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Review article

Nanoemulsion: Concepts, development and applications in drug delivery

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ABSTRACT

Nanoemulsions are biphasic dispersion of two immiscible liquids: either water in oil (W/O) or oil in water (O/W) droplets stabilized by an amphiphilic surfactant. These come across as ultrafine dispersions whose differential drug loading; viscoelastic as well as visual properties can cater to a wide range of functionalities including drug delivery. However there is still relatively narrow insight regarding development, manufacturing, fabrication and manipulation of nanoemulsions which primarily stems from the fact that conventional aspects of emulsion formation and stabilization only partially apply to nanoemulsions. This general deficiency sets up the premise for current review. We attempt to explore varying intricacies, excipients, manufacturing techniques and their underlying principles, production conditions, structural dynamics, prevalent destabilization mechanisms, and drug delivery applications of nanoemulsions to spike interest of those contemplating a foray in this field.
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1. Introduction

Nanoemulsions are oil-in-water (O/W), water-in-oil (W/O) dispersion of two immiscible liquids stabilized using an appropriate surfactant [1]. The mean droplet diameter attained is usually <500 nm [2]. Small droplet size gives them a clear or hazy appearance which differs from milky white color associated with coarse emulsion (whose micron sized droplets partake in multiple light scattering) [3]. The word nanoemulsion is sometimes used interchangeably with submicron emulsion or mini emulsion; however it should not be confused with microemulsion. Nanoemulsions despite having the same droplet size range as microemulsions, differ tremendously in structural aspects and long term thermodynamic stability [4].

Nanoemulsions can be rendered into several dosage forms, like liquids [5], creams [6,7], sprays [8], gels [9,10], aerosols [11,12], foams [13]; and can be administered by equally varying routes like topical [14], oral [15], intravenous [16], intranasal [17], pulmonary [17] and ocular [18]. They possess higher solubilization capacity than simple micellar dispersions, greater kinetic stability than coarse emulsions and have found use in cosmetic [19] and pesticide industry [20] as aqueous base for organic deliverables. Their long-term physical stability is a direct consequence of small droplet size, which impairs conventional destabilization phenomena like creaming, sedimentation and coalescence. Often *brownian motion* is strong enough to offset gravity or viscosity induced kinetic instability. In parenteral form, nanoemulsions have been used to solubilize and protect drugs against harsh environmental factors (oxidation, pH, hydrolysis) [21], to target specific organs by exploiting enhanced permeability and retention effect [22], and to evade reticuloendothelial system [23]. When administered orally, miniscule size of droplets in nanoemulsion and their capability to solubilize very hydrophobic drugs, provides a pathway to drastically increase rate of drug dissolution and subsequently expected systemic bioavailability [24]. Drug release from nanoemulsion involves partitioning of it from oil into surfactant layer and then into aqueous phase. The solubilized drug moiety whilst diffusing out of oil comes in contact with surrounding water and undergoes nanoprecipitaion. This raises drug's surface area enormously; accelerating its dissolution in accordance with *Noye-Whitney's equation*. Dynamics of drug release can thus be influenced at each of these steps by subtly varying composition of nanoemulsion to obtain a sustained/controlled release device [25]. Other factors like capability to undergo direct paracellular/transcellular transport [26,27], prolonged gastric retention due to mucosal entanglement [28], also contribute in nanoemulsion mediated bioavailability enhancement. It has been reported that several nanoemulsions undergo direct lymphatic absorption thereby avoiding first pass metabolism to boost bioavailability and reduce dose of drugs which undergo hepatic transformation to a large extent [29]. Nanoemulsions can be used to effectively screen bitter or metallic after taste of drugs which cause nausea and associated non-compliance [30]. Also nanoemulsions typically require lesser surfactant than other colloidal dispersions, whilst still retaining many virtues and are accordingly expected to storm commercial dug delivery barrage (Table 1). Despite these benefits, there is still relatively narrow insight regarding development, manufacturing, fabrication and manipulation of nanoemulsions which primarily stems from the fact that conventional concepts of emulsion formation and stabilization only partially apply to them. This general deficiency sets up the premise for current perspective. We attempt to cover varying intricacies, structural dynamics, fabrication conditions and drug delivery applications of nanoemulsions to spike interest of those contemplating a foray in this field. There are disadvantages too with nanoemulsions which should be considered whilst rationalizing candidature of any drug. Primary amongst them is inability of nanoemulsions to solubilize substances which have high melting point. Also components to be used in development of nanoemulsions must be strictly non-toxic (preferably GRAS excipients) if intended for human use. This turns out to be a limiting issue. Nanoemulsions prepared by low energy methods frequently require large amounts of surfactants for stabilization of droplets; in such cases heavy usage of surfactant can cause biomembrane fluidization, ruling out their internal use. Price effectiveness of nanoemulsion manufacturing is also an issue that needs to be dealt with in advance, as expensive instruments are often involved.

A trivial rendition of differing types of nanoemulsions is given in Fig. 1.

2. Types of nanoemulsions

Depending on constituents and relative distribution of the internal dispersed phase/phases and the more ubiquitous continuous phase, nanoemulsions are termed as biphasic (O/W or W/O) or multiple nanoemulsions (W/O/W). Phase volume ratio (Φ) measures comparative volumes of internal and external phase comprising a nanoemulsion and determines its droplet number and overall stability. Normally, phase present in greater volume becomes the external phase. To predict type of nanoemulsion formed under given conditions, interaction of various components making up the nanoemulsion must be estimated. If chief surfactant is water soluble it favors O/W emulsification, and conversely if surfactant is oil soluble it favors W/O emulsification. Polar portion of an emulsifier is generally better barrier to coalescence than hydrocarbon region. It is therefore possible to make O/W emulsion with relatively high internal phase volumes. On the other hand W/O

Table 1
Nanoemulsions undergoing clinical trials.

Drug	Condition	Vehicle	Target	Goal	Route	Phase	Clinical trials.gov identifier	Status	Sponsor
5-Aminolevulinic acid	Lentigo maligna	BF-200 ALA, Ameluz® nanoemulsion in a light sensitizing gel	Establish efficacy of photodynamic therapy (PDT)	Histopathological and immunohistochemical curing of lentigo maligna within two months	Topical	IV	NCT02685592	Recruiting participants	Päijät-Häme social and health care
Diclofenac	Osteoarthritis of the Knee	3%-Diclofenac-nanoemulsion cream	Evaluate the safety and analgesic efficacy	Analgesic activity as measured by the WOMAC pain subscale, daily pain assessment, safety and tolerability	Topical	II	NCT00484120	Completed	Pharmos
Testosterone derivatives	Women libido, menopause	Nanoemulsion(Biolipid B2)	Establish efficacy of transdermal testosterone nanoemulsion (TNT) as a treatment for SSRI/SNRI-emergent loss of libido	Change in sexual function measured by the Sexual Self-rating Scale (SSS)	Transdermal	I	NCT02445716	Recruiting participants	University Potiguar
Propofol	Leukemia	Nanoemulsion	Compare safety and efficacy of propofol nanoemulsion and lipid emulsion for sedation in non-invasive ambulatory procedures	Classification of sedation level, loss of corneal-palpebral reflex, pain at injection, physician satisfaction	Intravenous	II, III	NCT01326078	Terminated due to commercial reasons	Cristália Produtos Químicos Farmacêuticos Ltd
5-Aminolevulinic acid	Basal cell carcinoma	BF-200 ALA nanoemulsion	Comparative efficacy in photodynamic therapy (PDT) versus Methylaminolevulinate	Histological lesion clearance, pain in VAS-scale	Topical	II	NCT02367547	Recruiting participants	Päijät-Häme social and health care
Curcumin	Atypical ductal breast hyperplasia	Nanoemulsion	Reduction in inflammatory changes in breast tissue in obese women at high risk for breast cancer	Modulation in pro-inflammatory biomarkers in plasma and breast adipose tissue	Oral	–	NCT01975363	Recruiting participants	Comprehensive Cancer Center of Wake Forest University
14C- cholesteryl oleate and 3H- cholesterol labeled low density lipoprotein (LDL)-like nanoemulsions	Diabetic dyslipidemia	Artificial lipid nanoemulsion	Clearance of cholesteryl ester and free cholesterol from intravascular sites in patients with advanced coronary artery disease	Plasma kinetics of both forms of cholesterol (free and esterified) in type 2 diabetes patients	Intravenous		NCT01010035	Completed	University of Sao Paulo General Hospital
Same as above	Diabetic dyslipidemia	Artificial lipid nanoemulsion	Clearance of free cholesterol and cholesterol ester in impaired glucose tolerance patient, asymptomatic for coronary artery disease to elucidate mechanisms involved in atherogenesis	Plasma kinetics of both forms of cholesterol in glucose intolerant patients	Intravenous		NCT01020578	Completed	University of Sao Paulo General Hospital
CoQ10	Ataxia-oculomotor Apraxia 1	Nanoemulsion	Correction or stabilization of albumin level with CoQ10 supplementation	Albuminemia, evolution of clinical criteria SARA and CCFS, oculomotor evaluation	Oral drops	III	NCT02333305	Recruiting participants	Assistance publique - hôpitaux de paris
NB-001	Recurrent herpes labialis	Cationic nanoemulsion	Healing of a cold sore will be lower in active treatment	Time to healing of the primary lesion complex, safety and tolerability	Topical	III	NCT01695187	Unknown	NanoBio Corporation
Cyclsporine	Dry eye syndromes	Nanoemulsion	Comparative study against marketed suspension	Evaluation of efficacy and safety, Corneal staining test	Ophthalmic drops	III	NCT00927459	Completed	Tekmira Pharmaceutical Corporation
BF-200 ALA (Ameluz®) with	Actinic keratosis	Nanoemulsion gel formulation	Efficacy of photodynamic therapy (PDT)	Patient response, assessment of pain during PDT	Topical	I	NCT01966120	Completed	Biofrontera bioscience GmbH

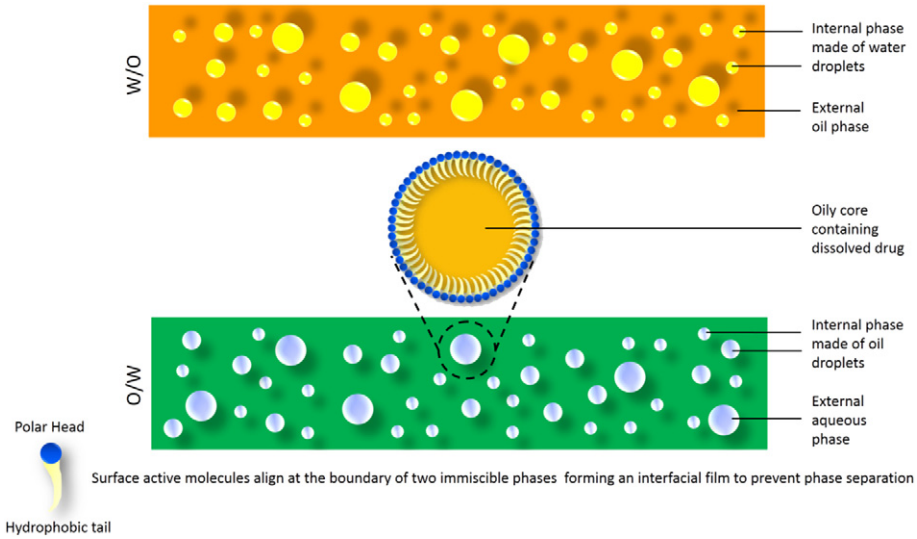

Fig. 1. Structure of an O/W or a W/O nanoemulsion. These are biphasic systems in which an optimized volume of internal oil phase is dispersed in bulk aqueous phase or vice-versa, with the help of an interfacially active compound, 'surfactant'. Employed phases are usually immiscible liquids and the drug content is generally solubilized in the internal phase. Size-wise, nanoemulsions are much finer than coarse emulsions which results in several outstanding advantages as discussed throughout the text.

emulsions invert easily if water content is raised and therefore continuous dilution of a nanoemulsion either with water or oil can reveal its type. Usually increasing Φ beyond 40% can lead to phase inversion of a W/O emulsion. Some oils fluoresce when excited with UV light and if incorporated in an O/W nanoemulsion, produce a dotted pattern of fluorescence upon illumination. Conversely, in case of a W/O nanoemulsion the entire field lights up. Conductivity tests can also be used to differentiate between O/W and W/O subtype of nanoemulsions [31]. In multiple nanoemulsions inner water phase is dispersed in an oil phase which in turn is distributed within a bulk aqueous phase within a single system. Owing to their structural characteristics, both hydrophilic (5-fluoro uracil) and hydrophobic (paclitaxel) compounds can exist concurrently in the inner and/or outer water phase as well as oil phase, respectively. O/W/O nanoemulsions are extremely rare and hence not discussed here.

3. Components of a nanoemulsion

3.1. Oil/lipids

Nanoemulsions generally contain 5–20% oil/lipid droplets in case of O/W emulsions, though it may sometimes be significantly larger (upto70%). Lipids/oils to be used in nanoemulsions are generally propositioned on solubility of drug. Reesterified fractions derived from soybean oil [22,32–34], sesame oil [34], cottonseed oil [35], safflower oil [36], coconut oil [7], ricebran oil [37] [labeled as long chain triglycerides (LCT), medium-chain triglycerides (MCT) or short chain tri glycerides (SCT) depending on their chain lengths] are used either alone or in combination to formulate nanoemulsions. D-α-Tocopherol (vitamin E) family has been extensively used as a carrier in nanoemulsions [16, 38,39]. Oleic acid and ethyl oleate have also been used in oral, topical and parenteral nanoemulsions [40,41]. Efforts have been put into finding suitable marine oils (salmon oil) for emulsification purpose [42]. These marine oils are polyunsaturated i.e. possess more than one double bond in their structure, with several therapeutic benefits and have proven to be a safer alternative for atherosclerotic patients who cannot tolerate regular LCT and MCT. Type of oil used in formulation of nanoemulsion sometimes determines bioavailable fraction of active constituent. McClements and Xiao have investigated influence of formative components and droplet size on bioavailability of curcumin nanoemulsion. Bio-relevant testing revealed that maximum systemic availability was attained in nanoemulsions made with LCT and MCT which were digested to an appreciably lesser extent than those made with SCT [43].

