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Exploring oral nanoemulsions for bioavailability enhancement of poorly water-soluble drugs

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Introduction: More than 40% of new chemical entities discovered are poorly water soluble and suffer from low oral bioavailability. In recent years, nanoemulsions are receiving increasing attention as a tool of delivering these low-bioavailable moieties in an efficient manner.

Areas covered: This review gives a brief description about how oral nanoemulsions act as a tool to improve the bioavailability of poorly water-soluble drugs. The recurrent confusion found in the literature regarding the theory behind the formation of nanoemulsions is clarified, along with the difference between nanoemulsion and lyotropic 'microemulsion' phase. This paper gives a clear-cut idea about all possible methods for the preparation of nanoemulsions and the advantages and disadvantages of each method are described. A description of the stability problems of nanoemulsions and their prevention methods is also provided, in addition to a comprehensive update on the patents and research works done in the arena of oral nanoemulsions.

Expert opinion: Low-energy emulsification techniques can also produce stable nanoemulsions. It is guaranteed that oral nanoemulsions can act as a potential tool for the delivery of poorly water-soluble therapeutic moieties in a very efficient manner.

Keywords: aqueous solubility, bioavailability, nanoemulsion, oral delivery

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1. Introduction

Aqueous solubility and permeability are the most significant parameters affecting drug bioavailability. As per Biopharmaceutics Classification System (BCS) class II and class IV, compounds are poorly water soluble, that is, aqueous solubility less than 100 μg/ml. Class II compounds are characterized by high permeability in spite of their low solubility. BCS class IV compounds are characterized by both low solubility and poor intestinal permeability, are generally poor drug candidates (unless the dose is very low). Greater than 40% of failures in drug development are due to poor biopharmaceutical properties, mainly due to poor dissolution or poor permeability [1]. The barriers facing lipophilic drug absorption are aqueous solubility, limited absorption site, unstirred water layer, intestinal drug-metabolizing enzymes (cytochrome P450 3A4 and P-glycoprotein) and first-pass hepatic metabolism [2]. Now a lot of research is going in this field to prevent the elimination of these potent moieties taken from the drug delivery domain. The present review gives a brief description about how oral nanoemulsions (NEs) act as tool for improvement of bioavailability of poorly water-soluble drugs. The authors have tried to clarify the recurrent confusion found in the literatures regarding the theory behind the formation of NE. The difference between NE and lyotropic 'microemulsion' phase

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has been clarified. This review gives a clear-cut idea about all possible methods for the preparation of NE and the advantages and disadvantages of each method have also been described. A description of the stability problems of NE and its prevention methods is also provided. The review also put forward a comprehensive update on the patents and research works done in the arena of oral NE.

2. Mechanism of absorption of lipophilic moieties

Most of the orally administered drugs enter the systemic circulation by direct absorption into the portal blood. But highly lipophilic drug may reach the systemic blood circulation by intestinal lymphatic system. The overall bioavailability of lipophilic drug is composed of the portion absorbed through the portal blood plus the component absorbed through the lymphatic system. A study by Dahan *et al.* proved that lymphatic transport pathway has a major role in the absorption of lipophilic drugs. They investigated the absorption characteristics of vitamin D_3 followed by oral administration in rats. Collecting the lymph through a cannula implanted in the mesenteric lymph duct reduced the absolute bioavailability of the vitamin from 52 to 12.6%. It showed that 75% of the absorbed vitamin D_3 was associated with lymphatic transport pathways, whereas only 25% was absorbed directly to the portal blood [3].

After oral administration, dissolution of the drug molecule in the intestinal milieu is a prerequisite for the absorption process. Since the water solubility of a lipophilic molecule is poor, the aid of surfactants provided by biliary secretions is essential to solubilize the lipophilic drug and allow its absorption. The bile fluid is secreted by the liver and is stored and concentrated inside the gallbladder. The most important organic solutes of the bile are bile acids, phospholipids and cholesterol. These biliary secretions assist the solubilization of the lipophilic drug by forming submicrometer mixed micelles in which the lipophilic molecule is solubilized and reaches the absorptive membrane of the enterocyte. The solubilization of the lipophilic molecule occurs mostly in the upper part of the gastrointestinal (GI) tract, where pancreatic fluids and biliary lipids are secreted and aid this solubilization process. Therefore, the absorption of these molecules usually takes place from the small intestine.

For a lipophilic drug, the main route for entry into the intestinal lymphatics is transcellular pathways using the physiological intestinal lipid transport system. Lingual lipase and gastric lipase are responsible for the beginning of hydrolysis of limited amounts of triglycerides, to form the corresponding diglyceride and fatty acid inside the stomach. Due to the shear movement of the stomach and the passage through the pyloric sphincter, these products are turned to the form of a crude emulsion. Lipids in the duodenum facilitate the secretion of bile salts, biliary lipids like phospholipid and cholesterol ester and pancreatic fluids into the duodenum. They absorb to the o/w interface and increase the stability of emulsion with a reduced droplet size. The enzymatic hydrolysis is completed by the action of pancreatic lipase produce the corresponding two monoglycerides and two fatty acids.

These digestion products are arranged in bile salt micelles and solubilize the coadministered lipophilic drug. Micelles are not absorbed as such, and the drug dissociates from the micelles before absorption into the enterocyte.

After its entry in to the enterocyte, the long-chain fatty acids travel to the endoplasmic reticulum, and they re-esterify to form triglyceride-rich droplets. Then these droplets assemble to form a lipidic core of a lipoprotein called as chylomicron. The chylomicron in association with the lipophilic drug carries the drug into the lymphatic circulation. Then the chylomicron is packaged in the Golgi apparatus and secreted from the basolateral membrane of the enterocyte into the intracellular space. Due to its large size, the chylomicron cannot permeate into the blood capillaries, so it is absorbed into lacteal (a porous mesenteric lymph vessel) and travels with the lymph until it get drained into the systemic blood circulation. So if we can increase the process of association of chylomicron with drug, we can also increase the lymphatic transport [2,3].

