interface giving rise to fairly coarse drops. During the process of emulsification by ultrasound,cavitation or formation of cavities by liquid and their subsequent collapse occur accompanied by intense hydraulic shocks.(figure 1.1)the exact mechanism of droplet break up is unknown. Postulations are that the droplet oscillates at a natural frequency until it bursts.

ISSN 2319-1074

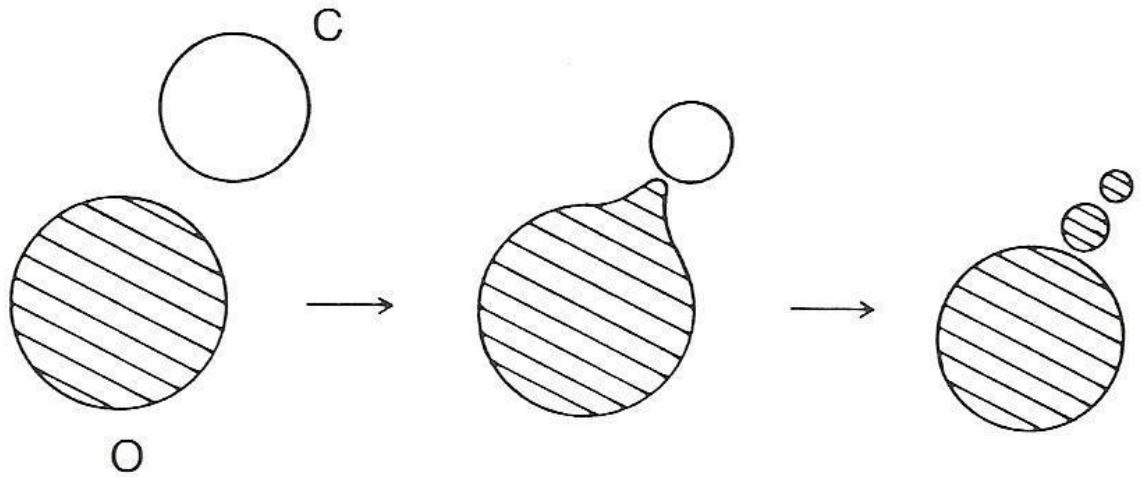


Figure 1. 1 Mechanism of Cavitation in Emulsification by Ultrasound (Cavity C collapses in the vicinity of droplet O)

Drug emulsions for parenteral delivery have been prepared by sonication,studied the development of a parenteral emulsion formulation for lorazepam using sonication. Jafari and coworkers evaluated the efficiency of sonication and microfluidization in the production of d-limonene oil-iniwater nanoemulsions and observed that the size of the emulsions decreased with increasing sonication time and also produced emulsions in the size range of 150-700nm.

Emulsification using high pressure homogenizers.

Homogenizers may be used in one of two ways:

1. By mixing ingredients in the emulsion and then passing those through the homogenizer to produce the final product and
2. Produce a coarse emulsion first using a different method then passing it through a homogenizer.

The purpose is to decrease droplets size and obtain a greater degree of uniformity and stability.the homogenizers operate at pressures of 1000 to 5000 psi to produce some of the finest dispersions obtainable in an emulsion.

The production of small droplets(submicron) requires application of high energy. Nanoemulsion formation requires input, generally from mechanical devices or from the chemical potential of the components. The methods using mechanical energy(high shear stirring,high-pressure homogenizers and ultrasound generators)are designed as dispersion or high energy emulsification methods[12].

Microfluidization[13]

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.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.

ISSN 2319-1074

Phase Inversion Method

Phase inversion in emulsions can be one of two types: transitional inversion or catastrophic inversion[10].These methods make use of changing the spontaneous curvature of the surfactant.