3.2. Surfactants and co-surfactants

Surfactants are amphiphilic molecules which stabilize nanoemulsions by reducing interfacial tension, and prevent droplet aggregation. They tend to rapidly adsorb at oil water interface and provide steric or electrostatic or dual electro-steric stabilization. A common surfactant employed in nanoemulsions is lecithin (phosphatidylcholine) derived from egg yolk or soybean [44]. Surfactants like sodium deoxycholate (bile salt) [11,36] and cremophor EL (Polyoxyl-35 castor oil) [36,45] have been used in marketed parenteral products. Tween 20, 40, 60 and 80 (Polyoxyethlene sorbitan monolaurate) [46–48], Span 20, 40, 60 and 80 (Sorbitan monolaurate) [49,50], Solutol HS-15 (polyoxyethylene-660-hydroxystearate) are also regularly used [51]. Other common surfactants include those belonging to poloxamer family [52], sodium dodecyl sulfate [53,54], amphiphilic proteins like casein [55,56], β-lactoglobulin [57], polysaccharides (e.g., gums, starch derivatives) [58], and PEG containing block copolymers [59]. Selection of a surfactant/surfactant blend not only influences size and stability of nanoemulsion but sometimes also determines its toxicity, pharmacokinetics and pharmacodynamics [40,60–62]. For instance desirable concentration of surfactant in parenteral nanoemulsions is pretty narrow, e.g. poloxamer 188 if used in a concentration > 0.5% has renal toxicity potential [63]. Surfactants may also be used as decorative templates to attach ligand for active targeting of certain cancers [64]. Sometimes, co-surfactants are used to complement surfactants, as they fit suitably in between structurally weaker areas, fortifying the interfacial film. Co-surfactants that are commonly used include propylene glycol, polyethylene glycol, ethanol, transcutol IP, glycerine, ethylene glycol and propanol [21].

3.3. Preservatives, antioxidants and chemoprotectants

Preservatives employed in nanoemulsions should meet criteria like low toxicity, stability to heat and storage, physical and chemical compatibility, reasonable cost, ease of availability, acceptable odor, taste and color and should have a broad antimicrobial spectrum. Microorganisms thrive in both oil and water, and consequently selected preservative should attain effective concentration in both the phases. Use of preservatives in parenteral nanoemulsions is more or less avoided due

to their toxic potential. Acid and acid derivatives viz. benzoic acid, sorbic acid, propionic acid, dehydro acetic acid can be used as antifungal agents in formulation. Alcohols like cholorobutanol and phenoxy-2-ethanol are routinely used in ophthalmics. Phenolics and quarternary ammonium compounds serve as broad spectrum preservatives [31]. Emulsified oil and lipids are subject to autoxidation upon exposure to air; many drugs used in nanoemulsion are also highly susceptible to oxidative degradation. Upon oxidation, unsaturated oils give rise to rancidity [65]. If oxidation is to be avoided it is advisable to employ synthetic lipids which lack the sensitive acyl group. This however is not always feasible, so an extra component namely an antioxidant is added. Antioxidants offer oxidative stability to formulation by acting either as: *Reducing agents* - e.g. ascorbic acid, sodium bisulfite, metabisulfite, thiourea and sodium formaldehyde or *Blocking agents* e.g. ascorbic acid esters, butyl hydroxytoluene and tocopherols or *Synergists* e.g. ascorbic acid, citranoic acid, phosphoric acid, citric acid and tartaric acid. Nanoemulsions are usually transparent which implies that entire spectrum of radiation including visible and UV rays can easily penetrate oil layers and catalyze photodegradation of drug molecule. Inclusion of chelating agents, pH stabilizers, UV protectants etc. is therefore sometimes required to counter environmental degradation.

3.4. Manufacturing nanoemulsions

Tailoring methods for nanoemulsions can be categorized into high-energy or low-energy or a combination of low and high energy [1,66]. Sequence of excipient and bioactive addition has a bearing on the final outcome. A lipophilic drug is dispersed directly or with assistance of an organic solvent in the oil phase, whereas hydrophilic drugs go into the aqueous phase. Other excipients are drafted in as per requirement. Most important criterion in manufacturing nanoemulsion is obtainment of desired droplet size with monomodal distribution. This ensures uniformity of properties and provides a good starting point for further fabrication. A brief inspection into factors which affect droplet dynamics follows next.

3.5. Droplet dynamics

3.5.1. Effect of shear

Amount of shear applied directly influences droplet size. A fundamental relationship supervising how droplet of a liquid is sized down in another immiscible liquid was developed by Taylor [67]. If dispersed phase viscosity (η_d) is negligible in comparison to continuous phase viscosity (η_c), Taylor's equation is written as:

$$r \sim \frac{\gamma}{(\eta_c \sigma)}$$

where σ is the shear rate applied, r is the attained radius of droplet and γ is the acting interfacial tension. Application of high shear rates by a second immiscible liquid on the dispersed phase stretches interface of a droplet from spherical to ellipsoid to flat dumbbell shaped entities (Fig. 2). Driven by interfacial tension this creates capillary instability which ultimately results in pinching off of the original droplet into smaller satellite droplets causing size reduction.

3.5.2. Effect of surfactant

Taylor's equation highlights crucial role played by surfactants in lowering interfacial tension which in turn brings down overall requirement of shear. Ultimate size of droplets in a nanoemulsion is a resultant of droplet rupture and coalescence. Rate of rupture should always exceed that of recolaescence to induce effective size reduction. With inherent ability to lower interfacial tension due to their amphiphillic structure, most important role played by any surfactant is to stabilize newly formed droplets and ensure long term stability. Surfactants do this by *Gibbs-Marangoni* effect [68]. To imagine this effect at work, consider any system in which oil droplets (undergoing size reduction by any means possible) and surfactant molecules are distributed evenly throughout a continuous aqueous phase (Fig. 2). Upon size reduction, formation of two new droplets generates new surface area which disturbs thermodynamic equilibrium of the system by increasing surface energy. One way to reduce this excess energy is for the droplets to reattach. Whilst rushing towards each other in order to reattach, these drops tend to adsorb surfactant molecules in their path, effectively reducing interfacial tension. But this adsorption is not even, as surfactant molecules available in area of closest approach (for droplets) is lesser in comparison to bulk. Differential adsorption of surfactant sets up a gradient which draws more surfactant to this deficient region. Viscous drag created by sudden rush of surfactant molecules pulls attached water creating a hydrostatic influx which drives the two drops on course for reattachment apart. Strength of *Gibbs-Marangoni* effect is dependent on concentration of surfactant and Gibbs elasticity of the interface. Therefore if excess surfactant is employed during processing, rate of droplet coalescence becomes negligible and terminal size of nanoemulsion is dictated by rate of droplet disruption only. Since different surfactants follow different adsorption kinetics, their selection becomes an empirical step in designing of nanoemulsions. It has been reported that small molecule surfactants are more adept in forming finer emulsions [67].

3.5.3. Relative viscosity of oil and water

Relative viscosities of two phases i.e. dispersed (η_d) and continuous phase (η_c) has a strong influence on outcome of size reduction process. When relative viscosity is too high, droplets become resistant to breakup, and instead start rotating upon their own axis when subjected to shear. The oil type and oil volume fraction also affects droplet size; for instance nanoemulsions made with high viscosity oils are much larger than those made with simpler oils such as hexadecane. Over time, researchers have found an optimum viscosity ratio ($0.05 \leq \eta_d / \eta_c \leq 5$) which produces finest nanoemulsions. If dealing with very thick oils, droplet size can be reduced by raising viscosity of continuous phase [69]. Considering factors described above, it is apparent that final droplet size attained is a complex interplay between surfactant chemistry, applied shear, etc. This interplay must be taken into account whilst selecting or optimizing process for manufacturing nanoemulsions.

3.6. High energy emulsification method

High energy methods depend on mechanical devices to create powerful disruptive forces for size reduction. Disruptive forces are achieved via ultrasonicators, microfluidizer and high pressure homogenizers which are industrially scalable. Their versatility lies in the fact that almost any oil can be subjected to nanoemulsification. Major disadvantages include instrumental cost and generation of high operational temperatures which sometimes rules out thermolabile drugs.

3.7. High pressure homogenization (HPH) method

3.7.1. Microfluidizer

A microfluidizer (MicrofluidicsTM Inc., U.S.A.) concomitantly uses hydraulic shear, impact, attrition, impingement, intense turbulence and cavitation, to effect size reduction. Simplified working of microfluidizer is shown in Fig. 3. It forces feed material through an interaction chamber consisting of microchannels under influence of a high-pressure displacement pump (500–50,000 psi), resulting in very fine droplets.

Usually a coarse emulsion is passed repeatedly (sometimes up to 100 cycles) through a microfluidizer until desired size and dispersity is obtained. Impaction energy generated by collision of droplets dissipates in form of heat and requires cooling. *Weber number* is a dimensionless number in fluid mechanics which analyses pattern of fluid

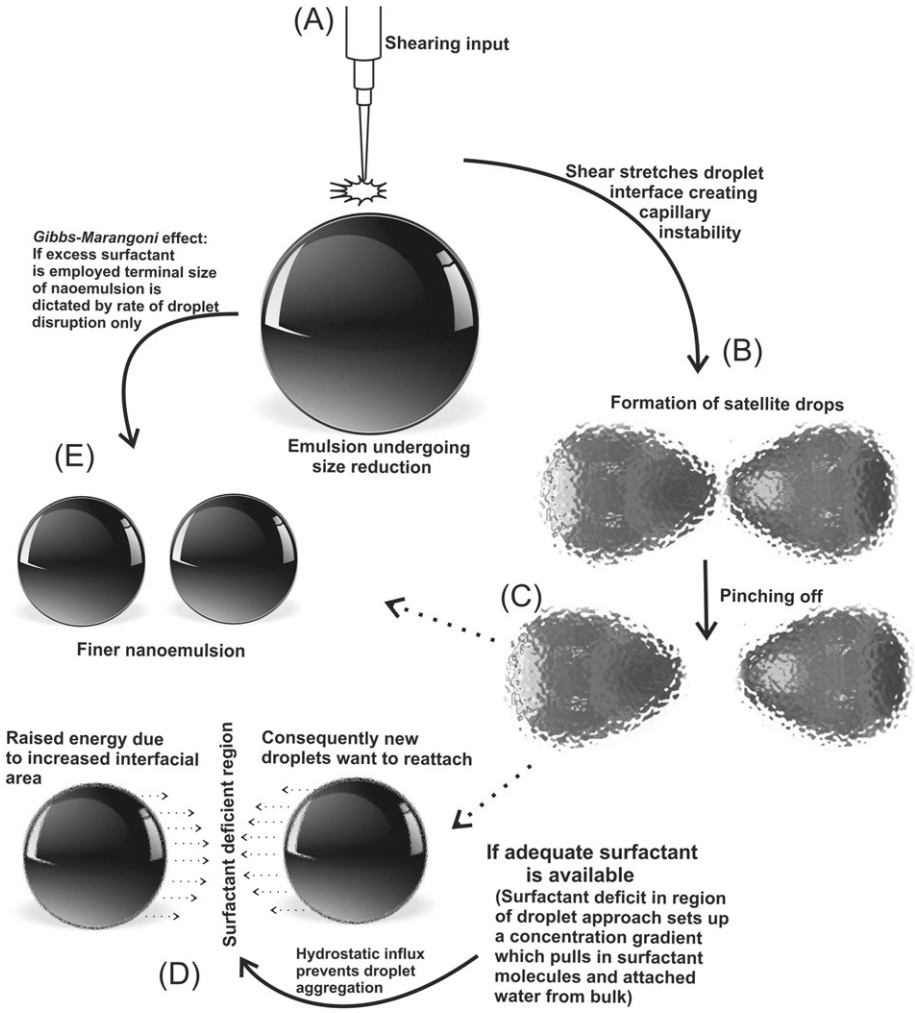

Fig. 2. *Gibbs-Marangoni* effect at work. (A) Size reduction of a larger emulsion droplet via stretching of interfacial surface which changes shape of droplet from spherical to ellipsoid to (B) dumbbell. (C) This continues till satellite droplets pinch off from each other to form two independent droplets. (D) However as a result, interfacial energy of nascent droplets is also raised and they acquire a spontaneous tendency to reattach. Whilst on the course of reattachment these nascent droplets only pick up surfactant molecules available in the bulk as the gap in between the two droplets which earlier was occupied by the larger droplet itself is still relatively devoid of surfactant. A concentration gradient is consequently set up which pulls in surfactant and attached water from the bulk and keeps finer droplets as independent entities.

flow and correlates homogenization efficiency with viscosity ratio of dispersed and continuous phase and can be a good starting point to gauge overall efficiency of high pressure homogenization [70]. Biggest advantage of this highly scalable process is zero contamination of feed material as reduction is effected by source material itself.