Lymphatic transport of a drug provides advantages including avoidance of hepatic first-pass metabolism, a potential to target specific disease states known to spread via the lymphatics and superior plasma profile of the drug. In many lymphatic absorption studies, a complex lipidic vehicle has been used [2]. The impact of the fatty acid chain length of the triglyceride in the formulation on the lymphatic transport of the lipophilic antimalarial drug halofantrine was investigated by Caliph and associates. Both lymphatic transport and total systemic exposure of halofantrine were enhanced by an increase in the fatty acid chain length of the coadministered lipid. This work shows that formulating with long-chain lipids, rather than shorter lipids, leads to an increased lymphatic absorption of the coadministered lipophilic drug [4].

A review by Christopher *et al.* details the mechanisms by which lipids and lipidic excipients affect the oral absorption of lipophilic drug emphasized on the capacity of lipids to enhance drug solubilization in the intestinal milieu, recruit intestinal lymphatic drug transport and alter enterocyte-based drug transport and disposition [5].

3. Techniques for enhancing absorption of lipophilic drugs

A lot of techniques are available for enhancing absorption of poorly water-soluble drugs, like lipid-based formulation approach, pro-drug approach, lymphatic transport pathways and cytochrome P450 3A4 and P-glycoprotein inhibitors [2]. Thus enhancement of aqueous solubility in such case is a valuable goal to successfully formulate them into bioavailable dosage forms. Therefore, great efforts have been made to improve oral bioavailability of poorly water-soluble drugs by increasing

their dissolution rate through various techniques. A range of novel strategies are currently being developed for efficient delivery of poorly water-soluble drugs, such as the formulation of amorphous solid form, nanoparticles, microemulsions, solid dispersions, melt extrusion, salt formation and formation of water-soluble complexes. Among all, the most accepted approach is the lipid-based formulation approach [6,7]. Since successful bioavailability enhancement utilizing lipid-based formulations has been accomplished with the immunosuppressive agent cyclosporine A (Neoral®, Novartis Pharmaceuticals Corp., East Hanover, New Jersey, USA), and for the two HIV protease inhibitors ritonavir (Norvir®, Abbott Laboratories, Illinois, USA) and saquinavir (Fortovase®, Roche Pharmaceuticals, Nutley, New Jersey, USA), consequently, considerable interest in lipid-based formulations has been aroused.

Lipid-based formulations enhance the absorption by enhancing solubilization, prolonging gastric residence time, stimulating the intestinal lymphatic transport pathway, altering intestinal permeability, reduced activity of efflux transporters and reduced metabolism.

Lipid-based formulations present a large range of optional systems such as solutions, suspensions, self-emulsifying systems and NEs. Among these approaches, oral NEs offer a very good result because NEs can improve the bioavailability by increasing the solubility of hydrophobic drugs and are now widely used for the administration of BCS class II and IV drugs [2]. Oral NEs use safe edible materials (e.g., food-grade oils and 'generally regarded as safe' (GRAS)-grade excipients) for fabrication of the delivery system [8].

The definition of NEs is different according to different authors. According to Graves 'NEs' are metastable dispersions of submicrometer droplets that have a significant surface tension, which form only when extreme shear is applied to fragment droplets strongly and are kinetically inhibited against recombining by repulsive interfacial stabilization due to the surfactant [9].

Nakajima and associates defined NEs as fine oil-in-water dispersions, having droplet covering the size range of 100 – 600 nm [10]. NEs are dispersions of nanoscale droplets formed by shear-induced rupturing according to Mason and associates [11]. NEs are a class of stable emulsions formed by a monolayer of phospholipids composed of surfactant and vegetable oil suspended in water with mean particle diameters of approximately less than 100 nm. The stability of NE makes them amazing, and they are regularly referred to as 'approaching thermodynamic stability' [12-15].

In a broad sense, we can consider NEs as a class of multiphase colloidal dispersions. Macroemulsions or lyotropic liquid crystalline phases are also similar to NEs in nanoscale structure and composition, even though such systems are somewhat different from classical NEs. Microemulsions form spontaneously and contain equilibrium structures of two different liquid phases and surfactants, they exist as lamellar sheets or hexagonally packed columns or wormlike micellar phases and, moreover, they are thermodynamically stable. NEs also possess a relatively high kinetic stability for many years. The colloidal state is a type of metastable state and will remain stable until some high-energy factors are there to break this high-energy colloidal state.

4. Theory behind the formation of NE

The attractive interactions between the molecules of the two liquid phases are different since an interfacial tension, σ, exists between the two liquids in their contact point [16,17]. The energy required to create an additional interfacial area 'A' between the two liquid phases is σA. Interfacial tension always acts to minimize the interfacial area. Therefore, the interface between two immiscible liquid is like a planar sheet at their contact point (**Figure 1A**). Usually the oil phase remains in the upper side since it has a low density than the water phase. And so the system is under thermodynamic equilibrium in the absence of any surfactants. When we add surfactants, they remain at the interface so as to reduce the interfacial tension (**Figure 1B**). Surfactants that are highly soluble in any one of the phases especially in the dispersed phase can reduce the interfacial tension significantly. If oil–water interface that is coated with surfactants brought in close to each other, a thin film of water will remain at the interface. Therefore, the interface repels each other due to the like or similar charges of the surfactants (**Figure 1C**). At a very low volume fraction of dispersed phase (Φ), the droplets remains spherical with a radius of 'a', the curved interface exerts a pressure on the molecules inside the droplet, called the Laplace pressure [18].

$$\text{Laplace pressure} \prod_L = 2\sigma/a$$

Since the Laplace pressure is inversely proportional to the radius, smaller droplets experience a higher Laplace pressure (**Figure 2A**) than larger ones (**Figure 2B**). So to deform a droplet, the applied shear must overcome Laplace pressure [19]. Therefore, a larger shear stress (τ) is to be applied to rupture the droplets into smaller size.

$$\text{shear stress}, \tau = \eta_c \gamma'$$

where η_c is the viscosity of the continuous phase and γ' is the shear rate.

