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REVIEW ARTICLE

Challenges and Future Prospects of Nanoemulsion as a Drug Delivery System

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Abstract: Nanoemulsion has the potential to overcome several disadvantages in drug formulation. Loading poor water-soluble drugs in the appropriate nanoemulsions enhances their wettability and/or solubility. Consequently, this improves their pharmacokinetics and pharmacodynamics by different routes of administration. Associated with the optimum nanodroplets size or even combined with key components, the droplets act as a reservoir of drugs, enabling nanoemulsion to be multifunctional platform to treat diverse diseases. A number of important advantages, which comprise nanoemulsion attributes, such as efficient drug release with appropriate rate, prolonged efficacy, drug uptake control, low side effects and drug protection properties from enzymatic or oxidative processes, have been reported in last decade. The high flexibility of nanoemulsion includes also a variety of manufacturing process options and a combination of widely assorted components such as surfactants, liquid lipids or even drug-conjugates. These features provide alternatives for designing innovative nanoemulsions aiming at high-value applications. This review presents the challenges and prospects of different nanoemulsion types and its application. The drug interaction with the components of the formulation, as well as the drug mechanistic interaction with the biological environment of different routes of administration are also presented.

Keywords: Nanoemulsion, drug delivery system, poor water-soluble drug, drug solubility, review.

1. INTRODUCTION

Nano-sized carriers are recognized as efficient drug delivery systems for poorly water-soluble drugs, which represent about 40% of newly discovered drug substances. Among the novel approaches, nanoemulsions have emerged as potential alternative drug carriers [1]. This type of surfactant-lipid-based formulation is capable of interacting with the body's natural barriers enabling drug absorption due to its composition and functionality.

Thus, oil-in-water nanoemulsions may overcome the drawback of low solubility of those drugs by improving the bioavailability, increased drug stability, and lower side effects, providing a wide range of applications [2].

In addition to the solubility enhancement, nanoemulsions are also a promising active drug targeting carrier for tumor cells [3], macrophages [4], and to overcome the blood-brain barrier [5, 6]. The flexible attribute of nanoemulsion extends through its manufacturing process options, which comprise both high and low energy processes, enabling narrow-sized droplet formation by mechanical and spontaneous physicochemical mechanisms, respectively [7].

Nanoemulsions are a resourceful platform, which can be formulated with a wide range of surfactants and liquid lipids. This enables alternatives to provide development of innovative drug delivery-system with high level of applicability.

Several nanoemulsion delivery systems have been marketed since last decade including Restasis®, a non-ionic ophthalmic nanoemulsion of cyclosporine A and Cationorm®, a cationic ophthalmic nanoemulsion, both preservative-free and recommended for treating dry eye syndrome [8], Neoral®, non-ionic nanoemulsion of cyclosporine as oral immunosuppressive agent and Diprivan®, a propofol nanoemulsion used as an intravenous anesthetic agent [1, 9]. Despite those advantages, nanoemulsions pose several challenges to the formulators. These include identification and understanding of the sources of variability in the nanoemulsion development, which highly affects the safety and the therapeutic efficacy of the drug product and its stability. The aim of this review is to present the challenges and prospects of different nanoemulsion types and their application.

2. NANOTECHNOLOGY AND LIPID NANOPARTICLE TYPES

Nanotechnology includes engineered products or materials at the nano-scale, ranging from 1 nm to 100 nm, or even reaching a micrometer (1,000 nm) scale, exhibiting physical or chemical properties or biological effects attributable to its dimension(s), where unique phenomena enable novel applications [10]. Nano sized particles offer better stability, high efficacy and less toxicity when administered, compared to their macroscale drugs in the pharmaceutical field. Among them, lipid nanoparticles gained special attention due to their higher degree of biocompatibility, biodegradability and versatility [11]. They are classified into liposomes, solid lipid nanoparticles, nanostructured lipid carriers and nanoemulsions, according to the specific composition and physicochemical characteristics.

Nanoemulsions are nanoscale dispersions of two immiscible phases, consisting of oil-in-water (O/W) or water-in-oil (W/O) phases according to the surfactant type. For poorly water-soluble drugs, they can also be expressed as spherical nanodroplets with hydrophobic liquid cores, stabilized with surfactant shells [1] (Fig. **1**). Differing from microemulsion which enables its spontaneous formation, nanoemulsion cannot be spontaneously formed and an introduction of some energy (mechanical or chemical) is required. Therefore, nanoemulsions are kinetically stable but thermodynamically unstable, with Ostwald ripening being the main factor of their instability [7, 12, 13]. This phenomenon of oil droplet growth over

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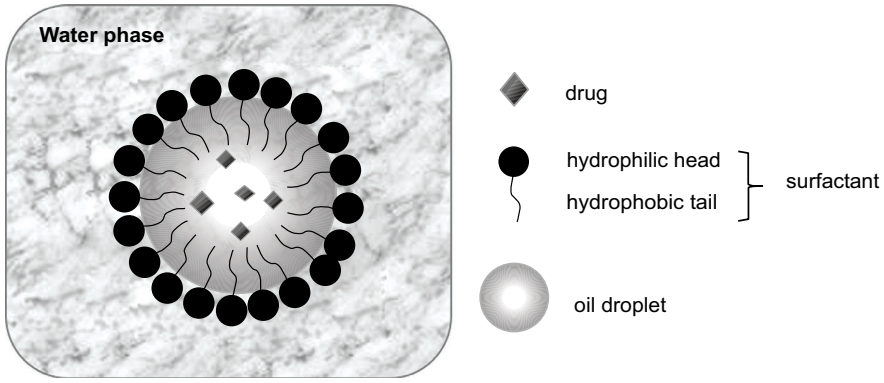

Fig. (1). Schematic illustration of oil-in-water nanoemulsion delivery system.

time occurs owing to the diffusion of small internal oil drops, from smaller to larger ones, in an aqueous medium [7, 14, 15]. Nanoemulsions can be classified in different types according to the surfactant selection (non-ionic, cationic or anionic). They can also be classified as, polymer associated nanoemulsions, and drug-conjugated as functionalized nanoemulsions. For the non-ionic nanoemulsions, the ethoxylated non-ionic surfactants enable the curvature change of the lipid-water interface during the phase inversion process, yielding narrow droplet sizes by low energy process. The polymeric non-ionic surfactants (*e.g*. poloxamers) provide steric stabilization, also called polymeric stabilization. Cationic and anionic nanoemulsions are composed, respectively, of cationic and anionic surfactants as the main emulsifier; nevertheless, both present higher toxicity than the non-ionic surfactants. The stabilization of cationic and anionic nanoemulsions is provided by electrostatic repulsion of the droplets due to the charged surfactant adsorbed on the lipid-water interface. Electrostatic stabilization combined with steric stabilization can be achieved using polymeric non-ionic surfactant associated with ionic surfactants [16]. For polymer-associated nanoemulsion, a range of polymers can be used providing steric and/or electrostatic stabilization depending on its characteristics and method preparation. A polymer or monomer can be added into the oily or aqueous phase [14, 16, 17] or, the surface of pre-formed nanoemulsion can be coated with this component [16]. Additionally, nanoemulsion can be formulated by inclusion of conjugated drug. Usually a drug covalently coupled to other molecules such as proteins is added to the composition. The conjugated drug offers characteristics different from its original form, yielding a functionalized nanoemulsion to enable specific targeted drug delivery to the cells, overcoming the problems associated with the free drugs [18]. The advantages and disadvantages of different types of nanoemulsion, their fabrication processes and applications are described in the following sections.

3. NANOEMULSION ATTRIBUTES IN A DRUG DELIVERY SYSTEM

Nanoemulsions are versatile drug delivery systems for poorly water-soluble drugs, which exhibit low bioavailability in their original form. Several attributes of nanoemulsions to overcome those drawbacks are listed below.

3.1. Drug Solubilization

Under correct selection of liquid lipid and surfactants, nanoemulsions have the ability to solubilize large amounts of hydrophobic drugs, providing high drug loading in the oil core of the nanosystem, acting as a drug reservoir [1, 19, 20].

3.2. Bioavailability

The physicochemical properties of nanoemulsions can be easily customized by different factors such as process selection, oil/surfactant composition [8], as well as the surface modification for specific delivering to cells or organs, through active and passive targeting mechanisms [1, 21]. Nanoemulsions provide a water-based formulation to poorly water-soluble drugs [20], and also owing to their small size, they have a large surface area that easily interacts with the body [22, 23]. This large surface area benefit the breaking rates of the formulation under oral administration, providing improved bioactive agent release, rapid and wider distribution of the drug [23], and also a prolonged efficacy by parenteral administration [21].