3.7.2. Piston gap homogenizer

Piston gap homogenizers work on principle of colloid mills. A coarse emulsion is made to pass through a narrow gap (of dimension < 10 μm) between a fixed stator and a rapidly moving rotor. Size reduction is caused by high shear, stress and grinding forces generated between rotor and stator [71]. The upper ceiling of droplet size can be ascertained by fixing dissipation gap to required size, which implies that a yield will not be obtained unless and until emulsion is ground down to a size which is equal or lower to that of the gap between rotor and stator. A basic visualization is displayed in Fig. 4.

3.7.3. Ultrasonication

Ultrasonication methods depend on high-frequency sound waves (20 kHz and up). They can be used to form a nanoemulsion in situ or reduce size of a pre-formed emulsion. Bench-top sonicators consist of a piezoelectric probe which generates intense disruptive force at its tip [72]. When dipped in a sample, ultrasonic waves produce cavitation bubbles which continue to grow until they implode (Fig. 5). This implosion sets up shock waves, which in turn create a jet stream of surrounding liquid, pressurizing dispersed droplets and effecting their size reduction [73]. Investigation into operational parameters has revealed that droplet size decreases with increasing sonication time and input power [74]. Probes in an ultrasonicator are available in variety of dimensions which affect their functionality. Usually narrower probes are preferred for working on small volume batches. Relative placement of probe in the sample, i.e. depth to which it is dipped alters pattern of wave reflection and pressure distribution and consequently it should not touch any solid surface. Procedurally, a coarse emulsion is prepared by addition of a homogenous oil phase to aqueous phase under mechanical stirring. The emulsion is then subjected to ultrasonication at different amplitudes for short time cycles until desired properties are obtained for nanoemulsion. Compared to other high energy methods, ultrasonication requires least energy expenditure. One serious downside of this technique is contamination induced by probe. For scale up applicability, commercial homogenizers based on sonication have been developed, in which nanoemulsion is made to flow through a special column capable of producing ultra-sonic waves.

4. Low energy emulsification method

Nanoemulsions prepared by low-energy emulsification methods were developed after studying cumulative behavior of oil, surfactants,

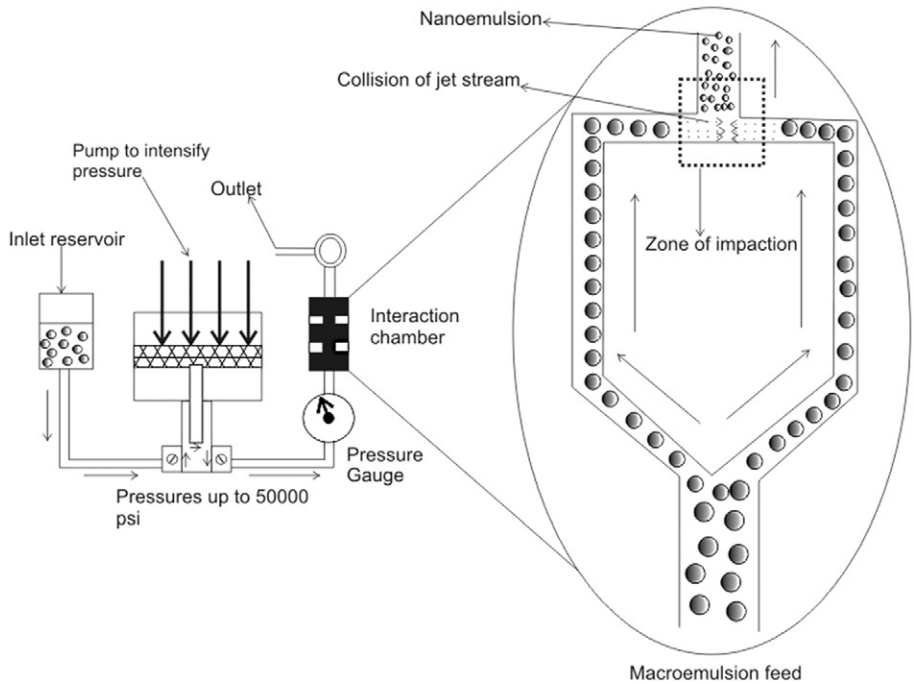

Fig. 3. Setup and functioning of a microfluidizer. The feed material i.e. coarse nanoemulsion is passed through microchannels of the interaction chamber under intense pressure. Multiple mechanisms including inter droplet impaction, attrition, shearing, cavitation, etc. act together to effect size reduction. Nanoemulsion is subjected to several passages through the instrument to ensure size uniformity.

co-surfactants, drug, aqueous component, hydrophilic lipophilic balance of utilized oil surfactant blend, and operative temperature [75]. Low-energy methods include spontaneous emulsification [76], phase inversion [77] and the less utilized catastrophic phase inversion method [78]. A key character of these methods is utilization of energy stored in the system to produce ultra-fine droplets. Low energy methods are sometimes limited by oil type and emulsifiers that can be used.

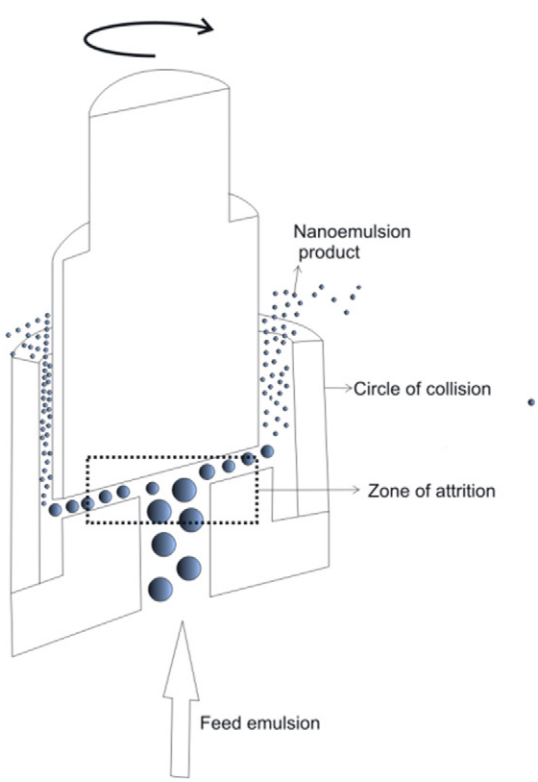

Fig. 4. A through section of piston gap homogenizer. Faster moving rotors accelerate production output; however ultimate size of nanoemulsion depends on the clearance between rotor and stator. Piston gap homogenizers are sometimes used to pre-condition a sample before it is subjected to microfluidization, in order to increase the latter's efficiency and form an important part of industrial set up.

4.1. Spontaneous emulsification

Spontaneous emulsification is akin to nanoprecipitation method utilized in manufacturing polymeric nanoparticles. However, instead of polymer, oil is used. The procedure involves preparation of two phases, one a hydrophilic surfactant containing aqueous phase and other an organic or oil phase such as mygliol containing a drug, an oil soluble surfactant such as Span and a partially water miscible organic solvent such as acetone or ethyl acetate. Organic phase is added drop wise to aqueous stirred phase (although the reverse i.e. adding water to oil is equally feasible in case of W/O emulsions) to form small nanoscale emuslions.

4.1.1. Why does spontaneous emulsification occur?

For a process to be spontaneous, overall change in ΔG should be negative. Since nanoemulsions traditionally have a positive finite interfacial tension it has to be offset by increased entropy generated by diffusion of oil droplets. Energy of emulsification which is expended in development of new surface area is thus provided by accompanying turbulence. In some cases small amount of external energy may still be required (supplied by a magnetic stirrer), yet the actual process of emulsification occurs spontaneously. To further elaborate; suppose oil is dissolved in a water miscible organic solvent like acetone which is to be emulsified into aqueous surfactant solution. Upon introduction, acetone in organic phase and water move towards each other. This leaves behind tiny droplets of oil, which are immediately covered up by surfactant molecules. Mild magnetic stirring is helpful in setting up tiny convection currents which consistently distribute oil droplets in bulk so that any new surface generated by solvent diffusion is immediately covered by surrounding surfactant molecules. To prepare very small droplets, it is usually necessary to use a high ratio of water miscible component-to-oil in the organic phase prior to mixing.

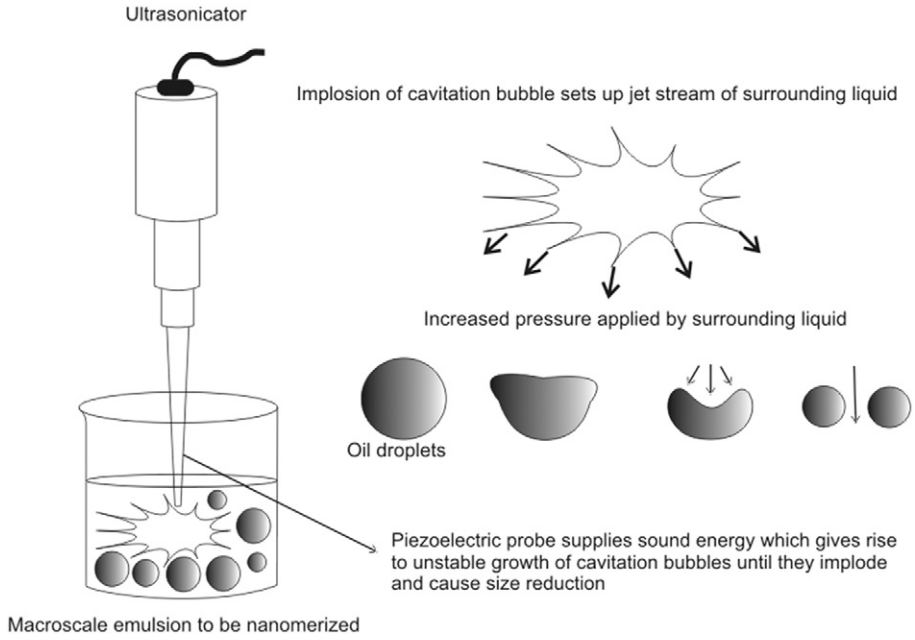

Fig. 5. Emulsion droplet size reduction via ultrasonication. Supplied electricity is converted into ultrasonic waves by the piezoelectric probe. These intense waves set up cavitation bubbles which grow in an unstable manner and ultimately implode to generate stream of surrounding liquid which shears the droplets into smaller droplets. Ultrasonicators are amongst the most common lab scale instruments of nanoemulsion production.

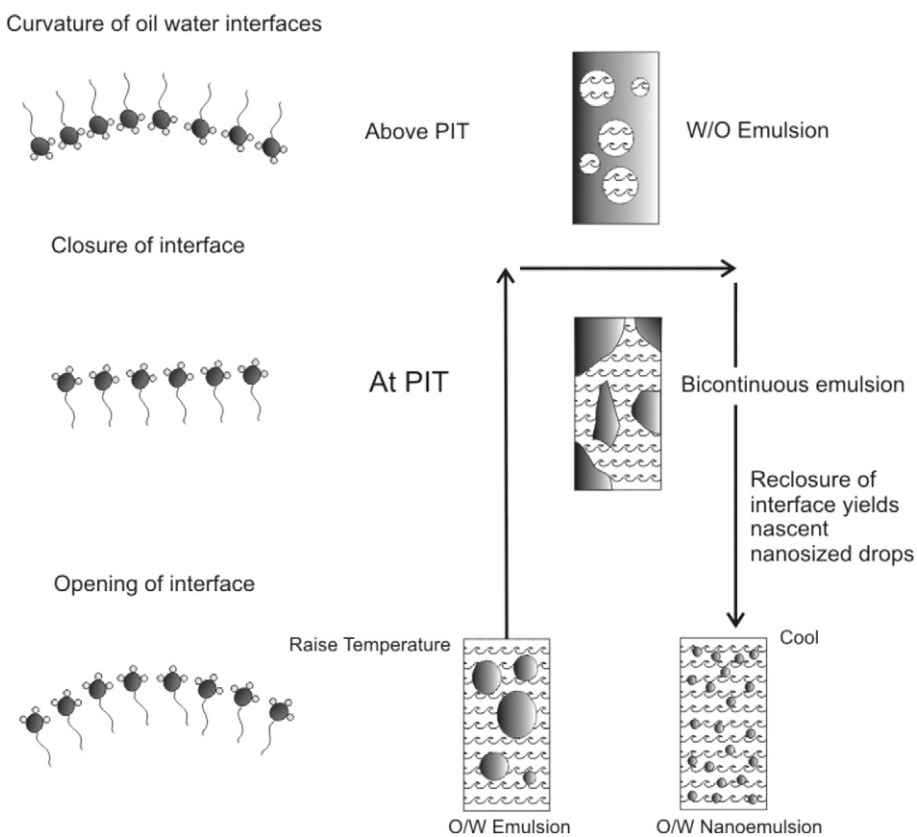

Fig. 6. Schematic representation of phase inversion temperature method for nanoemulsification. PIT method relies on change in optimum curvature of interfacial film or solubility of surfactants with changing temperature. Under normal circumstances, due to hydrophobic hydrophobic attraction, surfactant molecules tend to spontaneously associate with each other in water and form monolayers that have a curvature which allows most efficient packing. When this system is heated, the interfacial film starts contorting as surfactant molecules begin to dehydrate. At PIT, solubility of surfactant in the oil and water phases is approximately equal. Exceeding PIT leads to exclusion of surfactant from aqueous phase and preferential oil solubilization leading to phase inversion in allegiance with Bancroft rule. Once temperature is brought down sharply, the interfacial film opens and closes again but to form finer droplets thereby creating nanoemulsions which have long term stability.