For non-ionic surfactants, phase inversion can be achieved by changing the temperature of the systems,forcing a transition from an oil-in-water(O/W)emulsion at low temperature to a water-in-oil(W/O)emulsion at higher temperatures(transitional phase inversion). During cooling,the system crosses a point of zero spontaneous curvature and minimal surface tension,promiting the formation of finely dispersed oil droplets. Catastrophic inversion is a transition in the spontaneous radius of curvature that can be obtained by changing the volume fraction of the phase. By successively adding water into oil, initially water droplets are formed in a continuous oil phase. Increasing the water volume fraction changes the spontaneous curvature of the surfactant from initially stabilizing a W/O emulsion to an O/W emulsion at the inversion locus. This process is well known for short-chain surfactants which form flexible monolayers at the oil-water interface,resulting in a biocontinuous microemulsion at the inversion point.

Phase inversion in emulsions can be one of two types: transitional inversion induced by changing factors which affect the HLB of the system,e.g. temperature and/or electrolyte concentration. catatrophic inversion,which is induced by increasing the volume fraction of the disperse phase. Transitional inversion can also be induced by changing the HLB number of the surfactant at constant temperature using surfactant mixtures.

Factors to be considered during preparation of nanoemulsion[14]

Three important conditions:

1. Surfactants must be carefully chosen so that an ultra low interfacial tension(<10-3Mn/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 nano droplets 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.

Characterization of emulsion

Characterization of nano-emulsions is of most importance in order to ensure the production of emulsions which fall within the desired droplet size range,viscosity and charge and are stable with time.Several techniques have been developed to characterize emulsions such as particle size analysis,polydispersity and zeta potential determination, differential scanning calormetry,nuclear magnetic resonance NMR,HPLC,viscosity and surface tension determination.some of these methods will be highlighted below.

Particle size determination

Particle size of emulsion can be determined using several techniques.Some of the major techniques are hydrodynamic chromatography,photon correlation spectroscopy,spectroturbidimetry,field flow fractionation,sensing zone, electron microscopy and sedimentation.

Photon Correlation Spectroscopy

Photon correlation spectroscopy is also known as dynamic light scattering.The principle of operation is based on the intensity of the light scattered from dispersions of particles and macromolecules. This intensity fluctuates with time and are too rapid and shift too little to be evident to the human eye.The pace of the movement of particles is inversely proportional to the

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particle size can be detected by analyzing the time dependency of the light intensity fluctuation scattered from particles when they are illuminated with a laser beam.The time dependence of the light scattered from a very small region of solution over a time range from 10^{th} of a microsecond of the millisecond is measured.These fluctuations in intensity are related to the rate of diffusion of particles in and out of the region (Brownian Motion),and the data can be analyzed to directly give the diffusion coefficients of the particles doing the scattering. The data are processed to give the size of the particles(radius or diameter)[16,17,18] which is based on the theoretical relationship between the Brownian motion and the size of the spherical particles.this is based on the Stokes-Einstein equation.

$$D_o = \frac{kT}{3\pi\eta d} \text{ ----------Equation 2}$$

Where Do is the diffusion coefficient,η is the viscosity of the suspending medium and d is the particle diameter.The instrumentation for photon correlation spectroscopy consist basically of a light source,which is either argon or heliumneon laser, a spectrometer consisting of an optical system for defining the scattering angle and limiting the number of coherence areas,a detector(usually a photomultiplier),a signal analyzer which is a digital autocorrelator and a computer for processing and displaying the data.Instrument such as Malvern Zetasizer Nanoseries(Malvern Instruments UK) and Nicomp particle sizer(Particle Sizing Systems,CA)are available for measurement of particle size.

Electron Microscopy Techniques

Scanning electron microscopy and transmission electron microscopy are important techniques in emulsion characterization.Microscopic techniques allow determination of both particle size and distribution,and observation of particle shape.In addition,other parameters, such as morphology or surface roughness can be observed[15].