3.3. Formulation Stability

Depending on composition and manufacturing process selection, nanoemulsions may be an excellent system to overcome instability of poorly water-soluble drugs. Their nano-scaled particle size protects the formulation against sedimentation and creaming. Instability problems due to Ostwald ripening phenomenon can be overcome by using highly water-insoluble oils, or by using steric or electrostatic repulsion elements on the droplet surface [24].

3.4. Drug Protection

The incorporation of a chemically labile drug into the oil core may protect it against oxidation, enzymatic degradation or hydrolysis, making nanoemulsions to an ideal platform as a drug delivery system [1, 19, 20, 23].

3.5. Safety

A prolonged efficacy, dose reduction due to the use of nanoemulsions can yield reduction of common side effects [21]. Owing to non-ionic surfactants that are widely employed in the nanoemulsion compositions in lower concentration compared to *e.g*. a microemulsion system, it may reduce toxicity. A study of the influence of nanoemulsions on intracellular reactive oxygen species (ROS), which normally cause deleterious oxidation of biomolecules by other nano sized formulation, showed no induced oxidative stress in human BJ5ta cells [25].

3.6. Sterilization

The selection of a suitable sterilization method is crucial to guarantee safe application of the nanoemulsion for ophthalmic or parenteral administration [1], since the use of filtration by a 0.22-μm-size pore membrane or moist heat sterilization are limited due to possible filter pore blockage and nanoemulsion instability, respectively [8]. Moist heat sterilization was applied to a lipid nanoparticle formulation composed of tripalmitin, lecithin, tween 20 and sodium deoxycholate, indicating the impact of the emulsifier and the liquid lipid type for good nanoemulsion stability under this sterilization process [26]. Successful sterilization of nanoemulsion containing Capmul MCM, didodecyldimethylammonium bromide, Poloxamer 188 and phospholipid was shown by moist heat steriliza-

tion [27], and an ion-sensitive *in situ* ocular nanoemulsion gel of terbinafine by gamma radiation sterilization [28].

3.7. Wide Applicability

The high versatility of the nanoemulsions offers extensive potential applications which include oral delivery, parenteral, transdermal, ophthalmic and cancer targeted drug delivery. Their use as imaging components for cancer therapies have also been reported in recent studies [1]. Further data are presented in subsequent pages in this article.

One of the main disadvantages of O/W nanoemulsions is their instability mainly due to the Ostwald ripening as mentioned before. One study reported a new strategy for improving stability against Ostwald ripening by adding non-ionic amphiphilic polysaccharide (derived from dextran), without the need of ultrahydrophobic components [29]. Another recent study introduced a new biocompatible polymeric emulsifier, polyglycerol-block-poly(ε-caprolactone), which was highly effective in stabilizing O/W nanoemulsions through the formation of a semi-solid interphase. O/W nanoemulsions formed by different oils in this system presented excellent stability against mechanical stresses generated during repeated freeze/thaw cycles [30].

Another limitation of O/W nanoemulsions, i.e. compared to a highly concentrated nano sized formulations such as a nanocrystal (which is constituted primarily of the drug component itself), is the lower drug-loading capacity. Therefore, an effort to boost drug loading of nanoemulsions requires extensive understanding of the selected manufacturing process, the interactions between the nanoemulsion components, as well as the behaviour of the dissolved drug in the oil core.

4. NANOEMULSION MANUFACTURING PROCESSES

In our previous work, we presented the different manufacturing processes and a detailed mechanism governed by high and low energy production of nanoemulsions [7]. In a general way, nanoemulsion productions are divided into two main processes: high and low energy processes. High-energy processes, (those requiring mechanical energy input), include high shear stirring, ultrasounds, high-pressure homogenisation, membrane emulsification and microfluidization. High shear stirring, used generally in combination with other processes, consists of breaking down larger droplets into the smaller droplets by mechanical force. Ultrasound is based on the implosion of the droplets by a series of mechanical depressions and compression, resulting in cavitational forces. In high-pressure homogenisation, nanodrops are produced by passage through a narrow slot of a homogenizer under high-pressure, which involves shearing, collision and cavitation force. The membrane emulsification and microfluidization involve the passage of two immiscible fluids in channels by a high-pressure pump, in which droplet sizes are controlled by the size of pores or channels, enabling formation of uniform and controlled internal phase nanodroplets [14, 16, 17].

The low energy process where physicochemical energy is required involves processes such as phase inversion temperature (PIT), phase inversion composition (PIC) and spontaneous emulsification (or Ouzo effect). Nanoemulsions formed by phase inversion temperature and phase inversion composition methods are based on the spontaneous curvature change of surfactants, by temperature or composition transition during manufacturing, respectively. The spontaneous emulsification is based on the specific and rapid diffusion of an organic solvent from the oily phase to the aqueous phase, by a dispersion and condensation phenomenon [14,16]. The D-Phase emulsification method, compared to the PIT and PIC, was presented as a recent and an alternative means for overcoming the need of strict hydrophilic-lipophilic balance (HLB) adjustment dictated in the PIT and PIC methods. D-Phase emulsification method requires the polyol as the fourth component for the creation of a low interfacial tension phase (isotropic phase), enabling the final narrow droplet formation [31, 32].

The advantages of high-energy processes include flexibility in the process adjustment and broad formula composition choice, whereas the disadvantages include the higher cost investment on equipment. The advantages of a low energy process is the low equipment cost compared to the high energy processes, although the disadvantages consists mainly of the need for strict adjustment of the phase composition in order to lower the interfacial surface tension of the phases [14, 16, 17].

Several process application studies were also presented in our previous work, and some recent studies sparked consideration. A high-speed stirring study in an ART MICCRA DZ7 rotor-stator system was used for nanoemulsion production as an alternative for high-pressure homogenisation. Nanoemulsions with 135 nm and narrow size distribution were obtained by this system, showing to be a fast, cost-effective and suitable process for large-scale production [33].

The stability of nanoemulsions was analysed by several methods, based on drop size distribution information. The experiments were conducted using a set of alkanes with different chain lengths and physical properties. Nanoemulsions were formed by ultrasound, phase inversion composition and Ouzo methods. The authors showed that despite that, the Ostwald ripening is a dominant phenomenon for nanoemulsion at higher surfactant concentrations (i.e. 8.0 g/L), at low surfactant concentrations (i.e. « 4.0 g/L) coalescence was identified as the dominant growth mechanism of droplet sizes. Although still vulnerable to Ostwald ripening and flocculation in the long-term shelf life, the phase inversion composition method was found to be the most stable method in this study [34].

A different method based on stirred media mills was evaluated resulting in 25-nm-sized nanoemulsions obtained in a hexane - Tween 85 - water system. The lowest oil-to-emulsifier ratio, as well as the processing temperature, below the lipid solidification temperature, was considered to be the most advantageous conditions for the small droplet sizes in this system [35].

As a complementary reflection, taking into consideration that there is no high temperature required for lipid melting nor high pressure input during nanoemulsion production compared to solid-state nano formulations, one may consider that nanoemulsions are more suitable to most manufacturing processes. This may be reflected in the optimization of the processing time, moreover the feasibility to the labile drugs, and higher reproducibility for large-scale production.

5. NANOEMULSION - DRUG INTERACTION WITH OIL COMPONENTS

O/W nanoemulsion delivery systems are mainly composed of drugs solubilized in the oil phase, which is dispersed in the water phase. Different oil types can influence the drug solubilization as consequence of difference in density, viscosity, and polarity [36]. Therefore, apart from process conditions, the drug behavior in different oil components is also crucial during design of an effective delivery system. Thus, nanoemulsions possess in their core the oil phase as the main component, which may highly affect the bioavailability of the poor water-soluble drug, by increasing the drug absorption from the gastrointestinal (GI) tract and drug transportation via systemic circulation [2, 19, 22]. Several studies have been performed to evaluate the performance of drug-oil interaction in the nanoemulsion delivery systems as shown in the following sentences.