4.2. Phase inversion method

Phase inversion temperature (PIT) methods form nanoemulsions by exploiting changes in aqueous/oil solubility of surfactants in response to temperature fluctuation (Fig. 6). It involves ordered conversion of a W/O to O/W emulsion or vice versa via an intermediary bicontinuous phase. Usually an oil, water, and surfactant blend is heated past a predetermined temperature, termed as PIT (specific for the utilized formulation blend), and then cooled rapidly. Temperature change from low to high leads to opening and reversal of interfacial structure causing phase inversion. When this is followed by rapid quenching, interfacial structure closes again trapping oil or water. This is a bottom up process and nascent droplets remain stable over a considerable period of time due to rich surfactant coverage. Since input of heat is necessary, PIT methods may rule out utilization of thermosensitive drugs. Also good mutual solubility of water, oil, surfactant and drug is a prerequisite to facilitate smooth phase transition. Any destabilization is governed by Ostwald ripening only.

5. Kinetics of drug release

Variety of stimuli, including pH, temperature change, enzyme activity and ionic strength of the surrounding media can trigger drug release from nanoemulsion droplet. Release of drug from nanoemulsion in passive cases is dictated by *Fick's first law* and is given by the following equation [79].

$$t_{1/2} = \frac{0.0585 r^2 K_{OW}}{D}$$

Where ($t_{1/2}$) is the time required for half of drug to diffuse out of oil, r is droplet radii, D is the diffusion coefficient of drug (an intrinsic property) and K_{ow} is oil water partition coefficient. Very lipophilic drugs may only be released when lipid or oily components have been digested away. Nanoemulsions however have very high oil water interfacial area in comparison to traditional emulsions and are therefore expected to be digested more rapidly. Small droplet radius of nanoemulsion also implies that diffusion times of drug across oil may be fairly rapid. Consequently, nanoemulsions are sometimes fixed in a structured vehicle such as organogel to provide a tortuous pathway delaying drug release [24]. Another technique for controlling drug release is coating of nanoemulsions by rigid and thick layers of polymer, to form layer by layer nanocapsules using emulsion as a decorative template [80]. Surfactants also modulate drug release kinetics. A tightly packed interfacial layer can serve as a barrier turning nanoemulsion into a nanoreservoir, and can act as principal rate controlling mechanism.

6. In-vitro characterization of nanoemulsion

Adherence to a strict droplet size is a perquisite whilst fabricating nanoemulsions, and size estimation is mandatorily performed following formulation. Droplet size influences many properties. Larger, more spherical drops will typically flow easier than smaller or distorted droplets which tend to stick together. Uniformity of droplet size distribution is measured by polydispersity index; nanoemulsions are generally referred to as 'monodisperse' if polydispersity index is <0.2. Particle size analyzers measure droplet radius using photon correlation spectroscopy (PCS) or laser diffraction. PCS has limitations though, in terms of overall derivable information. It sometimes misses out on smaller populations, which differ substantially from average population. It is also impossible to differentiate blank droplets (which do not possess any drug molecule), surfactant aggregates, liposomes, micelles, nanoparticles or one colloidal form from other. Additionally, shape of oil droplets is taken as a perfect sphere which is not always the case. Furthermore, dilution of a sample is often required, which alters its native state. Therefore, for exact visualization (globule size, volume fraction, shape,) electron microscopy (SEM, TEM, cryo-TEM, freeze-fracture), neutron and X-ray scattering are applied to substantiate data obtained via PCS. SEM produces considerably deep two dimensional images and is beneficial in identifying topography, contours and morphology of a droplet. Freeze-fracturing can add value to SEM data, wherein rapidly frozen nanoemulsion droplets are ruptured open, to observe their interior which allows exact identification, localization and capturing of relative symmetry of constitutive lipids, surfactants, and co-surfactants [81]. To study real time droplet dynamics, nanoemulsions are fixed in glassy solids undergoing phase transition and then visualized under an electron microscope or directly analyzed using small-angle X-ray scattering [1]. TEM is also employed for observing nanoemulsion characteristics. It gives a wholesome picture, since it can capture coexisting structures (such as coated droplets, blank vesicles, any deviation from spherical structure, presence of aggregates, etc.), and microstructural details of the nanoemulsion itself. Grapentin et al. have comparatively utilized PCS and cryo-TEM to evaluate stability of perfluorocarbon nanoemulsions for up to one year. Results suggested that PCS alone produced misleading results with respect to stability however its combination with cryo-TEM gave greater insight into evolution of droplet dynamics, with both techniques assisting one another [82]. Optical characterization tools further include refractive index measurements, static light scattering, diffusing-wave spectroscopy etc. Refractive index of a developed nanoemulsion (measured by a refractometer) attains special significance when it is intended for ophthalmologic administration. Ophthalmic formulations should be as transparent as possible, to preclude any discrepancy between normal and post administrational patient vision. Diffusing-wave spectroscopy is used to analyze thick concentrated samples as it is not constrained by multiple light scattering. Zeta Potential is used for gauging charge on nanoemulsion surface, which provides clues towards its long term stability and in some cases interaction with target matrix. It is determined indirectly using principle of electrophoretic mobility. As a rule of thumb zeta potential values > +30 mV or <−30 mV are considered as good indicators of long term stability. Nanoemulsions with lower zeta potential may eventually aggregate and even phase separate. Manipulating zeta potential is therefore a method of enhancing emulsion stability. Using a combination of zeta potential measurements, absorption spectroscopy, and fluorescence quenching it is possible to delineate conformational changes occurring in the molecules making up a nanoemulsion with respect to alterations in drug loading pH, temperature and other processing variables [83].

7. Stability in nanoemulsions

7.1. Droplet aggregation

Instability affecting nanoemulsion is usually mediated by droplet aggregation which causes growth of droplet size, implying that all special characteristics imparted due to nano scale are lost. Greater aggregation may ultimately result in phase separation which causes irreversible damage. Working with classical Newtonian mechanics an empirical approach towards energetics driving aggregation process has been drawn [84]. Overall interaction (I_E) between droplets can be described by a sum total of van der Waals (I_{VDW}), electrostatic (I_{el}), hydrophobic (I_h) and steric (Is) interactions occurring in between droplets, although there are be numerous other factors also which influence level of interaction.

$$I_E = I_{VDW} + I_{el} + I_h + I_s$$

Whilst designing a nanoemulsion, interaction (I_E) between droplets should be reduced to a minimum by calibrating all the factors stated above and plotting a potential energy curve against inter-droplet distance. The minimum in this potential energy curve coincides with distances to which any two droplets could approach each other without

interacting and can give a crude idea of the overall stability of nanoemulsion. As a rough guide, higher zeta potential is a guaranteed indicator of stability.

7.2. Ostwald ripening

Ostwald ripening (OR) is a diffusive phenomenon, which leads to growth in droplet size; coarsening of emulsion and ultimately phase separation. Ostwald ripening is loosely interchangeable with flocculation and is especially evident in nanoemulsions. In Ostwald ripening, larger droplets grow at the expense of smaller ones (Fig. 7).

Ostwald ripening is driven by magnitude of Laplace pressure, Π_L, which is pressure difference across a curved interface. $\Pi_L = 2\ \gamma/a$; where γ is the surface or interfacial tension and 'a' is the surface area of droplet. In case of an individual droplet, whose surface area has been reduced due to size reduction, Π_L jumps through the roof and sets up a chemical gradient. Ostwald ripening of nanoemulsions can therefore be very rapid in comparison to regular emulsions/dispersions as differences in Laplace pressures are much greater [67]. Kinetics of Ostwald ripening has been explained on the basis of Lifshitz, Slezov, and Wagner theory (LSW theory). LSW theory suggests that, in an emulsion consisting of a single oil type with quantifiable aqueous solubility, the number average radius of a droplet undergoing Ostwald ripening increases with cube root of time. It is numerically elaborated as follows [85].

$$\omega = \frac{dr^3}{dt} = \frac{8}{9}\ \frac{C_\infty\ \gamma V_m\ D}{\rho RT}$$

here ω is the rate of Ostwald ripening, R is universal gas constant, T is the ambient temperature, γ is the interfacial tension at oil water interface, r is droplet radii of nanoemulsion, V_m is the molar volume of oil employed, C_∞ is saturation solubility of oil and D is the diffusion coefficient of oil droplets in continuous phase. Physical stability of nanoemulsions is assessed by measuring nanoemulsion size as a function of time using PCS. Ostwald ripening rates are obtained by plotting slopes of r_N^3 vs. time, where r_N is number average radius. Ostwald ripening can be purported as a primary destabilizing mechanism for nanoemulsions, if the so called, Ostwald plot (dr^3 versus time), derived from above equation, turns out to be linear [86].

7.2.1. Prevention of Ostwald ripening

Theoretically, employing an oil phase which has very low aqueous solubility can prevent Ostwald ripening forever. This however is not always feasible, and in practice, lipidic blends of MCT and LCT are employed to increase complexity of formulation in order to stall Ostwald ripening. One such approach is the trapped species method, wherein, a susceptible dispersed phase is trapped inside a normally Ostwald ripening insensitive phase. Internal osmotic pressure created by the trapped species counters Laplace pressure and reduces coarsening of nanoemulsion [87]. Delmas et al. have shown that Ostwald ripening of nanoemulsions, can be completely stopped even at very high temperatures by adding wax to the normally used oil blend of mono-, di-, and triglycerides [88]. The trapped species method though effective in reducing Ostwald ripening, limits size reduction possibility due to presence of an immobile species within the droplet whose size is being reduced. In order to overcome this drawback, another method namely evaporational ripening has been developed. Here, an O/W emulsion is formulated using an oil phase that typically consists of a polymer dispersed in a highly volatile solvent. When such a system is heated the polymer solvent vaporizes, leaving behind concentrated polymer droplets. Evaporation of volatile solvent provides an infinite sink to overcome internal osmotic pressure created by dense polymer droplets. Emulsion thus becomes finer with passage of time and OR can be effectively avoided for years [89]. Nam et al. in their efforts to prevent OR demonstrated that O/W nanoemulsions can be successfully stabilized by usage of surfactants which form a physically robust interphase. They used amphiphilic block copolymer poly (ethylene oxide)-poly (ε-caprolactone) (PEO-b-PCL) which is soluble in oil phase at higher temperatures, but recrystallizes as soon as the system is bought back to ambient temperature and prevents size growth due to Ostwald ripening [90].

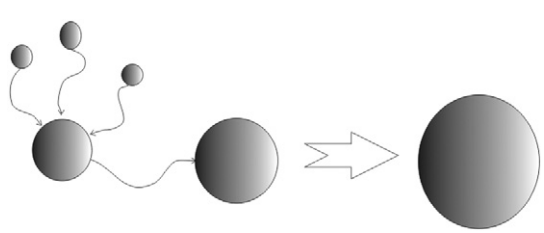

Fig. 7. Pictorial depiction of how Ostwald ripening leads to growth of droplet size. Smaller oil droplets have greater solubility than larger droplets and consequently they keep disappearing from dispersion only to desolvate on the surface of larger droplet. Nanoemulsions have prodigious capability to undergo Ostwald ripening due to their finer dimension.

7.3. Coalescence

Another aspect which creates instability in nanoemulsion is coalescence of droplets. Ostwald ripening and coalescence act simultaneously to accelerate destabilization of nanoemulsions. Coalescence is borne out of kinetic phenomena such as creaming, sedimentation and sometimes even random thermodynamic fluctuations which promote segregation, attachment or impingement of dispersed phase droplets. Sedimentation and creaming are reversible as droplets can be re-dispersed by simple shaking but coalescence leads to irreversible emulsion damage. Rate of sedimentation in an emulsion is governed by Stoke's law.

$$V_S = \frac{2(\rho_c - \rho_d)r^2 g}{9\eta_c}$$

where V_S is the terminal velocity of settling drop, ρ_c and ρ_d are individual densities of the continuous phase and dispersed phase respectively, g is acceleration due to gravity, r is radius of the droplet whose settling velocity is being monitored and η_c is viscosity of continuous phase. Fig. 8 depicts the actual processes which takes place in coalescence of two droplets to yield a droplet of larger size.

It can be inferred from Stoke's law that nanoemulsions with smaller radii should be resistant to destabilization phenomena such as sedimentation or creaming. Yet, coalescence is found to be a contributing factor in destabilization of nanoemulsion structure. It can be avoided by using a more hydrophilic surfactant, which tends to form a thicker hydration layer around interface, increasing its elasticity (it resists interface breakage and subsequent droplet attachment), or using a charged surfactant which can provide an electrostatic stabilization apart from the usual steric stabilization.