Since a large number of surfactant molecules are present in the continuous phase, they form a coating on the interface immediately when an additional interfacial area is formed and thus stabilizes the system from coalescence.

In the last century, Taylor developed a relationship between how an isolated droplet is ruptured further smaller by the applied shear stress. According to Taylor, droplet size is inversely proportional to shear stress.

$$a \propto \sigma/\tau$$

$$a = \sigma/\eta_c \gamma'$$

The size distribution of the ruptured droplets is determined by the history of applied shear, whether the shear is applied

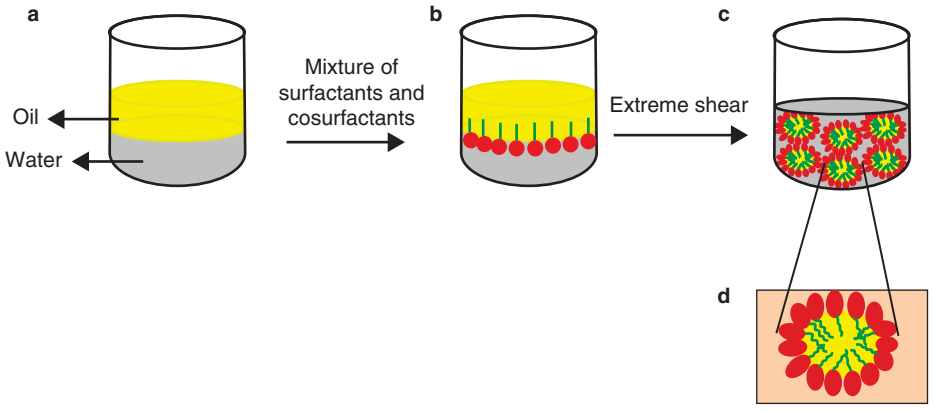

Figure 1. A. Oil and water exist as two separate layers initially. **B.** Addition of a surfactant that is soluble in the continuous phase remains at the interface. **C.** Due to extreme shear, oil droplets breakdown and surfactants remains coated on oil droplets and forms a stable emulsion. **D.** Surfactant-coated oil droplet.

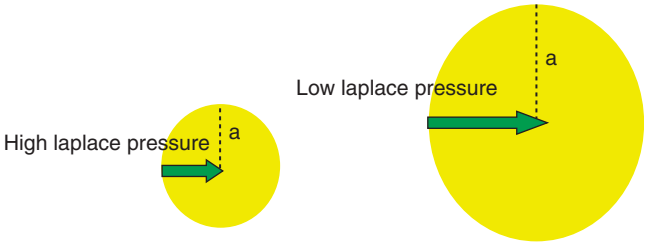

Figure 2. Relation between droplet radius and Laplace pressure.

to an emulsion having either high- or low-volume fraction of dispersed phase [19].

5. Methods of preparation of NE

Both high-energy and low-energy methods can produce stable NEs. High-pressure homogenizer or ultrasound generator can be used for the preparation of NE by high-energy emulsification method. Phase inversion methods such as phase inversion temperature (PIT) and phase inversion composition (PIC) are low-energy method for the preparation of NEs. Extreme emulsification methods such as microfluidic and ultrasonic techniques can be used to produce nanoscale dispersions of droplets of one liquid in another immiscible liquid by rupturing larger microscale droplets into nanoscale droplets [20]. The apparatus used for high-energy emulsification method should supply homogeneous flow and high energy in the shortest time in order to produce the smallest size. Classical 'metastable emulsions' [21-23] are formed by applying mechanical shear to the continuous phase in order to break larger droplets of the dispersed phase into smaller droplets. Low-energy methods such as PIT and PIC methods are collectively called as condensation method; they make use of stored chemical energy instead of mechanical energy in high-energy methods.

5.1 High-pressure microfluidic nanoemulsification method

High-pressure homogenizers are most widely used and accepted equipment since they meet all requirements [24]. To create a tremendously strong extensional flow for making NE by rupturing droplets in a concentrated emulsion, rapidly flowing streams of a premixed emulsion of droplet sizes typically less than 10 µm are forced through rigid stainless steel microchannels of dimensions typically closer to 100 µm. High-pressure air around 100 psi is mechanically amplified by a piston to produce liquid pressures that can reach as high as about 30,000 psi. Microscale emulsion droplets are taken at a rate of about 3 ml sec^{-1} into the channel and routed into an interaction area where an extreme extensional shear flow is created. Recirculation of the emulsion through the region of high shear is essential to maintain relatively low polydispersibility due to the in homogeneities of the pulsed microfluidic flow. Due to the higher shear rates, the emulsion is heated above room temperature. NE that leaves the region of extreme shear can be cooled by a heat exchanger without affecting the size distribution or stability. The volume rate of production of the NE is high about many liters per hour. The advantage of this 'high-throughput' microfluidic nanoemulsification method is the combination of extremely high shear rates, high-volume throughput of nanoscale droplets and reasonably uniform droplet size distributions [11,25-27]. Wang and coworkers developed stable NEs of some polyphenols such as epigallocatechin gallate and curcumin by high-pressure homogenizer using varying amounts of water, oil and emulsifiers [28]. Tagne and associates formulated a water-soluble NE of the highly lipid-soluble drug tamoxifen by microfluidization technique. The results suggested that NEs of tamoxifen, having mean particle sizes of 47 nm, inhibited cell proliferation 20-fold greater and increased cell

apoptosis fourfold greater in the HTB-20 breast cancer cell line [29]. Meleson and associates investigated the production of NE by a combination of extreme shear due to multipass, high-pressure microfluidic injection and systematic control of the emulsion's composition. At large droplet volume fractions (greater than 0.65), phase inversion is observed, rather than a reduction in the droplet size [17]. We can see that all the above-mentioned works could produce a stable NE in the size range of less than 100 nm and with a high zeta potential. So from the above studies, we can assure that stable NE can be prepared efficiently by high-pressure homogenization technique up to a certain droplet volume fraction.