Scanning Electron Microscopy

Scanning electron microscopy(SEM) is one of the most widely used techniques used in characterization of nanomaterials and nanostructures.the resolution of the SEM approaches a few nanometers and the instruments can operate at magnifications that are easily adjusted from -10 to over 300,000. SEM produces topographical information and the chemical composition information near the surface.In a typical SEM,a source of electrons is focused into a beam, with a very fine spot size of 5nm and having energy ranging from a few 100Ev to50kEv that is rastered over the surface of the specimen by deflection coils[16].As the electrons strike and penetrate the surface,a MSEM images of the emitted electrons are consequently produced on a cathode ray tube(CRT).

Transmission Electron Microscopy(TEM)

In TEM,electrons are accelerated to100 KeV or higher (up to 1 MeV),projected into a thin specimen(less than 200nm)by means of the condenser lense system,and penetrate the sample thickness either undeflected or deflected.[17] the greatest advantages that TEM offers are the high Magnification ranging from 50 to 10^6 and its ability to provide both image and diffraction information from a single sample[18].

Polydispersity Index

The second characterization of emulsion to be discussed is the polydispersity index. Photon correlation spectroscopy can also be used to determine the polydisoersity index of an emulsion

ISSN 2319-1074

using the Malvern Zeta sizer. This is necessary to characterize the particle size distribution of an emulsion. Polydispersity is the width of particle size. A narrow size distribution, corresponding to a polydispersity index between 0.1 and 0.2, is generally found with colloidal drug carriers. Larger polydispersity indices indicate a broad size distribution.

Zeta Pptential Determination[19]

Zeta potential is a measurement of surface potential. The magnitude of zeta potential gives an indication of potential stability of an emulsion.Zeta potential is an important parameters in determining the stability of an emulsion and other colloidal dispersion,zeta potential larger than about 25mV is typically required to stabilize a colloidal system. Zeta potential is determined by a number of factors,such as the particle surface charge density, the concentration of counter ions in the solution, solvent polarity and temperature[20,21].Zeta potential can be determined using the Malvern Zeta sizer or the Nicomp particle sizer. Zeta potential is determined by electrophoretic light scattering(ELS).The smoluchowski equation can be used to compute the zeta potential from electrokinetic mobility μ.

$\mu=\zeta\varepsilon/\eta$…….equation3.

where ε is the permittivity and η the viscosity of the liquid used[22].

Viscosity Determination

Viscosity is defind as a measurement of the applied stress per unit area required to maintain a certain flow rate or shear rate.In general,viscosity is the resistance to liquid flow,whereas fluidity is the reciprocal of viscosity or the coefficient of viscosity.Thicker liquids have higher the viscosities while thinner the liquids have lower viscosities or higher fluidity[23].Two type of viscosity may be specified dynamic viscosity μ and kinematic viscosity ν.Dynamic viscosity is the absolute viscosity of the emulsion and the kinematic viscosity is obtained by dividing the dynamic viscosity by the density of the liquid[24].viscosity is determined by several method such as rotational,falling or rolling ball,capillary tube or orifice and surface viscosity methods[25].

Therapeutic Applications

Ocular

Glaucoma is a serious eye disorder characterized by an increase in the intraocular pressure which leads gradually to loss of vision due to damage of the optic disk[26,27,28].Drugs used to treat glaucoma work broadly in one of two ways,either to reduce the production or to increase the drainage of aqueous humour.Dorzolamide hydrochloride is an about 20 times more potent than acetazolamide with regard to the inhibition of carbonic anhydrase isoenzyme[29].Anti-VGEF treatment approach have changed the time course and prognosis of AMD.They not only reduce the disease progression,but also induce a gain in vision in about 30% of the treated patients[30,31].Antisense ODNs can also specifically down regulate any protein,particularly VGEF[32,33].However, the major limiting step for the clinical application of ODN strategy in the treatment of eye diseases is the lack of appropriate and efficient delivery systems to the involved intravascular tissues[34,35,36,37].Topical application of ODNs does not result in efficient intraocular penetration.