7.4. Thermodynamic, centrifugation, pH dependent and rheological stability studies

Apart from regular stability and accelerated stability studies prescribed by ICH, nanoemulsions are subjected to special thermodynamic stability studies to ensure droplet integrity in case of temperature fluctuations. These tests include consecutive heating and cooling of nanoemulsions where they are exposed to multiple cycles of refrigeration (4 °C) and heat (45 °C). Nanoemulsions may be subjected to freeze- thaw cycle and monitored for size or phase changes. Kinetic instability such as creaming, settling or any other form of phase separation is ruled out by centrifugation of nanoemulsions at 3000–4000 rpm. Long-term deliberations include storage stability studies where nanoemulsions are stored in ambient or refrigerated conditions over a

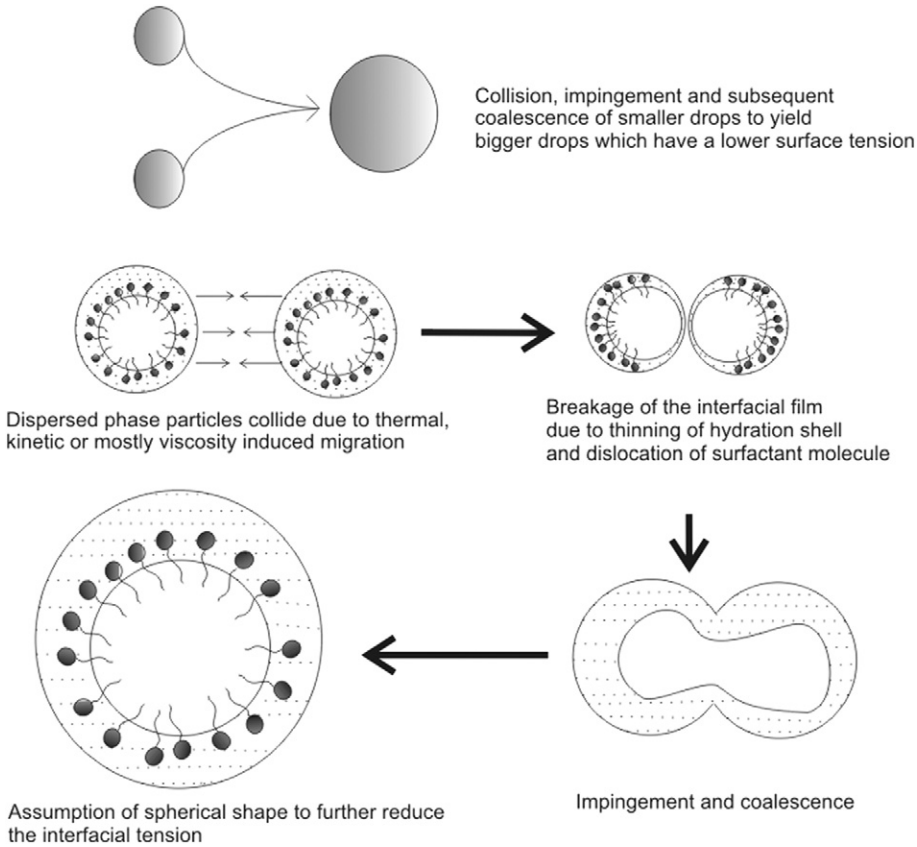

Fig. 8. Dynamics of droplets coalescence in nanoemulsions. Droplets whilst undergoing gravity, temperature, viscosity or sometimes *brownian motion* induced translocation have propensity to collide. If the collision is of sufficient magnitude these droplets actually impinge and attach forming loose floccules. These floccules re-disperse immediately when shaken but if allowed to stick for prolonged duration result in coalescence of smaller droplets to form coarser droplets. Phase separation in formulation almost always proceeds via coalescence and it consequently needs to be checked to ensure long term stability of nanoemulsions.

period of 3–6 months and evaluated periodically for their appearance, size, dispersity, zeta potential, drug content etc. Variations in said parameters does not necessarily indicate instability if they are within a prescribed range, however significant differences if present should be reported and used as a guiding principle for framing storage conditions or if required reformulating a stable formulation. Changes in pH often dictate in vivo amicability (painless administration of intramuscular injections, low irritancy of topical or ophthalmologic nanoemulsions is also dependent on formulation pH) and stability of housed drugs. Ramipril is sensitive to alkaline pH, and consequently its nanoemulsion is prepared and maintained at an acidic pH. Other examples include size variation, charge reversal, droplet fluidization, etc. induced by pH change. Therefore establishing pH requirements of a nanoemulsion system acquires significance. Nanoemulsions with a larger internal phase volume generally have higher viscosities. In order to transfer such systems, application of huge shear is necessitated which sometimes leads to rupturing of droplets. This may increase systemic viscosity even further by jamming smaller droplets in between larger droplets. Viscosity of nanoemulsion therefore requires prior calibration and should be evaluated at different shear rates at different temperatures using rotary or cone and plate viscometers.

8. Fate of nanoemulsion: in vivo

8.1. After oral ingestion

Upon oral administration, nanoemulsions enter gastrointestinal tract (GI tract) and are subjected to variety of environmental conditions. Persson et al. have come up with a theory that postprandial response is stimulated at least partially in such cases [91]. Stimulation of 'lipidsensing' mechanism in GI tract leads to secretion of gastric lipases which start fractional digestion of LCT or MCT making up the nanoemulsion, to yield simpler di-glycerides, mono-glycerides and free fatty acids. Small size of nanoemulsion droplets accelerates this lipase activity. Digestion of oily component frees up drug which usually undergoes nanoprecipitation. In other instances drug may just partition out of oil droplet into surrounding aqueous environment. Presence of oils and oil digestion products in GI tract stimulate secretion of bile and delay GI tract motility. Components of bile aid in solubilization of nanoemulsions by acting as endogenous surfactants and may form colloidal structures known as mixed micelles. Bile and preexisting mixed micelles further solubilize free drug and carry it across aqueous unstirred diffusion layer for absorption (Fig. 9).

Nanoemulsion droplets are sometimes absorbed intact via paracellular or transcellular pathways, or via M-cells present in *Peyer's* patches. Additionally collisional absorption also occurs, which involves accidental impact absorption of nanoemulsion droplet. Due to flexible nature of droplets, nanoemulsions tend to stick and squeeze through absorption barrier, bending and changing their contours according to gaps available in the packed bilayer [92]. Certain excipients such as tocopheryl polyethylene glycol 1000 succinate (TPGS) [93] and Labrasol® [94] used in formulating nanoemulsions have unique ability of inhibiting ATP dependent p-glycoprotein (P-gp) transporter and have been exploited to increase oral bioavailability of poorly soluble anticancer drugs like paclitaxel [95]. After absorption, nanoemulsion droplets may either enter systemic circulation via hepatic portal vein or alternatively be trafficked into perforated lymphatic endothelium. Drugs which enter mesenteric lymph are directly transported to systemic

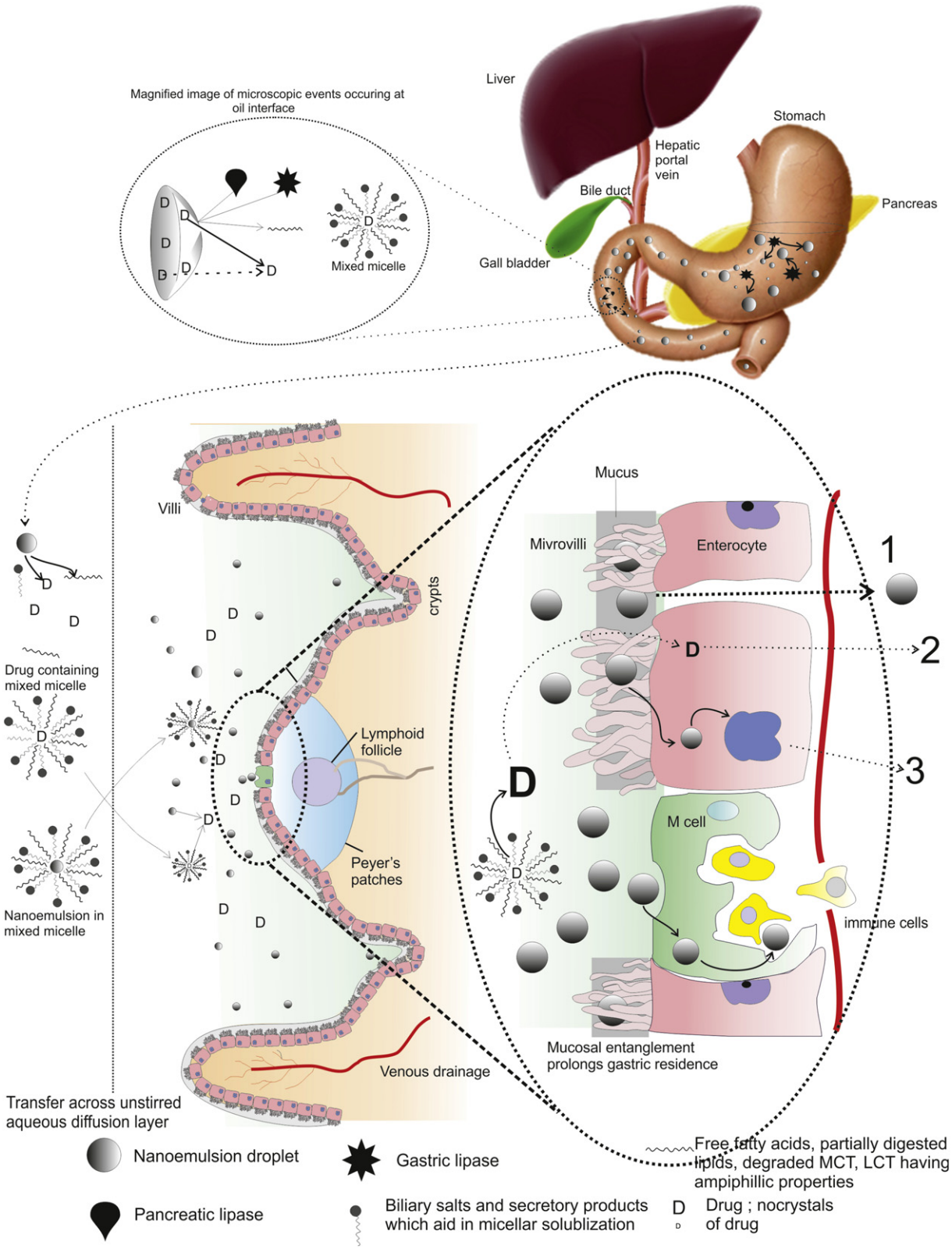

Fig. 9. An insight into events occuring inside GI tract which lead to absorption of nanoemulsion or its cargo. A nanoemulsion when administered orally might stimulate variety of lipid sensing mechanisms, which in turn induce secretion of gastric and pancreatic lipases and bile salts. Llipases digest oil component of nanoemulsions into simpler fractions accelerating drug release. Drug released in GI cavity is either precipated in nanomeric form or incorporated inside mixed micelles formed by liberated oil fraction or available bile salts. In either secenario intrinsic solubility of drug is enhanced and chances of it traversing the aqueous unstirred diffusion layer in vicinty of absorptive lining is raised several folds. (1) Thereafter a drug may be directly absorbed via conventional lipid solubilization and partitioning phenomena and become systemically available as per its biopharmaceutical properties which dictate preferential venous or lymphatic entry. A partially digested nanoemulsion droplet, or in cases where the oil is lipase resistant, an intact droplet might also be solubilized in a mixed micelle. (2) The droplet may exploit specific or non specific uptake mechanisms like paracellular or trancellular pathways, mucosal entanglement or enter M cells or other absorptive cells. (3) Once inside the absorptive cell, nanoemulsion droplet may be processed into apolipoproteins and channeled into lymphatic drainage.

circulation without undergoing hepatic first pass metabolism [92]. Therefore numerous mechanisms work in unison to offer several pathways which alter oral bioavailability of poorly available drugs when they are administered via nanoemulsion.

8.2. Fate of nanoemulsion upon intravenous administration

Following intravenous administration, nanoemulsions may be stimulated by continuous turbulence of ubiquitous hydrostatic drag of blood (which provides an infinite sink) to release their dissolved drug content. If released and solubilized, drug travels far and wide, extravasating with blood into various organs (Fig. 10). Sometimes nanoemulsions continue to circulate as foreign colloidal entities interacting with plasma proteins, red blood cells and circulatory immune cells (platelets, monocytes, leukocytes) [96, 97]. Erythrocytes form a major component of blood and thus may be susceptible to lysis if constituents of nanoemulsion droplet possess membrane disruptive action. It is therefore advisable in such cases to pre-validate hemolytic potential of a formulated nanoemulsion either by employing inert excipients, or by reducing amount of component/s contributing to hemolysis (cationic surfactants increase hemolytic potential) [98,99]. Nanoemulsions are subject to opsonisation and phagocytosis by circulatory macrophages and may be trafficked to perforated reservoirs such as spleen or liver, where immune cells usually reside [62,100]. Such inadvertent accumulation proves beneficial if liver or spleen targeting is the intended purpose of administering nanoemulsions [101], however it might be unwarranted in cases where sustained or heightened plasma concentration is required [102]. Larger nanoemulsion droplets with recognizable surface charge are often phagocytosed to a greater extent than finer neutral ones [103]. If a nanoemulsion droplet escapes plasma components, it may be carried with blood and enter venuoles and arterioles lining different organs, where, if size permits, they may gain access to interstitial fluid and finally to the cell type making up that organ. At cellular level, nanoemulsion droplet may be taken up via differing mechanisms: endocytic, phagocytic, pinocytic etc. [104] Once inside, droplets may be digested in lytic environment of vacuole, cytoplasm, etc. [105] to produce a small concentration flux which either acts on the molecular target of drug or facilitates back diffusion of drug into systemic circulation (albeit to a very small extent). This explanation accounts for passive targeting offered by nanoemulsions, which is accentuated in case of leaky vasculature lining up a tumor microenvironment and allows for nonspecific accumulation of nanoemulsion in its vicinity. For active targeting purposes, decoration of nanoemulsion surface has been attempted. Cholesterol-rich nanoemulsion droplets tend to acquire apolipoprotein character due to interaction with plasma proteins and readily bind to low-density lipoproteins (LDL) [106]. This characteristic has been exploited to target LDL receptor overexpressive cancer cells for delivery of several cytotoxics (carmustine, paclitaxel, and etoposide) [107–109]. Ye J et al. have published a series of reports describing a nanoemulsion containing paclitaxel cholesterol complex to target LDL overexpresssive triple negative and non-triple negative breast cancer. Their investigation into cellular uptake mechanisms revealed that developed nanoemulsion after intravenous administration was internalized by cancer cells through LDL receptor mediated pathway via clathrin-coated pits and pooled into lysosomes, and thereafter released into cytoplasm, consistent with internalization pathway and intracellular trafficking of endogenous LDL [110,111].