5.2 Ultrasonication method

Another method for producing NEs is by ultrasonic agitation of a premixed emulsion of microscale droplets. In this method, a vibrating solid surface agitates the premixed emulsion at ultrasonic frequencies about 20 kHz or larger. This high power produces extreme shear and cavitations that breaks up microscale droplets to nanoscale. The devices contain focusing horns and pointing tips. In most of the ultrasonic devices, recirculation is necessary like high-pressure homogenizers since the emitted sound field is normally inhomogeneous. Practically uniform droplet size distributions at dilute concentrations can be obtained if the emulsion is recirculated many times through the region of high shear [11]. According to Landfester, the efficiency of the dispersion process is strongly dependent on the ultrasonication time at different amplitudes. And also it was found that the more hydrophobic the monomer is, the longer will be the sonication time required [30-33].

Tiwari and associates designed oral NE formulations for enhancing bioavailability of hydrophobic drugs by sonication method. The formulated NEs had a particle size range in between 90 and 120 nm and zeta potential values ranging from -56 mV to +34 mV. Formulations of NEs were shown to generate enhancement in the oral bioavailability of paclitaxel, a model hydrophobic drug, relative to administration in aqueous solution [34]. The results of this study suggest that stable NEs with a uniform size distribution and high zeta potential can be formulated using ultrasonication technique. According to Ganta and associates, a prolonged ultrasonication or increased energy did not improve the result. They formulated paclitaxel and curcumin containing NE formulations using high-energy ultrasonication method and optimized the processing conditions and found that a 10 min ultrasonication (energy 21%, duty cycle 50%) resulted in an NE with particle size less than 200 nm [35]. According to Shekar and associates, emulsions prepared by ultrasonic technique were more stable for longer duration of time when compared with emulsions prepared by mechanical agitation, which can be attributed to the small droplet size that is thermodynamically stabilized [36]. From all these results, we can say that ultrasonication is a very good and most common method for the preparation of stable NE.

According to Anna and associates, hydrodynamic focusing of a liquid jet of the dispersed phase surrounded by the continuous phase in soft microchannels at low pressure can be used to produce very uniform submicrometer droplets [37]. When compared with high-pressure microfluidic method, the rate of production of nanoscale droplets is quite low. This low-pressure hydrodynamic focusing method is suitable for low-throughput specialty applications, which need a very high degree of monodispersity, with very small total droplet volume [11].

5.3 PIT method

According to PIT introduced by Shinoda and associates, fine dispersion can also be obtained by chemical energy, due to the phase transitions taking place through emulsification path [38]. Adequate phase transitions can be produced by varying the composition at constant temperature or by varying the temperature at constant composition [39,40]. This method is based on the changes in solubility of nonionic surfactants with temperature. The polyoxyethylene surfactants become lipophilic with increasing temperature due to dehydration of the polyoxyethylene chains. The surfactant monolayer has a large positive spontaneous curvature at low temperature. It forms an oil-swollen micellar solution phases or microemulsions of o/w type, which coexist with an excess of oil phase. As the temperature increases, the curvature becomes negative and water-swollen reverse micelles or w/o microemulsions will form and they coexist with excess water phase. At intermediate temperatures (the HLB temperature), the spontaneous curvature becomes close to zero. At this temperature, a bicontinuous-phase microemulsion containing similar amounts of water and oil phases coexists with excess water and oil phase [41-45]. Sole and associates used emulsion inversion point method to form NEs in the ionic system water/oleic acid–potassium oleate–$C_{12}E_{10}$/hexadecane. Potassium hydroxide solutions were added to oleic acid–$C_{12}E_{10}$/hexadecane solutions at constant temperature (25°C) in order to obtain NEs at 80% water concentration [46]. Pasternacki-Surian and associates investigated the effect of preparation temperature on the emulsification efficiency of perfluoro-3-butyltetrahydrofuran by low-shear emulsification method using polyoxyethylene oleyl ether as surfactant [47]. All these studies serve as a proof that sable NEs can also be produced by PIT method.

5.4 PIC method

PIC method, that is phase inversion composition method, involves the change in composition at a constant temperature. The preparation of w/o NEs by a low-energy method consisting of slow addition of the oil to surfactant/water mixtures is first reported by Usón and associates. The droplet size was a function of the surfactant mixing ratio and water concentration [48]. Porras and associates also

described the formation of water in oil NEs by the same low-energy method stabilized with mixtures of sorbitan ester surfactants. In experiments where the surfactant:oil ratio is constant, droplet size increases as water concentration increases. In addition, under conditions of constant water concentration, droplet size decreases when the surfactant: oil ratio increases [49]. These studies promise that stable NEs can be produced not only by high-energy methods but also by low-energy methods. **Table 1** gives comparison of different methods of preparation of NE.

A series of study done in our laboratory by Bali and associates proved that optimal and stable NE of can be formulated by this low-energy emulsification method [50,51]. Shafiq and associates formulated a thermodynamically stable and dilutable NE formulation of ramipril [52]. Azeem and associates developed stable NE of ropinirole with a very low polydispersibility index [53]. All these studies made use of low-energy emulsification technique for formulating stable NE for the delivery of poorly water-soluble actives.