Nanoemulsion of pterostilbene, an antioxidant component of blueberries was developed for nutraceutical purpose. Two different carrier oils, flaxseed and olive oil, was investigated aiming to determine the influence of the different oil types on the metabolism and bioavailability of this natural compound pterostilbene. A Caco-

2 cell permeability model was employed to evaluate the absorption of pterostilbene from the resulting micelle phases. It has an enhanced solubility in both carrier oils, however, differences in the metabolism patterns and a higher trans-enterocyte transport were observed for this polyphenol in olive oil based nanoemulsions [23].

Aiming to design stable resveratrol nanoemulsion system against UV-light exposure, grape skin extract (GSE), rich in resveratrol, was incorporated in a mixture of grape seed oil (digestible) and orange oil (indigestible), by spontaneous emulsification process. The ratio of orange oil-to-grape seed oil of 5:5 gave the optimum arrangement between emulsion size (driven by *e.g.* viscosity, interfacial tension and interfacial dynamics of the oils) and the stability (driven by the polarity of the oil component). The 220 nm nanomulsion, with a droplet size closest to the UV-light wavelength, showed higher resveratrol protection compared to others [37].

Ostwald ripening in O/W nanoemulsion was evaluated by the influence of lipophilicity values of different oils. The mean drop size was characterized over time (0 to 180 min), and as a general rule, a higher growth rate was shown with a short hydrocarbon chains. Nanoemulsion with C16 was stable for around 8 months [33].

In order to maintain the poor soluble drug ezetimibe (a selective cholesterol-absorption inhibitor) in the solubilised form, a screening test of combinations of six different oils and eight surfactants/cosurfactants was performed to design a suitable nanoemulsion. Oils from different categories such as long-, medium-chain triglycerides and synthetic monoglyceride oils were evaluated. Capryol 90 (proprylene glycol monocaprylate) was selected since it exhibited the highest drug solubility in the nanoemulsion system [38].

Potent anti-cancer bioactive components polymethoxyflavones (PMFs) extracted from citrus peels are highly hydrophobic molecules with poor solubility in both water and oil at room temperature. Aiming to improve their bioavailability, PMF-loaded nanoemulsions were prepared evaluating the influence of different carrier oils (corn oil, medium chain triglycerides, orange oil), emulsifiers (b-lactoglobulin and lysolecithin as highly anionic emulsifier, Tween 20 and 85 as non-ionic emulsifiers, and DTAB as cationic emulsifier), and cosolvents (glycerol and ethanol). Nanoemulsions less than 100 nm could be formed using high-pressure homogenisation employing all emulsifiers, except for DTAB (dodecyl trimethyl ammonium bromide). PMF crystallization and sedimentation were observed in all prepared nanoemulsions, although the crystal morphology, size and sedimentation speed altered according to the different oil types, emulsifiers and preparation methods. These results may provide important directions for the future development of poorly water and oil soluble bioactive components in a nanoemulsion delivery system [22].

The use of the bioactive component carotenoids in nutraceuticals is currently limited due to their poor water solubility, low bioavailability and chemical instability. The permeability of carotenoids (0.5 wt% in lipid phase) in O/W nanoemulsions using different types of carrier oils was evaluated by an *in vitro* GI model that simulates the mouth, stomach and small intestine. Nanoemulsions composed of Tween 20 as non-ionic surfactant, and three different carrier oils, such as corn oil that contains long-chain triglycerides (LCT), medium-chain triglycerides (MCT) or orange oil, generated particles lower than 200 nm. Calculation from the alkali titration of the released free fatty acids (FFA) showed that the rate and extent of free acid production in the intestine was in order of LCT ~ MCT » orange oil. The *in vitro* ß-carotene permeability was in the order of LCT » MCT > orange oil. The higher permeability of the LCT is explained by the fact that it is able to form mixed micelles larger enough to accommodate highly lipophilic molecule such as ß-carotene, than MCT. Concurrently, no mixed micelles were formed to solubilise ß-carotene in orange oil nanoemulsions [39].

Another recent study reported the impact of different carrier oils on the digestion of curcumin (0.15 wt% in lipid phase) O/W nanoemulsion stabilized by protein (b-lactoglobulin). An *in vitro* GI model was used to simulate the intestinal passage. Results showed the faster rate and extent of FFA release for MCT than the LCT containing nanoemulsions. This may due to the consequence of the resulting digestion products from MCT and LCT, which facilitate or hinder lipase activity, respectively. The discrepancy of these results from the precedent study may involve the difference of surfactant type (non-ionic and protein), the *in vitro* digestion conditions (fat content), and the number of stages of the *in vitro* GI model [40]. This demonstrates the importance of the right selection of oil / surfactant composition and *in vitro* digestion model for optimal nanoemulsion formulation development.

Silymarin, a hepatoprotective bioactive compound with poor aqueous solubility and low bioavailability, was incorporated into the O/W nanoemulsion to increase its oral bioavailability. Considering that higher oil solubility of this component will favour an overall stability of formulation with effective dose optimization, propylene glycol caprylate (Sefsol 218®) was selected as carrier oil, due to its high solubilization capacity of silymarin. *In vitro* dissolution studies showed higher drug release from nanoemulsions than bulk drug suspension, and after oral administration, both maximum plasma concentration (C_{max}) and area under the curve (AUC) were 4- to 6-fold higher than those of silymarin in suspension form. Pharmacokinetic studies showed better results (a 2-fold and 2.6-fold increase in the AUC and C_{max}, respectively) in nanoemulsion than the marketed formulation [2].

An interesting study as an indicative of the effectiveness of liquid core in the nanosized formulation (as the nanoemulsion) was performed comparing the behaviour of two lipid nanoparticle (LN) systems, using trilaurin and tripalmitin. Differential scanning calorimetry studies on formed LNs demonstrated that tripalmitin presented a “solid-like” state, whereas trilaurin formed a “fluid-like” core. After addition of the hydrophobic drug testosterone (TP) in the preformed LNs, from 0.2 to 1.4 wt %, it resulted in a large increase in the apparent hydrodynamic size of the tripalmitin LN. However, no change was observed in trilaurin LN even after 1-week stability, compared to their TP-free LNs. The size change in the TP-contained tripalmitin LN may be a consequence of the rearrangement in packing of the molecules comprising the LN, caused by adding the drug. The higher level of TP solubilization in the trilaurin LN compared to the solid state LN is explained by the presence of the liquefied-lipid core. [41]. This result may illustrate the nanoemulsion as one of most promising delivey system for poorly soluble drugs.

6. ROUTES OF ADMINISTRATION AND APPLICATIONS OF NANOEMULSIONS

Aiming to accomplish the main strategy for improving the solubility of a poor water-soluble drug, by increasing its efficacy and bioavailability, a drug substance need to overcome several natural barriers in the body. Different routes of administration and applications of various nanoemulsion types are presented in the following sections. An overview of those applications is shown in the Table **1**.

6.1. Nanoemulsions for Oral Route of Administration

6.1.1. Lipid Delivery System Pathway and Bioavailability

With the oral route being the most popular rout of administration, water solubility of poorly water-soluble drugs becomes a key parameter in drug formulation since it can lead to limited absorption in the gastrointestinal tract and limited bioavailability [42, 43]. Hence, understanding the complex sequences of physiological and

Table 1. Nanoemulsion drug delivery system application.