Ultimately nanoemulsions may undergo hepatic, renal or biliary clearance depending on their size and surface features. Ultrafine nanoemulsions which are sized lesser than 10 nm are subject to rapid glomerular filtration [112,113], however generalized conclusions regarding other dispositional processes is difficult to draw. We would

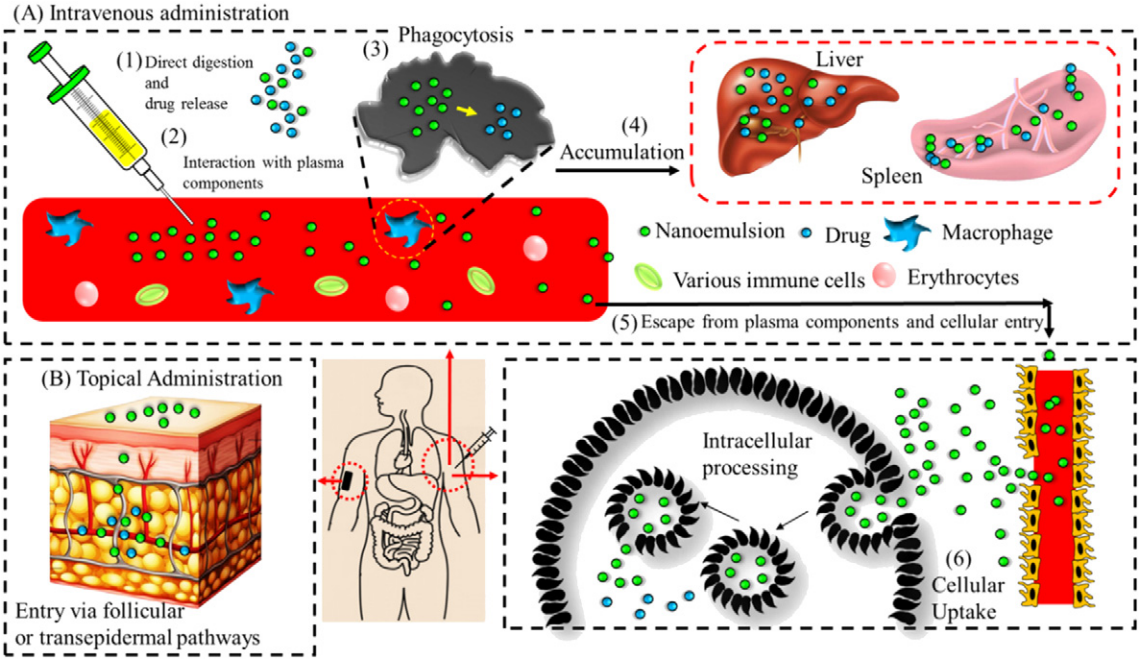

Fig. 10. (A) Fate of nanoemulsions after intravenous and (B) topical administration. (1) Post intravenous entry, a nanoemulsion under the turbulence of hydrostatic drag offered by blood might release its payload (which as per drug's intrinsic solubility and biopharmaceutic tendency will disseminate to its preferred site of residence). (2) Almost simultaneously the droplet also interacts with cellular entities making up plasma. (3) & (4) Depending on their size and charge, nanoemulsions might be preferentially phagocytosed leading to their hasty clearance from blood and accumulation into perforated organs. (5) If nanoemulsion droplets manage to avoid phagocytic uptake, they enter interstitial fluid lining up the capillaries and from there gain entry via specialized uptake pathways into cells making up the target region. (6) A nanoemulsion droplet may further be processed by intracellular machinery to empty its drug content.(B)Nanoemulsions when applied topically utilize either the transfollicular route or transepidermal route to deliver drug to varying depths of skin or into systemic circulation.

like to summarize by stating that trends drawn in the current section are by no means absolute and in some cases may be an extrapolation of the principles which apply to other nanocarriers.

9. Applications of nanoemulsion

9.1. Parenteral nanoemulsions

Parenteral nanoemulsions have varying applications. They are used to deliver drugs with lower bioavailability and/or narrow therapeutic indices. Chlorambucil, a lipophilic anticancer agent has been administered parenterally as a nanoemulsion (fabricated using ultrasonication and high pressure homogenization method) for treatment of ovarian and breast carcinoma [114]. Tagne et al. have developed a water soluble nanoemulsion of tamoxifen to increase its effectiveness in breast cancer [115,116]. TOCOSOL™ a vitamin E nanoemulsion containing paclitaxel was formulated using high pressure homogenization for treatment of various cancers like ovarian cancer, breast cancer etc. It was hypothesized that TOCOSOL™ would reduce toxic side effects of paclitaxel and it had shown great initial merit against metastatic breast cancer but unfortunately its Phase 3 trials did not meet primary endpoint [117]. This however, has not hindered further preclinical deliberations with TOCOSOL™ or undermined its formulation attributes like ultrafine (40–80 nm), neutral and stable droplets. TOCOSOL™ produces greater tumor suppression than plain drug in colon adenocarcinoma model solution and therefore warrants further exploration [117]. Taking note, our group has also attempted a slightly altered Vitamin E nanoemulsion of paclitaxel to accentuate inherent anti-proliferative and immunotherapeutic activity of Vitamin E. We found that drug loaded nanoemulsion substantially enhanced anticancer activity of paclitaxel by opening up alternative pathways of mitochondrial killing, pharmacokinetic improvement, and immunomodulation. Overall safety (measured by serum markers and organ histology) was also improved [16]. O/W parenteral lipid nanoemulsion of diclofenac has been investigated for treatment of arthritic conditions. Nanoemulsion containing diclofenac with mean droplet size of 200 nm was prepared by high pressure homogenization and ultrasonication. It was observed in vivo that diclofenac nanoemulsion provided sustained drug release allowing substantial dose reduction [118]. Nanoemulsions can be converted into stealth/long circulating nanoemulsions by coating or attaching a hydrophilic moiety such as PEG on to their surface which prevents identification, opsonisation and uptake by mononuclear phagocyte system (MPS). This can be exploited in targeting tumors by enhanced permeability and retention effect. For instance Hak et al. surface coated multifunctional nanoemulsions with PEG for its successful intravenous delivery [119]. They utilized paramagnetic and fluorescent lipids (for multimodal detection and imaging) to formulate nanodroplets which were then coated with a targeting ligand, RGD peptide, and PEG. It was assumed that PEG coating would prevent MPS uptake allowing preferential accumulation of nanoemulsion in tumor microenvironment, whereas RGD peptide would facilitate interaction of droplets with Rvβ3-integrin receptor present on tumor surface. For targeting diseases, which harbor pathogens inside macrophages like tuberculosis, leishmaniasis, MPS needs to be penetrated, pretty much contrary to principle of stealth nanoemulsions. Kansal et al. developed a layer by layer polyelectrolyte coated nanoemulsion bearing doxorubicin for intervention in visceral leishmaniasis (caused by a parasite which resides in MPS). They utilized phosphatidyl serine as a targeting ligand. It was found that phosphatidyl serine coated nanoemulsion was taken up massively by macrophages allowing selective payload delivery in deep (normally inaccessible) residence site of parasite [120]. Our group has also utilized a similar strategy to deliver amphotericin B to an intra-macrophage location via an O/W nanoemulsion template decorated with chitosan. Toxicity studies in macrophage amastigote system and efficacy study in infected hamsters suggested that developed nanoemulsion exceeded performance of marketed product (Fungizone) to augment antileishmanial property of amphotericin [80]. Nanoemulsion approach has been used to tackle drug resistance either by employing smart excipients like TPGS, or by using multi drug systems which potentiate primary anti-cancer entity. For instance Ganta et al. have coadministered paclitaxel and curcumin via a nanoemulsion to overcome drug resistance in ovarian cancer cells SKOV3. Efficacy of delivery system was established by quantitative cellular uptake studies [121]. Another group has developed a core-matched nanoemulsion using vitamin E and TPGS to co-deliver hydrophilic and hydrophobic cytotoxics, 5- fluoroucacil and paclitaxel, to overcome drug resistance in human epidermal carcinoma cell line KB-8-5 [122]. A vitamin E derivatized nanoemulsion carrying paclitaxel has been developed to tackle intrinsic or acquired drug resistance in ovarian carcinoma by inhibiting P-gp and altering levels of apoptotic and anti-apoptotic proteins, Bax and Bcl-2. Baicalein, a multidrug resistance reversal agent, has been co delivered with paclitaxel using a nanoemulsion platform to improve its in vitro cytotoxicity, cellular uptake and apoptosis [123]. Recently, 'high intensity focused ultrasound-responsive perfluorocarbon nanoemulsions' have emerged as a new class of smart multifunctional vehicles which exhibit theranostic properties and release their payload in a controlled manner [124]. Perfluorocarbons are fluorinated liquids which include perfluoropentane, perfluorohexane perfluorodecalin, perfluorooctyl bromide, perfluorotributylamine, perfluoro-15-crown-5-ether and were used mainly for liquid ventilation [125]. However fluorine-19 isotope in these fluorinated carbons enables quantitative fluorine-19 magnetic resonance imaging [83]. When stimulated ultrasonically these volatile compounds vaporize, transforming the nanoemulsion system into high-contrast microbubbles that cause drug release. There have been several studies detailing effect of acoustic and formulation parameters on contrast and effect provided by the droplets. In one such study Baghbani and Moztarzadeh synthesized perfluorohexane/alginate nanoemulsion (average size 55 nm) for co delivery of doxorubicin and curcumin to overcome multi drug resistant cancer. It was found that ultrasound irradiation significantly increased combinatorial cytotoxicity and in vivo tumor regression of doxorubicin and curcumin loaded perfluorocarbon nanoemulsion in comparison to pure drugs [126]. In another effort Rapoport et al. have formulated a paclitaxel-loaded perfluorocarbon nanoemulsion and obtained efficient tumor reversion in breast, ovarian, and pancreatic murine models under the influence of ultrasound [127]. Few examples of nanoemulsions that have been developed for parenteral use are enlisted in Table 2.

9.2. Oral drug delivery

Nanoemulsions are ideal vehicles for oral delivery of lipophilic drugs like antibiotics, hormones, steroids, cytotoxics, diuretics, antifungals etc. Nanoemulsions with their ability to coat drugs provide a platform for protecting them against hydrolytic enzymes or harsh pH and other environmental conditions. Nanoemulsions trapped in structured organogel have been developed to increase oral bioavailability of curcumin [24]. Candesartan cilexetil (CC), an antihypertensive, exhibits incomplete intestinal absorption due to its low aqueous solubility which ultimately reduces its oral bioavailability. Gao et al. have used Tween 80 and Solutol® HS-15 to develop an orally administered nanoemulsion of Candesartan cilexetil to placate this issue. Developed nanoemulsion increased peak plasma concentration of candesartan cilexetil 27 folds, whereas overall bioavailability increased 10 times in comparison to plain drug suspension. Clathrin-mediated endocytosis of nanoemulsion followed by lymphatic entry was proposed as the contributive mechanism responsible for bioavailability increment [136]. In another study Khandavilli et al. used labrasol and TPGS to develop a nanoemulsion of paclitaxel which enhanced its peroral bioavailability to >70%. Drug delivered via nanoemulsion was rapidly absorbed and steady state levels were reached within 30 min which persisted up to 18 h suggesting substantial absorption even from PgP rich distal ileal regions [95]. Nanoemulsions have also been developed using phase-inversion

Table 2
Examples of parenteral nanoemulsions.

Drug	Dispersed phase	Surfactant	Method	Purpose	Size (nm)	Ref.
Carbamazepine	Castor oil, MCT	Soy lecithin, Polyoxyl 35 castor oil, Tween 80	Spontaneous emulsification	Overcome poor solubility	150	[128]
Thalidomide	Castor oil, olive oil, soyabean oil, MCT	Twee 80	Spontaneous emulsification	Overcome poor solubility	200	[129]
Docetaxel	Oleic acid, Stearyl amine	Egg lecithin	Melt homogenization followed by ultra-sonication	Poor solubility, hydrolytic instability, and drug-induced side effects	190–230	[130]
Primaquine	Miglylol 812	Pluronic F68	Homogenization followed by HPH	Dose reduction, improved bioavailability, reduced toxicity	10–200	[101]
Fisetin	Miglylol 812, soybean oil, ethyl oleate	Labrasol, Lipoid E80	PIT method	Improved pharmacokinetics and anti-tumor activity	153 ± 2	[131]
Insulin	Self assembling protein complex	Poly vinyl alcohol	Spontaneous emulsification	Protection against enzymatic degradation	200–500	[132]
Clotrimazole	Soybean oil	Pluronic F68, cremophor, tween 20, tween 80	Spontaneous emulsification	Increased bioavailability	<25 nm	[133]
Paclitaxel and ceramide	Pine nut oil	Lipoid-80	Homogenization followed by ultrasonication	Increased cell uptake	200	[134]
Paclitaxel and Sulforhodamine B	Vitamin E	TPGS	Homogenization	Long circulation halflives increase theranostic capability	~100	[135]

method for oral delivery of protein drugs like bovine serum albumin (BSA). It was found that bioactivity, specificity and confirmational structure of encapsulated BSA was highly conserved in the system [137]. Xiaoyang Li have encapsulated insulin in a Labrafac® CC, phospholipid, Span™ 80 and Cremorphor® EL nanoemulsion by homogenization and coated it with chitosan alginate for oral delivery. Polyelectrolyte coating on nanoemulsion template provided a robust interphase capable of withstanding rigors of gastric environment. Conformity of insulin in coated nanoemulsion was established using circular dichroism. In vivo testing revealed that upon oral administration, insulin loaded nanoemulsion produced significantly greater and longer hypoglycemic effect than subcutaneously administered plain insulin solution [138]. Elsewhere, chitosan coated nanoemulsion have been grafted for enhancing oral protein absorption by exploiting mucoadhesive nature of polymer which enhances residence time of nanoemulsion droplet at absorptive site [139]. Highly active anti-retroviral therapy (HAART) when delivered via oral nanoemulsion has benefited therapy for AIDS. HAART despite its initial success is inefficient in the long run when administered conventionally. Viruses replicate and stay vital inside lymphocytes even if substantial anti-HIV drug concentration is attained in blood. Sometimes virus reservoirs are situated in deep anatomical locations like CNS which further reduces accessibility of drugs. Saquinavir (SQV) suffers from above shortcomings and acquires very low concentrations in brain. An O/W nanoemulsion of saquinavir, containing edible oil and Lipoid®-80 (100–200 nm) has been consequently developed and tested orally and intravenously in Balb/c mice. Maximum concentration and AUC values of saquinavir attained in brain were found to be five- and threefold higher for nanoemulsion than plain drug suspension, suggesting enhanced bioavailability following oral administration of nanoemulsions [36]. Some examples of drugs whose nanoemulsions have been developed for oral use are summarized in Table 3.