Parenteral

Carbamazepine (CBZ), and Ramipril are a widely used antiepileptic agent and antihypersentive drug respectively. CBZ has a slow and irregular gastrointestinal absorption due to its poor water solubility[39,40,41,42] developed a nanoemulsion for CBZ intravenous delivery.The spontaneous emulsification method was used to prepare different formulations containing CBZ. The physiochemical characteristics, such as drug content, zeta potential, and droplet size, were evaluated after 24 h and 3 months of preparation, demonstrating the feasibility of this emulsion

ISSN 2319-1074

for the i.v. route. Ramipril, is a potent antihypertensive drug that is a highly lipophilic, poorly water soluble drug with absolute bioavailability of 28–35%, when 5 mg of oral ramipril is compared with the same dose given intravenously[43,44,45]optimized nanoemulsion formulation of ramipril containing Sefsol 218, Tween 80 and Carbitol as oil, surfactant and cosurfactant, respectively. The absorption of ramiprilat from nanoemulsion resulted in 2.94-fold increase in bioavailability as compared to conventional capsule and 5.4-fold to that of drug suspension.

Cheliensisin A (GC-51) is a natural crystal compound extracted from *Goniothalamus cheliensis Hu* in Bull, Goniothalamus, Annonaceae. *In vitro* study suggests that GC-51 possesses a broad-spectrum efficiency, including inducing apoptosis, activation of caspase-3 and down regulation of Bcl-2 mRNAexpression [46]. A novel lead compound-based submicron emulsion was designed to act as the drug carrier for Cheliensisin A (GC-51)[47], which can be effectively delivered to solid tumors via parental administration route.

Topical

Many efforts have been made to develop and to improve delivery vehicles of pharmaceutical actives for dermal application.Examples for pharmaceutical carriers are liposomes, drug nanocrystals, and lipid nanocarriers, such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) or nanoemulsions. Each of this carrier system has advantages. Nevertheless not every carrier can be used for every pharmaceutical active, because the physical properties (e.g. insufficient solubility) of the compound might be a limiting parameter for an effective incorporation. Further approaches to improve the dermal action of actives are the additional use of compounds which do not act as a drug in particular, but promote or accelerate the therapy by supplying additional benefits[48].

Atopic dermatitis means “inflammation of the skin”. It is characterized by temporarily inflamed skin areas, typically next to skin creases, e.g. wrists, the front of the elbows and the backs of knees. The causes of the skin inflammation are not yet fully identified, thus up today the only efficient therapy is the treatment of the symptoms. A physically and chemically stable positively charged prednicarbate nanoemulsion was developed as a carrier system for the treatment of atopic dermatitis.Phytosphingosine was used to obtain the positive charge and also because of its supportive properties for the restoration of damaged skin[49].

Aspirin is widely used for their antipyretic, anti-inflammatory, analgesic and platelet anti-aggregation properties[50,51].studied the anti-inflammatory property of aspirin would enhance if delivered as nanoemulsion preparation.Nanoemulsion preparations of aspirin prepared with a Microfluidizer Processor were evaluated in the croton-oil-induced ear edema CD-1 mouse model using ear lobe thickness and the accumulation of specific in situ cytokines as biomarkers of inflammation.This study indicated that Nanoemulsion compared to aspirin suspension formulations increased the anti-inflammatory efficacy of aspirin in mice.This reduction in inflammation was associated with similar changes in the accumulation of auricular levels of proinflammatory cytokines and thereby suggests a possible method to reduce the adverse side effects associated with high-dosage level of aspirin.

Many lipophilic drugs, e.g.menthol, ibuprofen and ketoprofen are required to be delivered through skins at high concentrations for enhancing the topical uptake with low skin irritation[52,53].It is a challenge to enhance the permeation ability of drugs using drug delivery system with high drug-loading capacity,powerful permeation ability and low skin irritation via topical administration[54].However, the poor penetration and drug loading capacity of common products such as cream, gel or patch limited their topical therapeutic effects. The nanoemulsions containing camphor,menthol and methyl salicylate were prepared using high pressure

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homogenization with 600 bar and eight cycles, followed by being dispersed into carbomer 940-based gel matrix to form hydrogel thickened nanoemulsion[55].