Route of administration	Nanoemulsion (NE) type	NE composition	Manufacturing process	Drug / active principle	Therapeutic use	Result / prospect	References
Oral	Non-ionic NE	1% of WBO and 7.3% of a surfactant mixture of Span 80 (37.4%) and Tween 80 (62.6%).	High-speed blender (pre-emulsification) and ultrasonication	Wheat bran oil (WBO) rich in phenolic compounds	Nutraceutical	Good antioxidant and tyrosinase inhibitory activity	[36]
Oral	Non-ionic NE	Triacetin and Capryol 90 (1:1), Tween 80 and Transcutol P	Aqueous phase titration on the premixed oil / surfactant phases	Cilastazol	Antithrombotic activity	3.29-fold higher bioavailability	[51]
Oral	Non-ionic NE	Lauroglycol, Transcutol, Cremophor EL, deionized water, and λ-carrageenan	Ultrasound treatment	Aspirin	Anti-inflammatory and analgesic activities	Superior protection and less injury to the gastric mucosa, wide distribution of the drug throughout the intestinal tract, and increased drug-retention time in the desired region	[52]
Oral	Non-ionic NE	Lauroglycol™90, Transcutol HP®), Cremophore EL	Ultrasound treatment	Aspirin	Anti-inflammatory and analgesic activities	1.4- to 2.2-fold higher anti-inflammatory and analgesic effect	[53]
Oral	Non-ionic NE	Miglylol 812, egg lecithin, soyabean lecithin liquid, Poloxamer 188, glycerol, sorbitol / Capryol 90, Solutol HS 15 and Gelucire 44/14	Ultra-Turrax T 25 stirring and high pressure homogenisation / Self-emulsification	Primaquine/ clotrimazole	Malaria therapy	Efficacy at lower oral doses; increased oral bioavailability of the drugs loaded nanoemulsions	[54, 55]
Oral	Non-ionic NE	Capryol 90 (10%), Tween 20 (10%), and PEG 400 (16.67%)	Aqueous phase titration on the premixed oil / surfactant phases	Ezetimibe	Cholesterol and triglyceride lowering compounds	3.23-fold increase in bioavailability compared to drug suspension and a 4.77-fold increase in bioavailability compared to the conventional tablet	[38]
Oral	Non-ionic NE	Capryol 90 (10%, v/v), Cremophor EL (11.25%, v/v) and Transcutol P (33.75%, v/v)	Aqueous phase titration on the premixed oil / surfactant phases	Ezetimibe	cholesterol and triglyceride lowering compounds	Higher release and bioavailability of ezetimibe compared to the formulation of reference [38]	[56]
Oral	Polymer Associated NE	Medium chain triglyceride, (OSA)-modified starch, curcumin, chitosan and CMC (188:57:1.2:1.5:2)	High-intensity ultrasonic homogeniser / pre-formed curcumin nanoemulsion template	Curcumin	Antitumour	Stability and shelf improvement, minimum aggregation	[57]
Oral	Polymer Associated NE	Whey protein isolate, soybean protein isolate, beta- lactoglobulin, Labrafil M 1944CS, CaCl2	Mechanical mixing and high-pressure homogenisation	Fenofibrate	Cholesterol and triglyceride lowering compounds	Excellent stability and drug-loading capacity, which can be easily freeze-dried	[58, 59]

(Table 1) Contd....

Route of administration	Nanoemulsion (NE) type	NE composition	Manufacturing process	Drug / active principle	Therapeutic use	Result / prospect	References
Parenteral	Non-ionic NE	Squalane, Span-80, Tween-80, PBS, pH 7.4	High-pressure homogenisation	CHrPfs25	Malaria transmission blocking vaccine antigen	Highest antibody response; potential candidate as a Pfs25 vaccine adjuvant, as an alternative to existing aluminum salts	[64]
Parenteral	Non-ionic NE	Tween 20, phosphate buffer of pH 7.2	Ultrasound treatment	Neem oil; human serum albumin (HSA); bovine serum albumin (BSA)	Interaction of essential oil based NE with biological proteins	More stable complexes between BSA - neem oil NE compared to HSA; improving the efficacy of drug delivery, bio sensing and other clinical applications	[65]
Parenteral	Non-ionic NE	Curcuminoid extract, Tween 80, and water	Sonication	*Curcuma longa* Linnaeus	Inhibition mechanism of lung cancer cells (A549 and H460)	high stable NE; similar results of both NE and curcuminoid extract in the expression of proteins correlated with the cell cycle and apoptosis in these two cells.	[66]
Parenteral	Non-ionic NE	Miglyol® 812N/Labrasol®/Tween® 80/Lipoid E80®/water	Sonication	Fisetin	Antitumour	24-fold increase in fisetin relative bioavailability; antitumour activity at lower dose.	[68]
Parenteral	Non-ionic NE	sodium oleate, Polysorbate 80	Hot homogenisation	Risperidone	Antipsychotic	1.4-7.4-fold higher risperidone brain availability.	[69]
Parenteral	Polymer Associated NE	PEG-PDLA, perfluorocarbon	Sonication	Paclitaxel	Pancreatic anti-tumor	Higher therapeutic efficacy, lower drug resistance in tumors, lower systemic toxicity	[70]
Parenteral	Drug-conjugated NE	Vegetable oil, BSA-FA conjugate solution, PEGylated surfactant	High-pressure homogenisation	FA-functionalized PEGylated BSA + CORM-2	Antiproliferative effect on human cancer cells	FA-tagged protein NEs were preferentially internalized in the B-cell lymphoma cell line (A20 cell line)	[72]
Parenteral	Drug-conjugated NE	PEGylated surfactant, PBS, vegetable oil	High-pressure homogenisation	BSA-drug conjugates + methotrexate / BSA-drug conjugates + vancomycin	Potent anticancer agent / potent antibiotic	Effectiveness with improved half-life in systemic circulation	[18]
Parenteral	Drug-conjugated NE	PBS, vegetable oil, Poloxamer 407	High-pressure homogenisation	BSA NE + Poloxamer 407	Active targeting of folate receptor positive cells	5-fold higher internalization of tagged-nanoemulsions by cells	[25]
Topical	Non-ionic NE	Tween 20, oleic acid, propylene glycol	Aqueous phase addition on the pre-mixed oil / surfactant phases, sonication	Fennel essential oil	Prolonged antidiabetic activity	Superior permeation profiles for 24 h, high potential of reducing plasma glucose levels	[75]

(Table 1) Contd....

Route of administration	Nanoemulsion (NE) type	NE composition	Manufacturing process	Drug / active principle	Therapeutic use	Result / prospect	References
Topical	Non-ionic NE	Capryol™ 90, oleic acid, Tween 20	Aqueous phase addition on the pre-mixed oil / surfactant phases, sonication	Cumin essential oil	Systemic anti-oxidant and hepatoprotective activities	Best *in-vitro* and *in-vivo* antioxidant efficiency, high hepatoprotective potential	[76]
Topical	Non-ionic NE	Caprylic acid, propylene glycol, Tween 80, PEG 400, triethanolamine, Carbopol 940, Trypsin	Magnetic stirring	Meloxicam	Non-steroidal anti-inflammatory drug	Non-irritant, biocompatible, maximum inhibition of paw edema over 24 h	[77]
Intranasal	Non-ionic NE	Capmul MCM, Tween 80, PEG 400	Spontaneous emulsification process	Saquinavir mesylate	Anti-HIV	Higher permeation rate, no significant adverse effect, higher drug concentration in brain, larger extent transport of drug in the CNS	[6]
Intranasal	Polymer Associated NE	Capmul MCM, Tween 80, propylene glycol, Transcutol	Aqueous phase addition on the pre-mixed oil / surfactant phases, and subsequent addition of chitosan	Risperidone	Antipsychotic drug	Superior efficacy on brain/blood uptake ratio of risperidone	[82, 83]
Intranasal	Polymer Associated NE	Capmul MCM, Tween 80, ethanol and polyethylene glycol	Water titration method	Olanzapine	Antipsychotic agent	Highest drug targeting efficiency (DTE%) and direct nose-to-brain transport (DTP%), 2-fold higher DTP%	[5]
Ocular treatment	Cationic NE	Eutanol G, Lipoid S 100, cetylpyridinium chloride, glycerol	High-pressure homogenisation	Dexamethasone acetate and polymyxin B sulfate	Ophthalmic infection treatment	Amphiphilic cationic concept" offers highly ocular bioavailable solution for treating ophthalmic infections	[80]
Mucosal vaccine adjuvants	---	---	---	Local delivery, systemic delivery, mucosal vaccination	Mucosal and systemic immunization	Promising candidate as mucosal vaccine adjuvants: long-term release properties for antigens, non-invasive immunity and stability of antigens for mucosal and systemic immunization	[79, 84, 85]
Imaging-guided therapy	Non-ionic NE	Iron oxide nanocrystals, fluorescent dye Cy7	Spontaneous emulsification and sonication	Hydrophobic glucocorticoid prednisolone acetate valerate	"Theranostic" platform for image-guided therapy of cancer	Significant drug substance accumulation in tumors cells, potent inhibitory effect on the tumor growth profiles	[88]
Imaging-guided therapy	Non-ionic NE	Miglyol 810 N, DiD dye, PFPE, Pluronic® P105, Cremophor EL®	Microfluidizer	Celecoxib	Anti-inflammatory drug to target macrophages	Simultaneously delivery the drug to macrophages and monitor macrophage migration patterns by optical imaging	[4]

physicochemical phenomena, which the drug faces after being administered is the first step for developing an efficient formulation. In short, these emulsions under oral administration experience structural modification, resulting on flocculation and coalescence of droplets during the gastrointestinal passage [44].