5.5 Solvent displacement method

In this method, oily phase is dissolved in water-miscible organic solvents such as acetone, ethanol and ethyl methyl ketone. The organic phase is poured into an aqueous phase containing surfactant to yield spontaneous NE due to rapid diffusion of organic solvent. The organic solvent is removed from the NE by a suitable means, such as vacuum evaporation. In a work done by Bouchemal and associates, NEs were prepared using the spontaneous emulsification mechanism by mixing an organic phase and an aqueous phase. The organic phase is a homogeneous solution of oil, lipophilic surfactant and water-miscible solvent and the aqueous phase is hydrophilic surfactant and water. As per the study, the composition of the initial organic phase has great importance for the spontaneous emulsification process and, so, for the physicochemical properties of the obtained emulsions [54].

Spontaneous nanoemulsification has also been reported with the solution of organic solvents containing a small percentage of oil when it is poured into aqueous phase without any surfactant. According to Vitale and associates, emulsification also take place spontaneously by pouring, into water, a solution consisting of a small concentration of oil in a water-miscible solvent without the presence of surfactant due to Ouzo effect [55,56]. Diameter of the oil droplets depends on the ratio of excess oil to water-soluble solvent. In a review, Ganachaud and associates explained the preparation of metastable liquid dispersions by homogeneous liquid–liquid nucleation and its advantages as an alternative to ultrasonic and high-shear techniques. Disadvantages include the low oil content around 1% that can be dispersed and the solvent used should be soluble in water in all proportions and also the solvent should be removed [57].

6. Stability problems

There are two most important mechanisms that can destabilize the emulsion and cause the droplet size distribution to vary [26]. The first mechanism leading to destabilization of the emulsion is Ostwald ripening or molecular diffusion. Sole and associates found that the most probable breakdown mechanism of the NEs formed is Ostwald ripening [46]. According to Taylor, Ostwald ripening might be used as a tool to estimate the thermodynamics of solution of oils in water [58]. Ostwald ripening is due to polydispersity and also the difference in solubility between smaller and larger droplets so diffusive migration of individual dispersed phase molecules that are driven from smaller droplets, which have a higher Laplace pressure, to larger droplets, which have a lower Laplace pressure. Due to Brownian motion, the diffusion rate is higher than the sedimentation rate for smaller droplets [45,47,56]. The second is coalescence, caused by the rupturing of films of the continuous phase and the fusion of two droplets into a single larger droplet [20,26].

7. Prevention

Ostwald ripening can be suppressed by choosing a liquid for the dispersed phase that have very low solubility in the continuous phase [58-60]. The second one is the choice of surfactant, which do not result in the formation of lyotropic liquid crystalline 'microemulsion' phases. Surfactants with short-chain alkanes, alcohols, water and surfactants are known to form these phases [61]. A surfactant that provides a strong repulsion between droplet interfaces coarsening through coalescence can also be effectively eliminated even at large volume fractions of the dispersed phase provided that the critical disjoining pressure is not exceeded [21,62-66]. The third one is that the continuous phase should contain a significant excess of surfactant. These excess surfactants allow the new surface area of the nanoscale droplets to be rapidly coated. Extreme shear must be applied to rupture microscale droplets into nanodroplets. Typically, the stress level should reach the Laplace pressure of droplets having the desired size, usually in the range of 10 – 100 atm [11]. Ostwald ripening can also be reduced by the addition of a small amount of second oil that has a low solubility in the aqueous phase. According to a recent report, for an ethoxylated nonionic surfactant system, the Ostwald ripening rate can also be reduced by adding a second surfactant with the same alkyl chain length and higher degree of ethoxylation than the primary surfactant [56].

After oral administration, NE readily disperses to small droplet of less than 100 nm, which promotes wide distribution of the drug throughout the GI tract. Generally a good relationship has been established between solubility improvement and higher bioavailability for most of the BCS class II drugs. As a result bioavailability of the drug is increased, intra-subject and inter-subject variation of drug through oral route is minimized. It has been reported that oral NEs make

Table 1. Comparison of different methods of preparation of nanoemulsion.

Method	Pros	Cons	Ref.
High-pressure homogenization	High-volume throughput of nanoscale droplets and low polydispersibility	Recirculation is necessary for uniform size distribution. High-energy consumption and increase in temperature of emulsion during processing	[11]
Ultrasonic agitation	Very efficient in reducing droplet sizes	Recirculation is necessary for uniform size distribution. Appropriate for small batches only	[11,30]
Phase inversion temperature method	Economical and no need of sophisticated equipments	Coalescence rate is very fast if the cooling and heating process is not fast	[40]
Phase inversion composition method	Generates nanoemulsions at room temperature without the use of any organic solvent and heat. Kinetically stable nanoemulsions with small droplet size can be generated	Stability is less when compared with other methods	[48,49]
Solvent displacement method	Can yield nanoemulsions at room temperature and require simple stirring for the fabrication	Use of organic solvents, such as acetone, which requires additional inputs for their removal from nanoemulsion. A high ratio of solvent to oil is required to obtain a nanoemulsion with a desirable droplet size	[54]

the plasma concentration profiles and bioavailability of drugs more reproducible.

NEs possess various advantages such as they do not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with macroemulsions, and NEs have a much higher surface area than macroemulsions that make them an effective transport system. Since NEs are formulated with surfactants, which are approved for human consumption, they can be used orally. In the world of nanomaterials, NEs hold great promise since they can typically be formulated using considerably less surfactant than that is required for nanostructured lyotropic microemulsion phases [67,68].

8. Oral NE for improved delivery of therapeutic agents

NEs offer advantages of outstanding stability to encapsulate active compounds due to their small droplet size and high kinetic stability. Oral NE formulations are excellent for enhancing bioavailability of hydrophobic drugs.