Due to its antioxidant properties of vitamin E (tocopherol) is postulated to reduce the incidence and severity of disease states associated with oxidative stress, such as experimental atherosclerosis, cancer, chronic inflammation and neurological disorders[56].Vitamin E compounds have a direct effect against tumors and tumor cell growth[57]. In addition, it has been reported that certain isomers of tocopherol, γ -tocopherol, In particular, have anti-inflammatory properties[58,59,60,61] compared the transdermal application of a nano-sized emulsion versus a micron-sized emulsion preparation of delta tocopherol as it relates to particle size and bioavailability.nano-sized emulsion preparations provide a transdermal delivery system that increases the bioavailability of fat soluble substances such as tocopherol compared to micron-sized emulsion preparations that are produced containing the same compounds at equal concentrations.

Ketoprofen is an aryl propionic acid derivative has analgesic and antipyretic activities.This drug has been widely used for the treatment of rheumatoid arthritis and osteoarthritis[62].However it accompanies adverse side effects including gastrointestinal irritation usually when administered orally in chronic doses and also in acute administration in some case.Efforts to reduce the adverse effects have been attempted. One promising method is to administer the drug topically near to the effected site(s)[63] developed Ketoprofen in palm oil esters/water nanoemulsion was prepared by the spontaneous emulsification method. *In vitro* studies reveal that, sufficient percentage of ketoprofen was released and transferred through the artificial membrane, which in turn might result in good permeation when applied in skin.

Oral Delivery[64]

Nanoemulsion formulations offer the several benefits over conventional oral formulation for oral administration including increased absorption, improved clinical potency,and decreased drug toxicity.Therefore, Nanoemulsion have been reported to be ideal delivery of drugs such as steroids, hormones, diuretic and antibiotics. Pharmaceutical drugs of peptides and proteins are highly potent and specific in their physiological functions.However, most are difficult to administer orally.With on oral bioavailability in conventional (i.e. non-Nanoemulsion based) formulation of less than 10%, they are usually not therapeutically active by oral administration. Because of their low oral bioavailability, most protein drugs are only available as parenteral formulations.However, peptide drugs have an extremely short biological half life when administered parenterally, so require multiple dosing.A Nanoemulsion formulation of cyclosporine, named Neoral® has been introduced to replace Sandimmune, a crude oil-in-water emulsion of cyclosporine formulation.Neoral is formulated with a finer dispersion, giving it a more rapid and predictable absorption and less inter and intra patient variability.

Application of Nanoemulsions in Cosmetics[65]

Nanoemulsions have recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredients in particular skin layers.Due to their lipophilic interior, nanoemulsions are more suitable for the transport of lipophilic compounds than liposomes. Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation, or coalescence that are observed with macro emulsions.The incorporation of potentially irritating surfactants can often be avoided by using high-energy equipment during manufacturing.Nanoemulsions have attracted considerable attention in recent years for application in personal care products as potential vehicles for the controlled delivery of cosmetics and the optimized dispersion.

ISSN 2319-1074

Nanoemulsions in Biotechnology [66]

Many enzymatic and biocatalytic reactions are conducted in pure organic or aqua-organic media. Biphasic media are also used for these types of reactions. The use of pure a polar media causes the denaturation of biocatalysts.The use of water-proof media is relatively advantageous. Enzymes in low water content display and have- a. Increased solubility in non-polar reactants. b. Possibility of shifting thermodynamic equilibria in favour of condensations. c. Improvement of thermal stability of the enzymes, enabling reactions to be carried out at higher temperatures. Many enzymes, including lipases, esterases, dehydrogenases and oxidases often function in the cells in microenvironments that are hydrophobic in nature. In biological systems many enzymes operate at the interface between hydrophobic and hydrophilic domains and these usually interfaces are stabilized by polar lipids and other natural amphiphiles. Enzymatic catalysis in nanoemulsions has been used for a variety of reactions, such as synthesis of esters, peptides and sugar acetals transesterification,various hydrolysis reactions and steroid transformation. The most widely used class of enzymes in microemulsion-based reactions is of lipases.