9.3. Topical

It is a challenge to enhance permeation of several drugs intended for topical application. These are limited by poor dispersibility in topical vehicles like gels, creams, patches or possess skin irritant action. Nanoemulsions have been explored for topical uptake of such drugs. They provide a combination of penetration enhancement (by altering lipid bilayers) and concentration gradient by acting as tiny reservoirs of drugs. For instance, a nanoemulsion (made of soybean lecithin, tween and poloxamer) containing menthol, methyl salicylate and camphor was prepared by high energy method and incorporated in a hydrogel. The resulting formulation had high permeation rates [14]. In order to achieve deep skin delivery for tackling psoriasis, a nanoemulsion containing paclitaxel has been evaluated. In vivo pharmacokinetic studies following topical application revealed very high availability of drug in the skin, with minimum systemic escape [95]. Caffeine has been delivered topically via a W/O nanoemulsion for treatment of skin related cancer. Shakeel et al. dispersed caffeine in an aqueous solution and titrated it against Lauroglycol 90, Transcutol HP and isopropanol to arrive at a thermodynamically stable and safe nanoemulsion which was sized between 20 and 100 nm. Upon testing nanoemulsion against aqueous caffeine, they observed significant increase in permeability parameters, steady-state flux and permeability coefficient [159].

Nanoemulsions can be employed to deliver small molecules systemically via topical route. In an illustrative study, an O/W nanoemulsion (made with high pressure homogenization using soybean oil, phosphatidyl choline, Tween 80) containing α, δ or γ tocopherol was compared with their respective nanosuspensions. It was observed that systemic bioavailability along with antioxidant activity of δ and γ tocopherol increased 2.5 times when they were delivered as nanoemulsion [160]. Nanoemulsions of ketoprofen and celecoxib have been prepared with adequate stability to attain high skin permeation rate. When compared against regular drug containing gel, such formulations had substantially greater permeability and transepidermal flux [161]. W/O nanoemulsions have been used to deliver proteins or plasmids via transepidermal route. Their oil component is compatible with sebum present in follicular openings which act as alternate entry points. This opens up possibility of directing and confining potentially therapeutic transgenics to clinically active skin lesions. Wu eta al developed a system carrying plasmid pCF1CAT in olive oil employing spontaneous emulsification which had following characteristics: (1) significant entrapment of highly concentrated plasmid solutions (2) physical conservation of plasmid DNA due to simple manufacturing technique; (3) extended stability and (4) an acceptable safety profile [162]. Even a hydrophilic compound like inulin can be systemically delivered 5 to 15 fold better than its aqueous or micellar counterpart using a W/O nanoemulsion. Rate and extent of inulin transport across skin is dependent on hydrophile-lipophile balance of surfactant employed and availability of trans-folicular openings [163]. Tagne et al. have topically delivered dacarbazine via a nanoemulsion in xenograft nude mice inflicted with melanoma. When compared to regular micron sized suspension (5470 nm) the said nanoemulsion (131 nm) of dacarbazine caused 10 fold greater tumor reduction [164]. Another group has also demonstrated in vivo superiority of topically administered dacarbazine nanoemulsion in an epidermoid carcinoma model [165]. Hamouda et al. have worked on a unique nanoemulsion formulation which showcases broad-spectrum sporicidal activity against several pathogens and can be used as a topical anti-microbial. They used spontaneous emulsification

Table 3
Examples of nanoemulsions intended for oral use.

Drug	Dispersed phase	Surfactant	Method	Purpose	Disease	Size (nm)	Ref
Paclitaxel	Pine nut oil	Egg lecithin	Ultra-sonication	Increase oral bioavailability	Cancer	90–120	[140]
Dapsone	Isopropyl myristate	Tween 80, Span 80	Spontaneous emulsification	Higher dissolution rate	Antifungal/antibacterial	6–11	[141]
Candesartan	Soybean oil	Tween 80	Spontaneous emulsification	Increase oral bioavailability	Hypertension	35.5 ± 5.9	[136]
Primaquine	Miglylol 812	Poloxamer 188	Homogenization followed by HPH	Dose reduction	Malaria	10–200	[101]
Aspirin	Propylene glycol monolaurate	Cremophor	Ultra-sonication	Minimize adverse effects		–	[142]
Ramipiril	MCT	Tween 80	Spontaneous emulsification	Improve its solubility, stability and oral bioavailability	Hypertension	70–90	[143]
Silymarin	MCT	Tween 80	Spontaneous emulsification	Increase oral bioavailability		80–100	[144]
Colchicine	Isopropyl myristate	Tween 80	Spontaneous emulsification	Overcome poor water solubility, poor permeability, and rapid metabolism		41.2 ± 7.2	[145]
Avanafil	Labrafil, labrafac, miglyol 812 N	Tween 80 and cremophor EL	Direct trituration	3.2-fold increase in oral bioavailability	Erectile Dysfunction	13.89–25.67	[146]
Curcumin	Castor oil	Cremophor RH-40, Tween 80 and Pluronic F-68	Vortex and sonication	Increase in oral bioavailability for improved anti -cancer activity	Metastatic breast cancer	83.27	[147]
Cyclovirobuxine D	Oleic acid	Solutol SH15	Spontaneous emulsification	Improving bioavailability of poorly water-soluble drug	Arrythmias	64.80 ± 3.58	[148]
Piroxicam	Soybean or coconut oil	Tween 80	Vortexing followed by filtration	To improve dissolution, absorption and therapeutic efficacy of drug	Inflammatory diseases	30–100	[149]
Plasmid pcDNA3-EGFP	HIV-1 Tat peptide-oleoyl conjugate dissolved in Capmul MCM	Cremophor EL	Spontaneous emulsification	Investigate whether cell-penetrating peptides could amplify cellular uptake of plasmid DNA (pDNA) loaded nanoemulsions	–	35.5 ± 8.37	[150]
Dabigatran etexilate	MCT	Cremophor® RH 40	Spontaneous emulsification	For improving the dissolution and oral bioavailability of BCS class IIb drugs	Stroke and thromboembolism	−300	[151]
Repaglinide	Sefsol 218	Tween-80	Aqueous titration	Nanoemulsion as a carrier for persistent hypoglycaemic effect	Diabetes	76.23	[152]
Paclitaxel	Capryol 90	Tween 20	Spontaneous emulsification	Development of a safe oral formulation	Cancer	~200	[153]
Evodiamine–phospholipid	Ethyl oleate	Cremophor EL	Spontaneous emulsification	Improved absorption and in vivo pharmacokinetics	Variety of lifestyle ailments	30–90	[154]
Artemether	Coconut oil	Span 80	Ultrasonication	Improved oral bioavailability	Malaria	79	[155]
Insulin		Cremophor RH40	Homogenization in a tube rotator (Intelli-Mixer™)	Enhancing oral absorption and efficacy of insulin	Diabetes	30–100	[156]
Breviscapine	Ethyl oleate		Magnetic stirring	Enhancement of oral bioavailability	Platelet aggregation and ischemia	45.6	[157]
Docetaxel	Soybean	Lecithin, Pluronic F68	Hot homogenization followed by ultra-sonication	To develop stable, effective and safe alternative	Cancers	233.23 ± 4.3	[158]

Table 4
Examples of nanoemulsions developed for topical application.

Drug	Dispersed phase	Surfactant	Method	Purpose	Size (nm)	Ref.
Blank	Snake oil	Soyabean lecithin	Ultraturrax followed by HPH	Investigation of topical delivery potential	75–300	[170]
Turmeric oil	Turmeric oil	–	Spontaneous emulsification	Same as above against psoriasis	20–200	[171]
Lipophilic drugs	Soybean oil	Soyabean lecithin, Tween 80, poloxamer 407	Ultraturrax then HPH	Enhancing penetration	–	[14]
Tributyl phosohate	Soybean oil	Triton × 100	Spontaneous emulsification	Novel effective topical biocide	400–800	[172]
Ceramide	Sphingolipid	Lipoid	Ultraturrax followed by HPH	Alter skin permeability	210 ± 18	[173]
Antisense oligonucleotide	MCT	Lipoid E-80 Poloxamer188	HPH	Prevention of degradation up to 72 h	95	[174]
Nimesulide	Caprylic/capric triglyceride	Span 60	Spontaneous emulsification	Release of drug in viable layer of the skin	202–277	[175]
Tranexamic acid	Water	Tween 80	Spontaneous emulsification	Transfollicular transport		[163]

of soybean oil and tributyl phosphate in an aqueous external phase containing triton X to attain a droplet size in the range of 400–800 nm [166]. X8W60PC is yet another topical nanoemulsion, which has been devised for rapid antifungal action (within 15 min). Its spectrum includes *C. albicans*, *C. tropicalis*, *M. gypseum*, *T. mentagrophytes*, *T. rubrum*, *A. fumigatus* and *F. oxysporum* [167]. Certain nanoemulsion vehicles like glycerol monooleates (Peceol™), Caprylocaproyl macrogol-8 glycerides (Labrasol), possess innate antifungal activity and therefore potentiate topical antifungal activity of poorly dispersible compounds like amphotericin [168]. Recently a novel technique for delivering antigens from Salmonella enterica has been attempted via a topical nanoemulsion. Application of the said nanoemulsion onto bare skin induced a quantifiable antibody response which was significantly greater than other topical vehicles. A combination of occlusion, penetration enhancement and flexibility towards adapting transepidermal and transfollicular route were held responsible for effective delivery of antigen to immune inducer sites [169]. A brief list of nanoemulsions intended for topical use is given in Table 4.

9.4. Ocular and pulmonary drug delivery

Ocular administration of O/W nanoemulsions has been attempted to deliver water incompatible, environmentally sensitive, poorly absorbed (via trans corneal route) or poorly retained drugs. Special consideration is accorded to transparency, viscosity and refractive index of nanoemulsions whilst devising them for ophthalmic applications. Any nanoemulsion intended for ocular administration should always be titrated against different doses to evaluate its tolerability. Some drugs like antisense oligonucleotides which have shown great therapeutic potential at in vitro level in variety of ailments, but are limited by their dispositional susceptibility in vivo, are good candidates for localized delivery via nanoemulsions. Hagigit et al. have managed ocular antisense delivery using a cationic nanoemulsion made of DOTAP (a cationic lipophilic transfection agent) for treatment of retinal neovascularization. DOTAP forms a complex with negatively charged antisense nucleotide and helps in penetrating negatively charged biological membrane which otherwise is not possible due to electrostatic repulsion. This cationic nanoemulsion (zeta potential $+56 \pm 2.6$ mV) with a droplet diameter of 95 ± 2 nm was adjusted to pH 7.4 to create a formulation which was ideally suited for ophthalmic delivery [176]. Temperature sensitive ophthalmic nanoemulsions, which convert into gel upon administration have been developed to improve permeability, retention time and overall ocular bioavailability of loteprednol etabonate. Different blends of Capryol 90, tween 80 and transcutol were titrated against aqueous Poloxamer 407 solution to form a corneal temperature sensitive sol gel transition system which extended mean residence time of drug considerably compared to its marketed formulation [177]. A nanoemulsion made by spontaneous emulsification of triacetin, isopropyl myristate, ethyl alcohol and Tween 80 has been found capable of increasing solubility, ocular retention and permeability of lutein. The drug is effective in macular degeneration but suffers from limited ocular retention and consequently warrants repeated administration [178].

Pulmonary route is an important means of drug administration. Pulmonary delivery has been envisaged for direct lung delivery of amphotericin B to treat pulmonary aspergillosis. To substantiate the purported hypothesis, amphotericin B was sonicated into two commercially available nanoemulsions (Intralipid® or Clinoleic®). Nanoemulsions of amphotericin B when delivered by an aerosol (developed using Pari Sprint jet nebulizer) enhanced lung deposition and pulmonary retention of drug. Additionally, first pass metabolism and systemic escape was also avoided which further improved therapeutic efficacy [11].