NE delivery systems are fast becoming primary approaches for innovative strategies in the prevention and treatment of cancer. Tiwari and associates designed oral NE formulations for enhancing bioavailability of paclitaxel a hydrophobic drug. Following oral administration, an appreciably higher concentration of paclitaxel was observed in the systemic circulation compared with control aqueous solution. The absorbed drug was found to be distributed in liver, kidneys and lungs [34]. Tagne and associates prepared a water-soluble NE of the highly lipid-soluble drug tamoxifen in order to improve the bioavailability [29]. Ganta and associates examined augmentation of therapeutic efficacy upon coadministration of paclitaxel and curcumin, an inhibitor of nuclear factor kappa B (NFκB), as well as a potent down-regulator of ABC transporters, in wild-type SKOV3 and drug-resistant SKOV3TR human ovarian adenocarcinoma cells. Paclitaxel and curcumin were encapsulated in flaxseed oil containing NE formulations and the results proved that this strategy has significant promise in the clinical management of refractory diseases, especially in ovarian cancer [35].

Even though most of the works have been done on anticancer drugs, studies are also reported to improve the bioavailability of different class of compounds for a wide range of disease conditions. Wang and coworkers developed NEs of some polyphenols in order to improve their solubility. Polyphenols have poor stability and low oral bioavailability. The results suggested that NEs improved the stability and oral bioavailability of polyphenols such as epigallocatechin gallate and curcumin [28]. So from this study, we can conclude that NEs

are promising novel formulations that can enhance the oral bioavailability of hydrophobic drugs.

Brüsewitza and associates developed two NE systems with Pluronic® P104 as primary emulsifier, Pluronic® L62 or L81 as secondary emulsifiers and Lauroglycol® 90 as amphiphilic oil phase. The apparent permeability of the BCS class III compound atenolol was enhanced 2.5-fold, of BCS class II compound danazol 3.2-fold and of BCS class I compound metoprolol 1.4-fold [69]. So from the Caco-2 experiments, we can say that NEs can impart influence on intestinal permeation of paracellular and transcellular transport of drug. According to the study, the solubilizers are not primarily responsible for the permeability enhancement, though they might contribute to the effect. The permeation is based on partition coefficient of the drug, surface rheology and fluidity of the interface. Vyasa and associates investigated a novel oil-in-water (o/w) NEs containing saquinavir (SQV), an anti-HIV protease inhibitor, for enhanced oral bioavailability and brain disposition. SQV concentrations in the systemic circulation administered in flaxseed oil NEs were found to be threefold higher when compared with the control aqueous suspension. The oral bioavailability and distribution to the brain, a potential sanctuary site for HIV, were significantly enhanced with SQV delivered in NE formulations [70]. Polyunsaturated fatty acid (PUFA)-rich oil containing NEs can enhance bioavailability and can be used efficiently for the brain targeting. Bali and associates developed an optimal and stable NE of ezetimibe. The release of drug from the NE was highly significant as compared with the drug suspension. The value of total cholesterol in the group administered with the optimized NE formulation was highly significant with respect to the group administered with the suspension of the drug. The plasma concentration time profile of ezetimibe from NE represented greater improvement of drug absorption than the marketed formulation and simple drug suspension. The shelf life of the NE was found to be 5.94 years at room temperature [50,51]. So the present study established NE to be a possible alternative for minimizing variation in bioavailability of ezetimibe.

Singh and associates developed an oral lipid NE of primaquine. The developed primaquine oral lipid NE showed effective antimalarial activity in 25% lower dose level as compared with conventional oral dose. So from the study it was found that lipid NE of primaquine exhibited improved oral bioavailability and was taken up preferentially by the liver with drug concentration higher at least by 45% as compared with the plain drug [71]. Shafiq and associates formulated a thermodynamically stable and dilutable NE formulation of ramipril a poorly water soluble, highly lipophilic drug with around 28 – 30% of variable oral absorption [52]. Azeem and associates successfully developed oral NE of ropinerol a highly lipophilic agent for improving its bioavailability [53]. The study illustrated the potential of NE dosage form in improving biopharmaceutical performance of atorvastatin. Mustafa and associates investigated oil-in-water (o/w) NE of atorvastatin for enhancing its oral bioavailability. The area under the curve and maximum plasma concentration of atorvastatin NE were found ninefold and fivefold higher, respectively, when compared with simple atorvastatin suspension. The present study illustrated the potential of NE dosage form in improving biopharmaceutic performance of atorvastatin [72]. These entire results serve as a proof of the concept that NEs can potentially serve as a tool for improving the bioavailability of highly lipophilic therapeutic moieties.

In a recent work, Gao and associates designed a candesartan-loaded NE by a modified emulsification-solvent evaporation technique to improve the intestinal absorption. The experimental results indicated that the formulation contains nanometer-sized droplets with negative potential and the absorption was significantly improved in intestinal tract compared with free candesartan solution. Moreover, candesartan NE could be internalized into the enterocytes by clathrin-mediated endocytosis pathway and thereafter transported into systemic circulation via both portal vein and lymphatic pathway. The overall results suggested that the NE was very effective for enhancing the oral absorption of insoluble candesartan and showed the great potential for clinical application [73].

These efforts provide evidence for the potential of NEs to improve delivery of poorly water-soluble drugs and support ongoing efforts to initiate clinical trials. Although the early efforts with oral NE have not reached clinical trials yet, the works so far provide encouraging evidence that a subset of these preclinical technologies may enter clinical evaluation in the future.