Antimicrobial Nanoemulsions[67]

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.This fusion is enhanced by the electrostatic attraction between the cationic charge of the emulsion and the anionic charge on the pathogen.When enough nanoparticles fuse with the pathogens,they release part of the energy trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death.

Nanoemulsions and Drug Targeting[68]

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

Drug delivery routes across human skin [69]

Drug molecules in contact with the skin surface can penetrate by three potential pathways: through the sweat ducts, via the hair follicles and sebaceous glands (collectively called the shunt or appendageal route), or directly across the stratum corneum .The relative importance of the shunt or appendageal route versus transport across the stratum corneum has been debated by scientists over the years and is further complicated by the lack of a suitable experimental model to permit separation of the three pathways. In vitro experiments tend to involve the use of hydrated skin or epidermal membranes so that appendages are closed by the swelling associated

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with hydration. Scheuplein and colleagues proposed that a follicular shunt route was responsible for the pre steady-state permeation of polar molecules and flux of large polar molecules or ions that have difficulty diffusing across the intact stratum corneum.

SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS)

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water nanoemulsion when introduced into aqueous phases under gentle agitation[70].While considerable efforts have been made to develop SNEDDS for solid lipophilic drugs, few of them have considered SNEDDS for liquid lipophilic components,e.g.essential oils in herbal medicines, to improve their stability and oral bioavailability. Moreover, a liquid formulation such as SNEDDS is probably more suitable to be used for essential oil to prevent or minimize its volatility, which is normally not achievable with the solid preparation. SNEDDS are characterized by high solvent capacity, small particle size and excellent stability. In addition, they can enhance permeation across the intestinal membrane, reduce or eliminate food effect and enhance drug bioavailability[71,72]. Improved drug bioavailability induced by SNEDDS is not merely a matter of solubilization or particle size reduction.The interplay between certain excipients and enzymes or transporters has raised much concern about effect of such systems on drug absorption and metabolism.

Cefpodoxime proxetil (CFP) is an orally absorbed, broad spectrum, third generation cephalosporin ester implicated in treatment of upper respiratory tract and urinary tract infections. The prodrug ester is hydrolyzed *in vivo* to its active metabolite, cefpodoxime. The low bioavailability of CFP is mainly attributed to the degradation of its ester side chain by cholinesterases present in the intestinal lumen. In addition, poor water solubility (400 µg/ml), which may also be responsible for its poor bioavailability, as dissolution is a rate-limiting factor in intestinal absorption of poorly water soluble drugs[73].Self-nanoemulsifying drug delivery systems (SNEDDS) were developed with the objective to overcome problems associated with the delivery of CFP, a poorly bioavailable high dose antibiotic having pH dependant solubility. Studies on ternary phase diagrams indicated that CFP and the pH of dilution medium significantly affect the area of the nanoemulsion formation for the selected system. SNEDDS of CFP could accommodate high dose of CFP (130 mg) and exhibited rapid release independent of pH of dissolution media.

Zedoary turmeric oil (ZTO), an essential oil extracted from the dry rhizome of Curcuma zedoaria, is a mixture of structurally diverse compounds which are volatile and unstable under ambient condition[74].Pharmacological and clinical studies indicated that ZTO exhibits a wide array of therapeutic activities, such as hepatoprotection, tumor suppression, antioxidation and anti-bacterial action.[75,76]. Developed a SNEDDS for formulating ZTO with improved aqueous dispersibility, stability and oral bioavailability. In the formulated SNEDDS, the essential oil ZTO itself could serve as a partial lipid phase with the dual advantages of increasing drug loading as well as minimizing the amount of the inert oils required. Following oral administration of ZTO-SNEDDS in rats,both AUC and C_{max} of germacrone (GM), a representative bioactive marker of ZTO, increased by 1.7-fold and 2.5-fold respectively compared with the unformulated ZTO.