The digestion process of fats, administered orally, typically begins in the stomach. This process consists of the dispersion of lipids into finely fragmented emulsion particles, by action of surface-active materials (i.e. gastric lipases) at the lipid-water interface [44-46]. At this stage, it is expected that a drug is dissolved in the lipid, preventing undesirable drug precipitation [47]. Next the gastric content is emptied into the small intestine, where in the presence of bile salts, colipase binds to the surface of fat droplets providing an attachment site for lipases. Therefore, this process produces the final mixture for fat digestion, fatty acids and 2-monoglyceride [39, 48, 49]. During this process in the gastrointestinal tract, structural changes in the emulsion occurs, generating bile salt micelles and lamellar vesicles composed of phospholipids in the aqueous phase [45, 47, 48], and finally absorption by the enterocytes occurs [44, 46]. Therefore, those hydrophobic components of the nanoemulsion, which constitute the carrier of poor water-soluble drugs, when they are incorporated and solubilized into the mixed micelles, function like a drug reservoir to be passively transported to epithelium cells [39].

The bioavailability of nanoemulsion is expected to be higher than conventional emulsion taking into account the higher surface-area-to-volume ratio of the nanoemulsion. Since lipid digestion consists of an interfacial phenomenon comprised of lipase adsorption on the lipid droplet surface, the droplet size reduction favours an increase of interfacial area, and consequent increase of lipid digestibility and release [44, 46, 50]. Other than the particle size, the nature of the interface of lipid droplet seems also to interfere with the lipid hydrolysis during gastrointestinal process. The displacement of the small surfactant components by bile salts and phopholipids allows the efficient attachment of lipase on the oil droplet surface. Therefore, the characteristics of interfacial layer components such as Tween 20, protein and phospholipids may alter those attachment phenomena, thus the lipid digestion efficiency. However, more research on *in vitro* digestion models still needs to have better conclusions [39, 44, 49, 50]. The physical state of the fat constituting the lipid delivery system is also important regarding the rate and extent of lipid digestion. Studies with solid-state emulsions and liquid-state emulsions using an *in vitro* digestion model showed a higher rate and extent of lipid digestion in liquid-state emulsion compared to the solid-state emulsion [44, 49].

6.1.2. Nanoemulsion Applications in Oral Administration

6.1.2.1. Non-Ionic Nanoemulsions

For nutraceutical purposes, a formulation of O/W nanoemulsion containing wheat bran oil (WBO) rich in phenolic compounds was developed by using the response surface methodology. The emulsification method, the oil and surfactant concentration, as well as surfactant type were investigated on the droplet size and stability of the nanoemulsion. A combination of high-speed mixer (pre-emulsification) and ultrasonication resulted in the optimal condition reached by 1% of WBO and 7.3% of a surfactant mixture of Span 80 (37.4%) and Tween 80 (62.6%). This optimized nanoemulsion showed good stability over time, as well as good antioxidant and tyrosinase inhibitory activity [36].

A poor oral bioavailability therapeutic agent, cilastazol (CLZ), which is well known for its antithrombotic activity, was studied. Amongst various surfactants, co-surfactants and oils, the combination of triacetin and Capryol 90 (1:1), Tween 80 and Transcutol P were selected to obtain O/W nanoemulsion with droplet size of 93.72 nm and polydispersity index (PDI) of 0.278. The optimized nanoemulsion showed a 3.29-fold higher bioavailability in rats compared to CLZ suspension [51].

The effect of aspirin nanoemulsion (NE) in gastric tissue was studied compared with conventional aspirin. Conventional aspirin formulation induces pronounced oxidative damage and triggers the release of reactive oxygen species harmful to the stomach. A total of 24 male rats were used in the study. The effects of the aspirin were determined by the measuring the TNFα, iNOS, prostaglandin E2, and malondialdehyde levels, and also the glutathione, glutathione reductase, glutathione peroxidase, catalase, and superoxide dismutase. The 30-mg/kg aspirin NE showed superior protection and less injury to the gastric mucosa, which may be caused by rapid emptying of the fine oil droplets from the stomach, wide distribution of the drug throughout the intestinal tract, and increased drug-retention time in the desired region [52].

Another study was using O/W nanoemulsion and water-in-oil-in-water (W/O/W) nano multiple emulsion formulations containing aspirin (60 mg/kg). Both formulations were generated by an ultrasound process, and the anti-inflammatory and analgesic activities were investigated. Compared to the reference suspension, the nanoemulsion showed a 1.4- to 2.2-fold higher anti-inflammatory and analgesic effects, while the nano multiple emulsion resulted in mild inhibitory effects in the different experimental animal model tests. These results suggest the use of nanoemulsion and nano multiple emulsion as dosage forms for treating various diseases associated with inflammation and pain [53].

An interesting research of malaria therapy, based on O/W nanoemulsion for primaquine and clotrimazole oral administration, reported considerably reduced drawbacks compared to the existing therapies. These therapies show severe side effects and emergence of resistance to anti-parasitic drugs, as a consequence of complex and prolonged drug administration regimens. The oral nanoemulsions of both drug substances showed increased efficacy at lower oral doses when compared to their suspension forms. The efficacy was attributed to an increased oral bioavailability of the nanoemulsions [54, 55].

As mentioned before in section 5 an optimized and stable O/W nanoemulsion (NE) of ezetimibe was achieve. The drug absorption of ezetimibe NE in Albino Wistar rats resulted in a 3.23-fold increase in bioavailability compared to drug suspension and a 4.77-fold increase in bioavailability compared to the conventional tablet [38].

The same authors studied in the subsequent year, a new ezetimibe formulation containing O/W nanoemulsion formulated with Capryol 90 (10%, v/v), Cremophor EL (11.25%, v/v) and Transcutol P (33.75%, v/v), as oil and surfactant phases, respectively. This new optimized version presented higher release and bioavailability of ezetimibe compared to the previous formulation. According to the authors, it may be due to the presence of Cremophor EL known to be a potent inhibitor of P-gp over Tween 80, and Transcutol P as a permeability enhancer [56]. This enhanced version of ezetimibe nanoemulsion demonstrates the versatility of the nanoemulsion as a carrier system to be easily reformulated, according to the trends or needs.

6.1.2.2. Polymer Associated Nanoemulsions

Curcumin nanoemulsions, stabilized with octenyl-succinic-anhydride (OSA)-modified starch, were used as templates (core materials) and coated with an ultrathin polymeric film using a partially deacetylated chitosan (degree of deacetylation: 93.4%, with average molecular weight of 100 kDa), and Na-carboxymethyl cellulose (CMC), as cationic and anionic polyelectrolytes, respectively. A high-intensity ultrasonic homogeniser was applied to distribute these polymeric multilayer shells around the pre-formed curcumin nanoemulsion template. The aim of this invention was to overcome the drawbacks associated with the conventional nanoemulsions, such as stability and shelf life, by the creation of a barrier between the oily core and external environment. At appropriate sonication conditions, the final polymeric multilayer nanoemulsion

was measured with a mass ratio of the medium chain triglyceride (density: 0.940 g/mL), (OSA)-modified starch, curcumin, chitosan and CMC of 188:57:1.2:1.5:2. The increase in mean diameter, polidispersivity index and zeta potential of this final multilayer nanoemulsion were 159.85 ± 0.92 nm, 0.140 ± 0.01 and -17.2 mV, respectively. Additionally, minimum aggregation was observed at 4 °C for 4 weeks [57].

Biocompatible biopolymers composed of whey protein isolate, soybean protein isolate or beta-lactoglobulin were employed to develop stable nanoemulsion templates [58]. Shell crosslinking was carried out by incorporation of the inorganic crosslinker (Ca2+), which binds to the adsorbed proteins at those o/w interfaces in a one-step process. Fenofibrate, a highly lipophilic drug that is clinically used to lower lipid levels, was incorporeted into this novel shell-crosslinked nanocapsule system based on nanoemulsion templates with excellent stability and drug-loading capacity. The Powder X-Ray Diffraction (PXRD) and Differential Scanning Calorimetry (DSC) test of the freeze-dried nanocapsule indicated the solubilized state of the drug in the lipid core. Upon contact with water, it was easily dispersed and re-established into the original form [59].