9. Formulation aspects

NEs for the bioavailability enhancement of poorly water-soluble drugs could be formulated as liquid oral dosage forms. The important criterion for selection of the materials was that all the components are pharmaceutically acceptable for oral application and fall under GRAS category. The selection of oil phase depends on the nature and dose of the therapeutic moiety. The molecular weight and molecular structure of the oil are normally selected based on the intended application. Molecular weight should be large enough to hinder Ostwald ripening. Some of the oils that are commonly used are capryol, sefsol, triacetin, castor oil and PUFA-rich oils such as safflower oil and soybean oil. Selection of surfactants and its concentration depends on the solubility of drug in the oil phase. The surfactant type and concentration in the aqueous phase are chosen to provide good stability against coalescence. The surfactant chosen should be able to lower interfacial tension to a very small value to help in the dispersion process during the preparation of the NE, provide a flexible film that can readily deform around droplets and should be with appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired NE type. An important criterion for selection of the surfactants is that the

required HLB value to form o/w NE is greater than 10. The right blend of low and high HLB surfactants leads to the formation of a stable NE. Labrasol, cremophor, Peceol, tween 80, tween 20, Labrafil, Labrafac and so on are examples of commonly used surfactants. The right blend of low and high HLB surfactants leads to the formation of a stable NE. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of single surfactant, usually necessitating the addition of a cosurfactant. The presence of cosurfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form NE over a wide range of composition. Ethanol, PEG 200, PEG 400, propylene glycol and so on can be used as cosurfactants [74,75].

Solubility studies were done to investigate the solubility of drugs in various oils, surfactants and cosurfactants. In order to find out the concentration range of components for the NEs, pseudo-ternary phase diagrams are constructed using water titration method at ambient temperature. Different phase diagrams are prepared with varying weight ratios of surfactant to cosurfactant. Different formulations will be selected from the NE region of each developed phase diagram so that the required dose of drug could be incorporated into the oil phase.

Physical stability tests such as centrifugation, heating cooling cycle, freeze thaw cycle and long-term thermodynamic stability tests are essential to eliminate the unstable or metastable formulations. NEs can be characterized by droplet size analysis, viscosity determination, refractive index and zeta potential analysis, and so on. *In vitro* release studies of NE can be compared with *in vivo* evaluation of the optimized NE. Based on the results obtained, *in vitro–in vivo* correlation can be established using suitable statistical methods. Accelerated stability studies can be carried out to study the effect of temperature and humidity [50,51].

10. Characterization of NE

Droplet size of the NE was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles. To explore the structure and behavior of NEs, more advanced techniques such as dynamic light scattering (DLS) [76], X-ray or neutron scattering [77], atomic force microscopy or cryo-electron microscopy [78] are required. The droplet volume fraction describes the relative degree of concentration of the droplets and the droplet structure is typically reported in terms of a size distribution of droplets, usually measured using DLS from a diluted and filtered sample [79].

The droplet positional structure can be determined only through neutron or X-ray scattering methods of the liquid emulsions or by electron microscopy techniques, including cryo-transmission electron microscopy and cryo-fracture scanning electron microscopy [78]. Even though cryo-electron microscopic methods can provide interesting static real-space images of droplets at very high magnifications, droplet dynamics cannot be studied. Some mechanical shear or 'rheological' properties of NEs are also affected by the nanoscale size of the droplets. At very high dispersed phase volume fraction where the droplets begin to deform, the elastic shear modulus of repulsive emulsions of all sizes is proportional to the Laplace pressure of the undeformed droplets. This shows that NEs could have exceedingly large elastic moduli [62-64] Electrical conductivity measurement can be done to check the stability and assert the nature of the formulation. A higher conductivity is attributed to a larger percentage of water, which allows more freedom of mobility of ions [68]. Refractive index measurement can be done to check the transparency of the formulation. Surface zeta potential measurement allows prediction of surface properties of NE. Viscosity measurement also gives an idea about stability and in addition can be used to ensure better delivery of the formulation [59]. Dispersibility test can confirm the efficiency of self-emulsification of oral NE after administration. Thus, with infinite dilution, there is every possibility of NE to phase separate, leading to precipitation of a poorly soluble drug. Dissolution studies ensure the release of drug from NE formulations [52]. The impact on oral absorption can be examined by Caco-2 monolayer model of the small intestine. It gives an idea about the degree of intestinal permeability of the therapeutic agent [69].

11. Patented oral NEs

In recent years, significant efforts have made to provide improved versions of drugs to address the issues related to bioavailability. There is a battle for patents for everything related to 'nano,' which has created an overflow of excessive broad nanopatents because broad nanopatents are generally awarded for pioneering inventions. A number of patents have been reported highlighting the importance of delivering drug effectively as NEs by oral route [79].

Liu Sha and associates developed a method for preparing oral chondroitin sulfate NE, wherein the proportion of the oil phase to the surfactant is 45:1 – 1:7, and the proportion of the surfactant to the cosurfactant is 4:1 – 1:4. The oral chondroitin sulfate NE improved the bioavailability of the chondroitin sulfate, reduced the toxic and side effect of the chondroitin sulfate and fully exerted the efficacy of the chondroitin sulfate. The present invention relates to compositions and methods for enhancing the bioavailability. The patent covers a wide range of ratio of oil to surfactant to protect the patent [80]. Jiyu and associates developed an ivermectin NE of oil-in-water type consisting of ethyl oleate, tween 80 and 1,2-propylene glycol. The ivermectin NE greatly improved the effect of ivermectin in resisting parazoon, enhanced the dissolubility, safety and bioavailability and is a high-efficiency antiparasitic nanolevel medicinal preparation with convenient use and wide ways of administration. So we can say that tween 80 is a good surfactant along with propylene glycol cosurfactant for a stable NE [81]. Lehtola and associates

Table 2. Specific patents for oral nanoemulsion.