Tamoxifen citrate (TC) has been the clinical choice for the antiestrogen treatment of advanced or metastatic breast cancer for more than 20 years[77].It belongs to a class of non-steroidal triphenylethylene derivatives and is considered the first selective estrogen receptor modulator. It has a relatively low toxicity and is less harmful than most chemother.TC is a highly lipophilic drug of poor water solubility [78,79]. Furthermore, its oral bioavailability is mainly affected by the first-pass metabolism and P-glycoprotein (P-gp) pump efflux in the liver and intestine.The only

ISSN 2319-1074

attempt to improve TC oral bioavailability encompassed co-administration of TC with quercetin. Self-nanoemulsifying drug delivery systems of TC were prepared and in vitro evaluated.The formula was robust to dilution in different media using different dilution folds, exhibiting no signs of precipitation or separation.The globule size was unaffected in all applied media and dilution volumes and lied within appropriate range (130–150 nm). In vitro release in phosphate buffer (7.4) revealed a gradual release pattern with 100% release at 6 h. SNEDDS of TC showed a significant increase in release rate compared to the drug suspension under the same conditions.
Lacidipine (LCDP) is a calcium channel blocker used in the treatment of hypertension and atherosclerosis. It also possesses an antioxidant effect .The active Trans form is used in therapy. unfortunately, lacidipine is a highly lipophilic drug of poor water solubility and undergoes extensive first-pass hepatic metabolism with a mean absolute bioavailability of ~10% (range 3–59%). SNEDD systems of LCDP were prepared and in vitro evaluat. All the formulations showed good release profiles and exhibited a rapid rate of emulsification. D-optimal mixture experimental design was applied in order to rapidly obtain the optimal LCDP-loaded SNEDDS formulation so to contain minimum amount of surfactant, maximum amount of lipid, highest emulsification and dissolution rates. The stability of the optimized formulation was retained after storage at 40°C/75% RH for three months. The optimized SNEDDS formulation of LCDP showed a significant increase in dissolution rate compared to aqueous drug.

PATENTS RELATED TO NANOEMULSION [67]

Patents are the strongest form of intellectual property protection and are essential to the growth of a nanotechnology company. Similar to their importance to the development of the biotechnology and informational technology industries, patents will also play a critical role in the success of the global nanotechnology revolution., in fact patents are already shaping the nascent and rapidly evolving field of nanoscience and small technologies. As companies develop the products and processes of nantechnology and begin to seek commercial applications for their inventions, securing valid and defensible patent protection will be vital to their long term survivalg suspension in simulated gastric fluid and simulated intestinal fluid under the same conditions.

List of patent related to nanoemulsion

Patent Application Title	Patent App No.
Topical composition and method of detection and treatment	20120039814
Cancer vaccine compositions and methods	20110280911
Method of using nanoemulsion composition having anti-inflammatory activity	20110200657
Stable nanoemulsion for ultrasound-mediated of drug	20110177005

ISSN 2319-1074

delivery	
method of preparation of nanoparticle from nanoemulsion	20110135734
Nanoemulsion or emulsion for direct delivery	20110045050
Lyphilized nanoemulsion	20110015226

STABILITY ISSUES

The stability of any pharmaceutical product is defined as the capacity of the formulation to remain within defined limits for a predetermined period of time (shelf life of the product). The first step to designing any type of stability testing program is to specify these limits by establishing parameters defined in terms of chemical stability, physical stability and microbial stability. Next, methods must be established to evaluate these parameters [80].

Emulsions are thermodynamically unstable exhibiting flocculation and coalescence unless significant energetic barriers to droplet interactions are present.Emulsions are sensitive to coarsening phenomena like coalescence and Ostwald ripening since droplet size is not uniform and concentration gradients drive mass exchange, thus inducing changes in droplet sizes.Forced degradation studies involve subjection of emulsions to harsh conditions such as elevation of temperature. Emulsion characteristics are determined before and after the tests. As discussed in the experimental section, stability of emulsions were determined using the stress tests and accelerated stability studies of emulsions based on ICH guidelines were also determined.Stress tests included centrifugation and freeze thaw cycles of emulsions.Emulsion characteristics were determined before and after the tests.