6.2. Nanoemulsions for Parenteral Route of Administration

6.2.1. Drug Uptake Pathway

Among the parenteral route of administration, the intravenous one directly delivers drugs into blood stream [57]. Hence, once the drug is introduced into the bloodstream, it is distributed systemically via the vascular and lymphatic systems, followed by distribution into tissues depending on the blood flow and, i.e., particle size [60].

Nevertheless, upon introducing into the circulatory system, the nanoemulsion containing the drug may interact with erythrocytes, plasma proteins (opsonins), immune cells (monocytes, platelets, leukocytes, and dendritic cells), and tissue resident phagocytic cells like Kupffer cells in liver, dendritic cells in the lymph nodes, macrophages, and B cells in the spleen [1]. The opsonins (*e.g.*, immunoglobulin γ, complement factors and fibrinogen) primarily binds to the surface of foreign particles or surfaces, attracting immune cells and macrophages. The uptaked drug by *e.g.* macrophages will be forwarded to endogenous clearance mechanism, affecting its circulation time and efficacy [1, 20, 60, 61]. Opsonization can be evaded by a number of strategies within the drug formulation development, such as by modifying size, charge, and hydrophilicity [20]. Thus, to achieve the desirable extravasation of the drug into distant cells or organs, it is crucial to consider the size, the charge of the particle, surface properties (hydrophilicity), as well as the diameter of the "window" to enter in the extracellular space of the desired cells [20, 62].

6.2.1.1. Size

Smaller particles are known to escape phagocytosis in the reticuloendothelial system [1], predominantly in the liver and spleen. Concurrently the systemic circulation drug should be large enough to prevent their rapid leakage into blood capillaries [25]. Particle sizes on the order of 1 - 20 nm show improvement in circulation half-time [60, 61], and the particles about 30 - 100 nm administered by local injection avoid leakage into capillaries. [60]. Particles larger than 100 nm are quickly captured by the cells of the mononuclear phagocyte system (MPS) [1] (Fig. **2**).

6.2.1.2. Charge

The surface charge also influences the behaviour of the drug substance, considering controlling clearance response. Positive charged particles show a greater degree of phagocytosis, followed by negative charged particles, and the lowest degree for the non-charged particles [1, 20].

6.2.1.3. Hydrophilicity

Owing to the hydrophobicity of the particle surface, the blood serum proteins easily adhere to that surface. A number of studies regarding the use of the hydrophilic PEG chain showed a substantial reduction in the rapid clearance of the particles into the MPS. PEGylation generated a steric repulsion effect, creating a neutral charged particle shield, reducing adsorption of opsonins and other serum proteins on the particle surface [1, 25, 60, 61].

6.2.1.4. Shape

Although their findings have been limited, some studies have reported on the influence of particle shape on drug delivery. Rod-like structures and filamentous micelles showed longer circulation time and higher cellular uptake efficiency compared to the other particle shapes [63].

After passing through the blood circulation barriers, nanoemulsions need to overcome multiple membranes to reach intracellular structures and to be delivered at the desired intra cellular site [1, 60]. The nanoemulsion are uptaken via phagocytosis, macropinocytosis, or receptor-mediated endocytosis. Phagocytosis takes in larger particle sizes up to 10 μm, pinocytosis involves ingestion of sub-micron particles and substances in solution. Receptor-mediated endocytosis is constituted by high number of receptors generating higher selectivity for specific uptake by cells, which transduce a signal to the intracellular space, or can also lead to internalizing the ligand and its attached nanoemulsion vesicle by endocytosis [60].

Interesting phenomena occur in tumor tissues to which a drug substance can be transported both by a passive or active targeting mechanism. The tumor tissues due to the physiological mechanisms such as rapid angiogenesis generate a specific microenvironment such as more acidic pH, abnormal basement membranes, 'leaky'

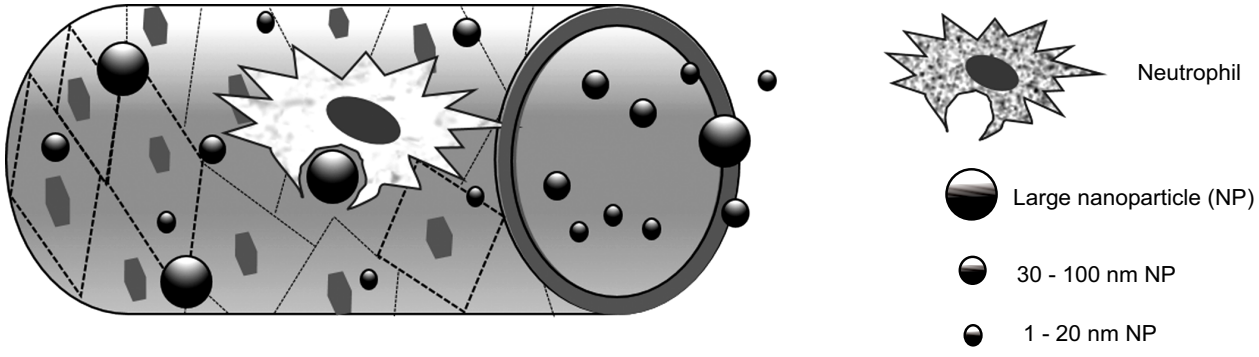

Fig. (2). Nanoemulsion sizes and clearance process [60].

endothelial cells, enhanced vascular permeability and poor lymphatic drainage. This enhanced permeability and retention (EPR) effect of the tumor environment provides favorable distribution and extravasation of the particular drug delivery systems into the tumor tissues [1, 60]. Besides this passive mechanism, it is possible to develop active targeting strategies using specific biological markers such as Arg-Gly-Asp (RGD) peptide, an antibody, or a nanoparticle-sized bioconjugates for cancer targeting [60].

6.2.2. Nanoemulsion Applications in Parenteral Administration

6.2.2.1. Non-Ionic Nanoemulsions

Aiming to develop a safe and immunologically more potent novel adjuvants and vaccine delivery systems, squalane-containing nanoemulsions (NE) and poly(D,L-lactide-co-glycolide) nanoparticles (PLGA-NP) using CHrPfs25 (a malaria transmission blocking vaccine antigen) were formulated. These preparations were evaluated via intramuscular (IM) route in mice, and the transmission-blocking efficacy of antibodies was analysed by standard mosquito membrane feeding assay using purified IgG from immune sera. Among different concentrations of NE and PLGA-NP, results showed the highest antibody response from CHrPfs25 formulated in 4% NE, compared to 8% NE and PLGA-NP. No further increases were observed by combining NE with monophosphoryl lipid-A (MPL-A) and chitosan. Further pre-clinical and clinical tests need to be performed; CHrPfs25 nanoemulsion may be a potential candidate as a Pfs25 vaccine adjuvant, as an alternative to existing aluminum salts [64].

Several spectroscopic studies were done aiming to better understand the protein-nanoemulsion interaction through their binding conformational alterations. Human serum albumin (HSA), which is considered to be the major soluble protein constituent of the circulatory system, delivering several nutrients in blood plasma, has excellent acceptor capacity with a wide range of molecules. Bovine serum albumin (BSA) has 76% sequence identity shared with HSA. Hence, the effect of neem oil nanoemulsion (NE) of different concentrations with both proteins was investigated using UV and fluorescence analysis. It was concluded that the binding mechanism of neem oil NE and serum albumins were governed by non-fluorescent ground state complex formation between them. FT-IR spectroscopy and circular dichroism spectral change studies suggest possible conformational changes in the alpha-helical and aromatic amino acid residues of the biomolecules. Also, this leads to the formation of more stable complexes between BSA - neem oil NE compared to HSA. This study provides information to develop protein-loaded nanoemulsions for improving the efficacy of drug delivery, bio-sensing and other clinical applications [65].

The inhibition mechanism of lung cancer cells (A549 and H460) by curcuminoid extracts and nanoemulsions prepared from *Curcuma longa* Linnaeus were evaluated. A high stable nanoemulsion composed of curcuminoid extract, Tween 80, and water, with 12.6 nm mean particle size was developed by sonication process. Both, curcuminoid extract and nanoemulsion treatments, demonstrated similar results in the expression of proteins correlated with the cell cycle and apoptosis in these two cells lines. However, the H460 cells were more susceptible to apoptosis than A549 cells for both treatments, and A549 cells showed a dose-dependent increase in cyclin B expression for both treatments while a reversed trend was found for H460 cells. In this study, the two cell apoptosis pathways, mitochondria pathway (intrinsic pathway) and death receptor pathway (extrinsic pathway), seems to be responsible for apoptosis of both A549 and H460 cells [66]. Azarmi *et al* showed that A549 cells are resistant to doxorubicin while H460 cell are not. However, drug loaded nanoparticles could overcome drug resistance. A similar mechanism might play a role in the curcurmin study [67].