Patent/ Application No	Title	Description	Ref.
101700224A	Method for preparing oral chondroitin sulfate nanoemulsion	The proportion of the oil phase to the surfactant is 45:1 – 1:7, and the proportion of the surfactant to the cosurfactant is 4:1 – 1:4. It can improve the bioavailability, reduce the toxic and side effects and fully exert the efficacy of the chondroitin sulfate	[77]
200810150354	Ivermectin nanoemulsion drug combination and preparation method thereof	O/w nanoemulsion consisting of ethyl oleate, tween 80, propylene glycol and ivermectin in double-distilled water. It enhanced the safety, dissolubility and bioavailability	[78]
WO105052A1	Novel oral formulations of ospemifene	The invention provides an improved drug formulation containing ospemifene, where the absorption of the drug is essentially increased and the variability in plasma level is essentially decreased	[79]
US0026988A1	Compositions and methods for human immunodeficiency virus vaccination	This invention relates to methods and compositions for the stimulation of immune responses. It provides methods of inducing an immune response to human immunodeficiency virus in a subject and compositions useful in such methods (e.g., a nanoemulsion comprising HIV or antigenic portion thereof)	[80]
WO035311A2	Antioxidant synergy formulation nanoemulsions to treat cancer	The microfluidized nanoemulsion improves the combination's cell membrane permeability by at least fourfold, which significantly increases the intracellular concentration of typically cell-impermeant antioxidants and/or systemic bioavailability. This synergistic combination has greater anticancer efficacy than the same combination applied as a free solution	[81]
WO016664A2	Compositions and methods for treating cancer with dacarbazine nanoemulsions	A uniform microfluidized nanoemulsion of dacarbazine improves the combination's cell membrane permeability and significantly increases the intracellular concentration of anticancer agents. As a nanoemulsion, dacarbazine has a greater anticancer efficacy than when applied as a free solution	[82]

developed novel oral formulations of ospemifene in the form of NE in which the absorption of the drug is essentially increased and the variability in plasma level is essentially decreased. This patent covers a promising study about bioavailability enhancement; that is, absorption is enhanced in the form of NE delivery when compared with oral solution [82]. This study suggested that oral NEs can ensure more reproducible plasma concentration profile.

Baker and associates designed methods of inducing an immune response to human immunodeficiency virus (HIV) in human subject and compositions useful in such methods (e.g., an NE comprising HIV or antigenic portion thereof) [83]. A uniform microfluidized NE containing a synergistic combination of two antioxidants and a cell membrane stabilizer was designed by Nicolosi and associates. The microfluidized NE improved cell membrane permeability by at least fourfold compared with conventional NE compositions, which significantly increases the intracellular concentration of typically cell-impermeant antioxidants such as tocopherol and/or systemic bioavailability. They proved that this synergistic combination NE has greater anticancer efficacy than the same combination applied as a free solution [84]. Nicolosi and associates also developed a uniform microfluidized NE of dacarbazine with a greater anticancer efficacy. These two patents describe stable NE formulation of anticancer agent and seem to be of very important since it can contribute to the treatment of cancer [85]. All the aforementioned efforts suggested that NEs can improve the oral delivery of poorly water-soluble pharmaceuticals. **Table 2** shows the specific patents for oral NE.

12. Conclusion

This review reveals the theory behind the formation, physical properties and structure of NEs. We have tried to clarify the recurrent confusion found in the literatures regarding the theory behind the formation of NE. From the literatures surveyed, it can be pointed out that low-energy emulsification techniques can also produce stable NEs. From the works done in the last few years, we can conclude that oral NEs can act as a potential tool for the delivery of poorly water-soluble therapeutic moieties in a very efficient manner. Some sections covered in this article are just going through the surface and so a lot of scientific discoveries are yet to be done to unwrap the mysteries behind the fabrication and utility of NE.

13. Expert opinion

After oral administration, highly lipophilic drug reaches the systemic blood circulation by intestinal lymphatic system. A lot of techniques such as lipid-based formulation approach, pro-drug approach and lymphatic transport pathways are available for enhancing absorption of poorly water-soluble drugs. Among lipid-based formulation approaches, oral NEs offer a very good result because NEs can improve the bioavailability by increasing the solubility of hydrophobic drugs so it can be widely used for the administration of BCS class II and IV drugs. NE possesses various advantages such as they do not show the problems of inherent creaming, flocculation, coalescence and sedimentation that are commonly associated with macroemulsions.

Different techniques including low-energy methods and high-energy methods are available for the formulation of NE. High-shear stirrer, high-pressure homogenizer or ultrasound generator can be used for the preparation of NE by high-energy emulsification method. Ultrasonic agitation of a premixed emulsion of microscale droplets can also produce nanoscale droplets. Hydrodynamic focusing of a liquid jet of the dispersed phase surrounded by the continuous phase in soft microchannels at low pressure can also be used to produce very uniform submicrometer droplets. Fine dispersion can also be obtained by chemical energy, due to the phase transitions taking through emulsification path as per phase inversion method. From the literatures surveyed, it can be pointed out that low-energy emulsification techniques can also produce stable NEs. So both low-energy and high-energy methods can be used to produce fine NE.

The two most important mechanisms that can destabilize the emulsion are Ostwald ripening and coalescence and these can be minimized or prevented by suitable choice of surfactant or a liquid for the dispersed phase that has very low solubility in the continuous phase or by the addition of a small amount of second oil that has a low solubility in the aqueous phase.

NE delivery systems are fast becoming primary approaches for innovative strategies in the prevention and treatment of cancer. From the works done in the last few years, we can conclude that oral NEs can act as a potential tool for the delivery of poorly water-soluble therapeutic moieties in a very efficient manner. The stability and oral bioavailability of polyphenols such as epigallocatechin gallate and curcumin are improved when formulated as an NE. Bioavailability and brain disposition of saquinavir are enhanced threefold when formulated as NE with flaxseed oil. Variation in bioavailability of ezetimibe is minimized in the form of NE when compared with drug suspension. A lot of anticancer drugs such as paclitaxel and tamoxifen were also formulated in the form of NE for better bioavailability. Similarly bioavailability of a lot lipophilic therapeutic moieties such as atorvastatin, candesartan, ropinerol and danazol was improved in the form of NE. Overall, NEs represent an intriguing new class of dispersions that may someday rival or surpass their microscale counterparts in commercial importance.

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