9.5. Intranasal drug delivery

A major hurdle in targeting brain is presence of blood brain barrier (BBB). It restricts entry of hydrophilic and high molecular weight molecules like peptides. However, olfactory vein in nasal mucosa provides a direct passage between nose and brain. This has been exploited by use of nanoemulsions loaded with anti-Alzheimer's, anti-parkinsonism, anti-psychotic drugs for targeting brain. Risperidone, an antipsychotic, exhibits low bioavailability due to extensive first pass metabolism. This warrants administration of huge doses, which brings about numerous side effects. To reach the brain in effective concentrations and to avoid any unnecessary side effects a strategy involving nanoemulsion has been implemented; that improves bioavailability by preventing first pass metabolism and facilitating blood–brain barrier transport. Risperidone was dissolved in capmul MCM, tween 80, transcutol and propylene glycol to (48%, w/w) to form an O/W nanoemulsion spontaneously. Ultra-fine globule size of the developed nanoemulsion (15.5–16.7 nm) ensured quick and effective risperidone delivery to brain following intranasal administration [179]. Nanoemulsions as a formulation unit have been employed as carriers for antigen presentation (vaccines) to dendritic cells either infiltrating or lying underneath the epithelial cell lining of nasal mucosa. Following enhanced intracellular uptake, and processing, antigen loaded nanoemulsion induce migration of stimulated dendritic cells to regional lymph nodes within a day. Das et al. have encapsulated an immunoadjuvant in an intranasally-deliverable O/W nanoemulsion (W805EC nanoemulsion) to complement an inactivated influenza vaccine. W805EC nanoemulsion adjuvant generated a strong immune response providing advantages over parenteral vaccination [180]. Orzechowska et al. have showed that epithelial cell death induced by W805EC nanoemulsion might have a role in its capability to enhance antigen uptake and presentation [181]. Yadav et al. have encapsulated a siRNA directed against TNF α in a cationic nanoemulsion for intranasal brain delivery to treat experimental neuroinflammation. The said nanoemulsion had greater transfection capability than commercially available transfection reagents and upto fivefold greater brain uptake than naked siRNA [182]. Few examples which showcase intranasal utility of nanoemulsions are mentioned in Table 5.

9.6. Nanoemulsions in commercial and clinical pipeline

Nanoemulsions available commercially and those undergoing clinical trials are pondered upon in this section. Nanoemulsions have been developed for total parenteral nutrition. Oil content (soy bean oil, egg phospholipids, peanut oil, glycerin, fat soluble vitamins) present in such parenteral nanoemulsions act as alternative sources of energy and supplementation to meet daily requirements of fat-soluble vitamin A, D_2, E & K_1 in critical patients who cannot consume fats orally (Intralipid®, Vitalipid® developed by Fresenius kabi). Dexamethasone palmitate a steroid used in treatment of allergic disorders, and skin conditions has been made available as a nanoemulsion (Limethasone®) by Mitsubishi Pharmaceuticals for benefit in rheumatoid arthritis. Flurbiprofen axetil, a non-steroidal analgesic, also indicated in rheumatoid and osteoarthritis arthritis is administered as a nanoemulsion too. In fact two competing products (Ropion and Lipfen) have been launched by Kaken Pharmaceuticals and Green Cross respectively. Another nanoemulsion manufactured by Mitsubishi Pharmaceuticals, bearing Alprostadil is available as Liple® for intravenous vasodilation in erectile dysfunction. Clevidipine a calcium channel blocker, with extremely short distribution and termination half-life is given as a nanoemulsion (Cleviprex® by The Medicines Company). Clevidipine is practically insoluble in water, but its nanoemulsification in soybean oil, egg phospholipids and glycerin yields an ultrafine milky dispersion which can be diluted infinitely and administered as a slow infusion capable of attaining therapeutic concentrations despite short half-life of drug. Astra Zeneca has developed a nanoemulsion Diprivan that contains 10 mg/ml propofol for inducing general anesthesia. Diazepam is

Table 5
Nanoemulsions employed in intranasal drug delivery.

Drug	Dispersed phase	Surfactant	Method	Purpose	Size (nm)	Ref.
Paliperidone	Labrafil M1944CS	Tween 80, Tween 20	Spontaneous emulsification	Compare efficiency of nanoemulsion and aqueous micelle system in schizophrenia	38.25	[183]
Selegiline	Grape seed oil	Solutol®, Labrasol®	High energy emulsification	Direct nose to brain delivery for improved bioavailability	60	[184]
Fluoxetine HCl	Capmul MCM	Labrasol®	Spontaneous emulsification	Selection and optimization of excipients for intranasal delivery	–	[185]
Quetiapine	Capmul MCM	Tween 80	Vortex and sonication	Higher transport efficiency and direct nose-to-brain transport	144 ± 0.5 nm	[186]
Paroxetine	Capmul MCM	Solutol HS	Spontaneous emulsification	Direct nose-to-brain transport, permeation enhancement	58.47 ± 3.02	[187]
Saquinavir mesylate	Capmul MCM	Tween 80	Spontaneous emulsification	Improved brain targeting of HIV, penetration of BBB	176.3 ± 4.21	[188]
Olanzapine	Capmul MCM	Tween 80	Spontaneous emulsification	Enhanced nasal diffusion and brain bioavailability	–	[189]

delivered via an intravenous nanoemulsion under the trade name Diazemuls (by Kabipharmacia). Kemira, a cosmetic company and its sister corporation TRI-K industries have come up with a NanoGel system which can accommodate variety of active constituents. They claim that NanoGel not only enhances penetrability of active compound but also minimizes epidermal water loss and keeps the skin moisturized for a longer period of time.

Biolipid B2® is a transdermal nanoemulsion that can be applied directly to the skin by a metered pump to deliver hormones systemically [190]. It has been used as vector for testosterone in middle aged men and women and has completed phase 2 trials in treating loss of libido. A topical 3% diclofenac nanoemulsion gel developed by Pharmos has successfully undergone phase 2 trial in older patients with osteoarthritis of knee. It is intended for multiple daily applications with the important benefit of sparing gastrointestinal lining from NSAID induced ulcers. BF-200 ALA (Ameluz®; Biofrontera AG) is an approved nanoemulsion-based gel containing 7–8% ALA (5-aminolevulinic acid: a photodynamic therapy precursor) for treatment of precancerous keratosis. It is also under phase 2 and phase 4 investigation for basal cell carcinoma and Lentigo maligna treatment. Owing to its greater lipophilicity, BF-200 ALA has deeper skin penetrability than the more hydrophilic bioactive ALA [191]. Daylight-mediated photodynamic therapy using BF-200 ALA is also effective in thin, grade I actinic keratosis [192]. A new breed of LDL like nanoemulsions(artificial lipid nanoemulsions) made up of fixed lipidic blends despite being devoid of proteins have been found to mimic intravascular behavior of circulating apolipoproteins. A prototype (14C- cholesteryl oleate and 3H-cholesterol labeled low density lipoprotein (LDL)-like nanoemulsion) when injected intravenously, serves as an excellent probe for clearance of cholesteryl ester and free cholesterol from intravascular sites in patients suffering from diabetic dyslipidemia. Another artificial nanoemulsion, consisting of a cholesteryl ester core stabilized by a phospholipid, has the ability to bind to LDL receptor and has been used as a vehicle for paclitaxel or nucleotides [193–195]. A topical nanoemulsion labeled as NB-001 is currently undergoing phase 3 clinical trial. It is an O/W cationic nanoemulsion (180 nm) consisting of highly refined soybean oil, purified water, ethanol, EDTA and two surfactants: polysorbate (Tween) 20 and cetylpyridinium chloride (CPC). Topical administration of NB-001 at least five times a day cures Herpes Labialis. For further examples of ongoing trials readers are directed to Table 1.

9.7. Road map for industrial scalability of nanoemulsions

Whilst scaling up nanoemulsions from few milliliters to few liters or more, deviations in physicochemical characteristics of nanoemulsions (droplet size, polydispersity index, zeta potential, etc.) are induced. These occur due to inadvertent multiplication of errors in pre-set process parameters and/or incompetency of process or operators in handling larger volumes, resulting in a low quality nanoemulsion with unwanted features such as aggregation, coalescence, creaming and in some extreme cases phase inversion. In microfluidization, main parameters dictating size reduction are pressure at which homogenization of liquid mixture occurs in the interaction chamber and number of cycles or passes a liquid mixture has to go through in the mixing chamber. To get an optimized product in less number of cycles, pressure has to be alleviated, which requires greater energy input, whereas if working at lower pressures (<10,000 psi) number of cycles must be increased, which makes the process time consuming. Even if a tradeoff between number of cycles and operating pressure is attained, need for pre-mix colloid sized dispersion (usually obtained by rotor-stator colloid mill, piston gap homogenizer) as inlet feed has to be satisfied which requires further investment [196]. A microfluidizer in addition to being energy intensive due to cumbersome and multi-channeled machinery has costly maintenance issues which imply cleaning is difficult. Therefore, simplified redesign of instrument has been attempted for aseptic production of nanoemulsions [197]. Currently, MF59®: a squalene nanoemulsion, which is widely used as an adjuvant with Fluad® vaccine (Novartis) is produced by industrial scale Microfluidizer M7250 at a rate of 0.3 L/min after five passes [198,199]. Liu et al. have recently demonstrated a scale up production method of celecoxib loaded perfluorocarbon nanoemulsion using cremophor EL and pluronic p105 as surfactants [200]. Briefly, the said nanoemulsion with size < 150 nm was produced at three levels; small scale (54 ml), medium or batch scale (270 ml) and large scale (1000 ml) and was found to be stable for 90 days without any detrimental changes in size, poly dispersity index and zeta potential. For small scale preparation, the final mixture was stirred at 250 rpm for 15 min and sonicated for 1 min in ice bath before microfluidization in pre-cooled Microfluidizer (M110S) for 10 cycles at pressure 17,500 psi. For production at medium scale and large scale, microfluidizer (M-110EH-30) with high shear force was used at 15,000 psi for five cycles and three cycles, respectively. Here pre-microfluidization processing of formulation was necessary to get a good final product. For medium scale production, mixing at stirring speed 350 rpm for 30 min and sonication for 2 min while for large scale production, mixing at 600 rpm for 30 min assisted with sonication for 5 min, was required. It was notable that after approximately 35 min of pre-treatment, ultimate productivity came around 0.3 l/cycle.

Recently there has been a surge in industrially feasible ultrasonication based production of nanoemulsions too. Many formulation scientists had never anticipated this development due to shortcomings associated with conventional ultra-sonic probes i.e. small surface area of tip (10 to 20 mm diameter), reduced peak to peak ultra-sound amplitude (μ_{pp}) which results in insufficient energy per volume of liquid, concern about metal contamination of pharmaceutical product

due to tearing of titanium alloy based acoustic horns (mainly from output surface) during cavitation at high amplitude and inherent heat production which sometimes rules out thermolabile drugs [201]. Conventional ultra-sonic probes (sonotrode horns) produce a mere 25 μm peak to peak (μ_{pp}) amplitude displacement; whereas 75–100 μm peak to peak (μ_{pp}) amplitude displacement is required to get very fine nanoemulsions [202]. Even high power ultra-sonic processors with conventional horns cannot generate >40 μ_{pp} amplitude. Therefore, a sonotrode has to be designed to get high power shear force by means of large radiating surface at low or same amplitude, to handle larger volumes. Working along these lines, researchers from Industrial Sonomechanics, LLC, New York and Allied Innovative Systems, LLC, Hillsborough, USA, have come up with the Barbell Horn Ultrasonic Technology (BHUT). They have crafted barbell shaped ultra-sonic horns capable of generating extremely high ultra-sonic amplitude (>100 μ_{pp}) [202]. BHUT contains a half wave barbell horn (HBH) with two high amplitude radiating surfaces and one transitional low amplitude generating surface which generate high intensity cavitation zones both above and below the barbell tip and hence, large cumulative cavitation zone volume. BHUT (ISP3000 ultrasonic processor) when compared with microfluidization (Microfluidizer M7250) for industrial scale production of MF59® had up to 8 fold greater productivity (2.5 l/min vs. 0.3 l/min) in one fifth time period (due to single pass and continuous flow production) with 12 times lesser power requirement (3 kW vs. 37 kW). Furthermore, scale up factor number (a dimensionless quantity describing efficiency of scale up process) of 55 was achieved during process translation from lab-scale to industrial scale. Streamlined equipment footprint, low power input, a flow through process, ease of cleaning, lower time consumption and large volume handling with effective size reduction (<200 nm) has put BHUT in a strong position for industrial manufacturing set up. Moreover, ultra sonication processes are partially self-sterilizing, making them more preferable for aseptic production of parenteral nanoemulsions [203,204]. Processors equipped with special cooling jacket have also been developed to tackle the problem of heating [205]. It therefore becomes imminent that in order to iterate a manufacturing process from lab scale to large scale, an intermediary batch scale needs to be necessarily set up as a check point.

10. Conclusions

Arguments made in this review suggest increasing influence of nanoemulsions in each and every aspect of drug delivery. Nanoemulsions are 1) inherently resistant to normal destabilizing mechanisms persistent in emulsions; 2) they are usually transparent which gives them a cosmetic appeal, and 3) present many opportunities of increasing oral bioavailability of strongly lipophilic drugs. Oral delivery was the principal concept which led to development of emulsions, and it is in this aspect that nanoemulsions are especially suited. Other routes of drug delivery are equally approachable via nanoemulsions. Their minute dimensions make them special candidates for innocuous intravenous entry. Nanoemulsions are expected to progressively become center of research and development. Nevertheless many challenges still need to be overcome, in order to ensure that nanoemulsions enter mainstream pharmaceutical market and reach from a laboratory bench side to an actual patient bed side. Principal amongst them are the cost implications for scaling up nanoemulsion production, quest for nontoxic solvents in formulation, and also enhancing toxicity database available for various excipients employed in fabrication of nanoemulsions.

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