The natural flavonoid fisetin (3,3′,4′,7-tetrahydroxyflavone) was incorporated into a nanoemulsion to improve its pharmacokinetics and antitumour therapeutic efficacy. A nanoemulsion with 153 ± 2 nm oil droplet diameter, composed of Miglyol® 812N/Labrasol®/Tween® 80/Lipoid E80®/water, was stable at 4 ◦C for 30 days. Although no difference compared to free fisetin was observed by pharmacokinetic studies in mice, when injected intravenously, the nanoemulsion showed a 24-fold increase in fisetin bioavailability than free fisetin when administered intraperitoneally. In addition, the antitumour activity of the fisetin nanoemulsion in Lewis lung carcinoma bearing mice occurred at lower doses (36.6 mg/kg) compared to free fisetin (223 mg/kg) [68].

The design of parenteral lecithin-based nanoemulsions intended for brain delivery of risperidone, an antipsychotic drug, was performed applying a general factorial experimental design. Risperidone-loaded nanoemulsions (mean size about 160 nm, size distribution <0.15, zeta potential around -50 mV), containing sodium oleate in the aqueous phase and Polysorbate 80, Poloxamer 188 or Solutol1 HS15 as co-emulsifier, were produced by hot homogenisation. Their ability to improve risperidone delivery to the brain was assessed in rats. A promising nanocarrier for brain selective delivery purpose was shown to be the Polysorbate 80-costabilized nanoemulsion with increased risperidone brain availability (1.4-7.4-fold higher) compared to other nanoemulsions and drug suspension. These differences in pharmacokinetic results, when administrated intraperitoneally, are probably due to their different droplet surface properties (different composition of the stabilizing layer), which determined the blood-brain barrier passage of risperidone [69].

6.2.2.2. Polymer Associated Nanoemulsions

An interesting study compared the therapeutic properties of polymeric micelles and nanoemulsions generated from micelles in pancreatic tumor bearing mice. The mice were treated with paclitaxel (PTX) loaded polymeric micelles, or corresponding perfluorocarbon, a halogen-substituted carbon nonpolar oil, nanoemulsions. Two structures of the polymeric block were compared: poly(ethylene oxide)-co-poly(D,L-lactide) (PEG-PDLA) and poly(ethylene oxide)-co-poly(L-lactide) (PEG-PLLA), on which the first generated micelles with elastic amorphous cores, while micelles with solid crystalline cores were formed in the second one. Micelles and nanoemulsions stabilized with PEG-PDLA copolymer demonstrated higher therapeutic efficacy than PEG-PLLA copolymer derivative micelle or nanoemulsion. This is probably due to the elastic physical state of the micelle cores (or droplet shells), allowing drug release via diffusion and/or copolymer biodegradation. PEG-PDLA stabilized formulations showed lower drug resistance in tumors than PEG-PLLA stabilized formulations, maybe due to the presence and preventive effect of copolymer unimers that were in equilibrium with PEG-PDLA micelles. Additionally, PEG-PDLA stabilized nanoemulsions showed lower systemic toxicity than corresponding micelles, suggesting higher drug retention in circulation [70].

A similar study was conducted comparing PTX loaded PEG-PDLA micelle with PEG-PDLA perfluorocarbon nanoemulsions. Polymeric micelle resulted in faster extravasation and tumor cell internalization than nanoemulsion, although the authors emphasized the need to optimize both drug retention and carrier diffusion parameters for a development of an ideal drug carrier [71].

6.2.2.3. Drug-Conjugated Nanoemulsions

Novel FA-functionalized PEGylated BSA nanoemulsions, loaded with CORM-2 (Carbon monoxide releasing molecule-2), were tested both, *in vitro* and *in vivo*. FA (folic acid) and FA conjugates are known to bind with high affinity to folate receptor (FR)-alpha and -beta and enter FR-expressing tumor cells by receptor-mediated endocytosis. For the nanoemulsion preparation, firstly CORM-2, which induces an antiproliferative effect on human cancer cells, was incorporated in the oil phase. This oil phase (vegetable oil) was emulsified with the aqueous phase containing BSA-FA (Folic Acid) conjugate solution and a PEGylated surfactant, by

high-pressure homogenisation. The obtained small and stable (FA)-tagged protein nanoemulsions were then evaluated in terms of specific uptake using a lymphoma cell line (A20 cell line). Results showed that the folic Acid (FA)-tagged protein nanoemulsions were preferentially internalized in the B-cell lymphoma cell line (A20 cell line), promoting them as promising nanocarriers for the selective delivery of drugs to a target cell population that express FR. Therefore, these functionalized nanocarriers constitute attractive alternatives to ameliorate the side effects and low efficacy of conventional cancer treatments [72].

A functionalized bovine serum albumin (BSA) nanoemulsion was produced with BSA-drug conjugates, either methotrexate (MTX), a potent anticancer agent, or vancomycin (VCM), a potent antibiotic, as a drug. BSA-folic acid (FA) conjugates were also produced resulting in effective FA-tagged nanoemulsion for specific FR-mediated targeting in a KB cancer cell line. BSA-drug conjugated nanoemulsions showed by *in vitro* analysis, effectiveness with improved half-life in systemic circulation, offering a good and flexible template for a wide range of medical applications [18].

Highly stable BSA nanoemulsions were produced by high-pressure homogenisation using a tri-block copolymer (Poloxamer 407). This copolymer presents a central hydrophobic chain of polyoxypropylene (PPO) and two identical lateral hydrophilic chains of polyethylene glycol (PEG). A linear correlation between this surfactant concentration and the resulting nanoemulsion's size was observed by TEM imaging. Further, the neutral and hydrophilic surface of the generated PEGylated nanoemulsion provides stealth particles that are less phagocytized, with a longer half-life in systemic circulation. The incorporation of BSA-FA (folic acid) conjugate solution in this system generated FA-PEGylated nanoemulsions which favors specific cell uptake mediated by folate, indicating 5-fold higher internalization of these tagged-nanoemulsions by cells than non-targeted PEGylated nanoemulsions. The absence of cytotoxicity associated with these attributes provides ideal characteristics to recommend this new functionalized Folic Acid (FA)-tagged protein nanoemulsion as a promising vehicle for targeted drug delivery into diseased tissues [25].

6.3. Nanoemulsions for Topical Route of Administration

6.3.1. Drug Uptake Pathway for Topical Administration

Drug penetration through the skin involves several challenges in this natural barrier that extends from the external to internal layers. Skin is composed of stratum corneum as the most external layer, followed by the epidermis, dermis, and subcutaneous tissue. There are three penetration pathways by topical applications, which consist of intercellular, hair follicle and transcellular pathways. The cement-like structure among the keratinized cells represents an effective natural barrier against external substances. The small particle size as well as the lipid nature of nanoemulsions favors efficient penetration of active compounds via topical application [73, 74].

6.3.2. Nanoemulsion Applications in Topical Administration

6.3.2.1. Non-Ionic Nanoemulsions

Fennel essential oil nanoemulsion (FEO NE) was employed as a transdermal drug delivery system aiming to achieve effective prolonged antidiabetic activity. HPLC analysis showed 64% loading efficiency for *trans*-anethole, with promising results in thermodynamic stability, conductivity, pH, particle size and zeta potential of the obtained nanoemulsion. FEO NE showed superior permeation profiles for 24 h, and also a high potential of reducing plasma glucose levels in rats which continued for 7-days after a single topical application of a dose of 120 mg/kg of FEO [75].

Cumin essential oil was loaded in transdermal nanoemulsion to acquire efficient and prolonged systemic antioxidant and hepatoprotective activities. Among the formulations that revealed good thermodynamic stability and physicochemical properties, the most promising one resulted in the best *in vitro* and *in vivo* antioxidant efficiency, provided high hepatoprotective potential and reserved rats' body weight loss after a period of seven days of a single transdermal application [76].