Phase behavior study[81]

This study is a characterization and optimization of ingredients (surfactant, oil phase and aqueous phase).Generally the study is necessary in case of nanoemulsion formulation prepared by phase inversion temperature method and self emulsification method in order to determine the phase of nanoemulsion and dispersibility.Study is done by placing the different ingredients of nanoemulsion by varying the concentration in glass ampoules and thoroughly homogenized at a certain temperature for a time until equilibrium anisotropic phase can be identified by polarized light.

Physical Instability

Instability of lipid emulsions can arise from changes in particle size of oil droplets leading to creaming and coalescence or from changes in pH, hydrolysis of emulsifier, or oxidation of oil. There are basically 5 ways in which the structure of a dispersion of liquid droplets in a continuous medium can change resulting in physical instability.

Creaming

Creaming is the upward movement of dispersed droplets relative to the continuous phase.There is no change in droplet size, but build up of an equilibrium droplet concentration gradient within the emulsion.This phenomenon occurs from external force fields, usually gravitational, centrifugal or electrostatic, acting on the system.The disperse phase, according to its density relative to that of the continuous phase, rises to the top or sinks to the bottom of the emulsion, forming a concentrated layer at the top of the emulsion. Sedimentation involves the same process but in the opposite direction [81].

Flocculation

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In flocculation of emulsion droplets, there is no change in droplet size or distribution but the buildup of aggregates of droplets within the emulsion.Flocculation results from the existence of attractive forces between the droplets.

Coalescence

Emulsion droplets within a close- packed array resulting from sedimentation or creaming, coalesce to form larger droplets.This results in a change in the initial droplet size distribution.

Ostwald Ripening

Larger emulsion droplets are formed at the expense of the smaller droplets. In principle, the system will tend to an equilibrium state in which all the all the droplets attain the same size. Ostwald ripening is associated with the difference in chemical potential for emulsion of different size.

Phase Inversion

This is the process where an emulsion changes for example from an o/w emulsion to a w/o emulsion.This may be brought about by a change in temperature or concentration of one of the components or by the addition of a new component to the system [82,83].

Chemical Instability

Chemical instability of an emulsion may occur as a result of drug degradation.Possible degradation pathways include hydrolysis, dehydration, isomerization and racemization, elimination, oxidation, photodegradation, and complex interactions with excipients and other drugs . The degradation pathway depends on the chemical nature of the drug.

Regulatory Guidelines for Evaluation of Emulsion Stability[84]

The International conference on harmonization (ICH) of technical requirements for registration of pharmaceuticals for human use regulates the stability test by which emulsions and other drug products should be evaluated.This includes stress testing, long term, intermediate or accelerated stability studies. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic instability of the molecule and validate the stability indicating power of the analytical procedures used.

ICH guidelines recommend stress test on a single batch of the drug substance. It should include the effect of temperatures (in 10°C increments (e.g., 50°C, 60°C, etc.) above that for accelerated testing), humidity (e.g., 75% RH or greater) where appropriate, oxidation, and photolysis on the drug substance.The testing should also evaluate the susceptibility of the drug substance to hydrolysis across a wide range of pH values when in solution or suspension. Photostability testing should be an integral part of stress testing [85].

ICH guidelines for accelerated stability studies includes testing at 40 °C ± 2 °C/75% RH± 5% RH for a minimum period of 6 months, intermediate studies at 30 °C ± 2 °C/65% RH ± 5% RH for at least 6 months and long term stability studies at 25 °C ± 2 °C/60% RH ± 5% RH or 30 °C ± 2 °C/65% RH ± 5% RH for a period of 12 months on at least three primary batches.

CONCLUSION

This review article covered all related nanoemulsion system including their application. Nanoemulsions have been prepared using different methods.

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