A nanoemulsion (NE) gel of meloxicam (MLX), a non-steroidal anti-inflammatory drug, was developed as transdermal delivery system. Percutaneous absorption studies on rat skin demonstrated a higher permeation of meloxicam from NE gel than the drug solution. MLX-NE gel showed to be non-irritant, biocompatible, and also provided the maximum inhibition of paw edema in rats over 24 h compared to MLX solution [77].

6.3.2.2. Polymer Associated Nanoemulsions

Eucalyptus oil nanoemulsion was impregnated into chitosan to develop a biopolymer film for wound management studies. The film with and without nanoemulsion was evaluated against *Staphylococcus aureus*, and higher antibacterial activity was obtained from the nanoemulsion-impregnated chitosan film [78].

6.4. Nanoemulsions for Mucosal Route of Administration/ Mucoadhesive Nanoemulsions

6.4.1. Drug Uptake Pathway

Composed of an epithelial layer, which varies by types, mucosal tissues yield a barrier for natural body cavities from external environment. Mucins, high molecular weight glycoproteins, heavily glycosylated (50 to 80%), which are responsible for adhesion phenomena, are the major component of mucus, usually secreted by goblet cells [43]. Intranasal and ocular routes, gained considerable attention in recent years due to its direct, efficient and non-invasive delivery system [79].

Intranasal administration pathway transports drugs by a well-vasculated cavity covered by thin nasal mucosa. The drug reaches systemic circulation without undergoing intestinal and hepatic metabolism, by the epithelial cell layer through transcellular, paracellular, carrier-mediated or transcytosis route. Targeting the blood-brain barrier (BBB) by directly transferring the drug from the nose to the central nervous system (CNS) is one of the particular benefits of this route, aside from local and systemic drug delivery [79].

The property of mucin, a component of the tear film, can improve the retention time of ophthalmic preparations. Thus, enhancing the bioavailability of the drug [43, 80]. Nevertheless, the natural barriers and the defence mechanisms of the eye are responsible for the reduced ocular residence time and the low bioavailability of the conventional products. The cornea and lacrimal film provide an efficient barrier against ophthalmic treatment [81].

Although several limitations, such as safety approval of components and application dosages for mucosal administration still remain, progress in nanoemulsion research offers interesting uses as mucosal drug delivery system, due to their small particle size, lipophilic-hydrophilic properties and composition flexibilities.

6.4.2. Nanoemulsion Applications

6.4.2.1. Non-Ionic Nanoemulsions for Intranasal Route

Saquinavir mesylate (SQVM) nanoemulsion (NE) was administrated by intranasal route to enhance central nervous system (CNS) targeting of this anti-HIV drug. NE composed of Capmul MCM, Tween 80, PEG 400 and SQVM was prepared by spontaneous emulsification process. SQVM-NE showed a higher permeation rate compared to a suspension administered to sheeps nasal mucosa. The nasal route showed no significant adverse effects, a higher drug concentration in brain was also observed compared to an intravenous injection of a suspension. Gamma scintigraphy imaging showed a larger drug transport into the CNS, when this SQVM-NE was administrated by intranasal route to rats [6].

6.4.2.2. Polymer Associated Nanoemulsions for Intranasal Route

Intranasal administration of nanoemulsions was highlighted as an innovative drug delivery system, to overcome some of the known drawbacks of the oral route of administration. The presence of a direct nose-to-brain transport pathway that bypasses the normal BBB pathway via the systemic circulations and the BCSFB (blood-cerebrospinal fluid barrier) has been reported, and an interesting intranasal drug delivery system was described in a review [43]: a mucoadhesive nanoemulsion containing risperidone, an approved antipsychotic drug, was prepared by the spontaneous emulsification method and subsequent addition of chitosan. Superior efficacy on brain/blood uptake ratio of risperidone was observed when a mucoadhesive nanoemulsion was used and compared to a non-mucoadhesive ones or a drug solution [82, 83].

Olanzapine, a novel antipsychotic agent, was loaded in nanoemulsions containing Capmul MCM, Tween 80, ethanol and polyethylene glycol. This olanzapine nanoemulsion (ONE) was coated with chitosan to prepare a mucoadhesive nanoemulsion (OMNE). The OMNE showed the highest drug targeting efficiency (DTE%) and direct nose-to-brain transport (DTP%) in rats among the tested formulations, followed by ONE and thirdly by olanzapine suspension (OS). OMNE showed nearly 2-fold higher DTP% than OS. These results demonstrated the benefit of mucoadhesive nanoemulsion formulation as effective brain targeting of olanzapine [5].

6.4.2.3. Cationic Nanoemulsions for Ocular Treatment

A positive charged, cationic nanoemulsion was formulated to deliver dexamethasone acetate (DEX) and polymyxin B sulfate (polymyxin B) for treating ophthalmic infection. Narrow droplet size-distribution and average droplet size below 200 nm were obtained by high-pressure homogenisation. The *in vitro* test demonstrated the mucoadhesion efficacy by electrostatic interaction between this cationic nanoemulsion and the negatively charged mucin, which coats the corneal surface. This innovative "amphiphilic cationic concept" offers highly ocular bioavailable solution for treating ophthalmic infections and an array of ophthalmic products [80].

6.4.2.4. Nanoemulsion as Intranasal Vaccine Adjuvants

Recent research highlights nanoemulsion as a promising candidate as mucosal vaccine adjuvants, although further studies are needed to better understand its safety and the mechanisms of mucosal immune response. The mucosal membrane is a large surface area for pathogens to enter, which also makes it a promising pathway for immunization. Nanoemulsion may provide long-term release properties for antigens, non-invasive immunity and stability of antigens for mucosal and systemic immunization [79, 84, 85]. A series of nanoemulsions composed of combination of cationic and nonionic surfactants, co-solvents and soybean oil was developed as mucosal vaccine adjuvants. The physicochemical properties of formulations containing cationic surfactants demonstrated to be a key factor to modulate nanoemulsion adjuvant activities. Thus, this may support the development of customized adjuvants for specific needs to trigger appropriate immune responses [85].

6.5. Nanoemulsion Applications for Imaging-Guided Therapy

The application of nanoemulsion composed of, *e.g.*, perfluorocarbon (PFC) in Magnetic Resonance Imaging (MRI) is an emerging concept as a non-invasive imaging analysis system. These nanoemulsions migrate to injured tissues by natural defense mechanisms such as phagocytosis, and acting then as marker agents [86, 87]. The association of targeting, therapeutic and diagnostic functions provides the so-called "theranostic" nanomedicine. These multifunctional nanoemulsions may act as a promising imaging therapy for tumor detection and treatments [88].

6.5.1. Non-ionic Nanoemulsions

The application of nanoemulsion in image-guided therapy showed enormous promise in cancer medicine in the past decade. A "theranostic" platform based on oil-in-water nanoemulsions, loaded with hydrophobic glucocorticoid prednisolone acetate valerate (PAV), iron oxide nanocrystals for MRI, and fluorescent dye Cy7 for near-infrared fluorescence imaging (NIRF), was developed and evaluated in a colon cancer mouse model. All of the PAV nanoemulsion-treated animals showed a significant drug substance accumulation in tumors cells by MRI and NIRF; in addition, a potent inhibitory effect was observed on the tumor growth profiles compared to the control nanoemulsion-treated animals, representing a flexible and unique theranostic platform for image-guided therapy of cancer [88].

A stable, non-toxic, theranostic nanoemulsion of celecoxib, an anti-inflammatory drug, was developed to target macrophages. The mouse inflammation model induced with complete Freund's adjuvant (CFA) showed greater accumulation of celecoxib nanoemulsion in the inflamed vs. control paw. This innovative system is able to simultaneously delivery the drug to macrophages and monitor macrophage migration patterns by optical imaging [4].

7. PERSPECTIVES

Rationally designed nanoemulsion formulations with benefit-cost ratio and low side effects are emerging to address the low bioavailability of poorly water-soluble drugs, allowing optimized and effective drug delivery systems. The challenges for future developments comprise the further understanding of the mechanisms that make nanoemulsion more efficient than conventional drug formulations. These challenges refer to the elucidation of the interactions between the drug and nanoemulsion's components; the investigation of the impact of the manufacturing process on the formulation composition and drug stability. Also, a better understanding of the influence of nanoemulsion formulation on drug release and drug uptake by different routes of administration is needed. Exploring these mechanistic insights, opens opportunities to enlighten future and rising wave of advanced nanoemulsion developments. These attributes confirmed nanoemulsion as a prospective drug carrier with extensive application to a broad array of opportunities.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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