



Teaser Nanoemulsions are among the most promising future drug carriers with remarkable potential for the brain delivery of a wide variety of drugs.



Nanoemulsions in CNS drug delivery: recent developments, impacts and challenges

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Despite enormous efforts, treatment of CNS diseases remains challenging. One of the main issues causing this situation is limited CNS access for the majority of drugs used as part of the therapeutic regimens against life-threatening CNS diseases. Regarding the inarguable position of the nanocarrier systems in neuropharmacokinetic enhancement of the CNS drugs, this review discusses the latest findings on nanoemulsions (NEs) as one of the most promising candidates of this type, to overcome the challenges of CNS drug delivery. Future development of NE-based CNS drug delivery needs to consider so many aspects not only from a physicochemical point of view but also related to the biointerface of these very small droplets before achieving clinical value.

Introduction

Neurological disorders contribute to 11% of the global burden of disease and this number is anticipated to rise in the coming years owing to an aging global population. Nowadays, significant research efforts have been devoted to the development of improved therapies for central nervous system (CNS) diseases. However, despite tremendous efforts, treatment of CNS diseases has been limited by the inability of the majority of neuroactive drugs to reach CNS compartments in sufficient amounts [1]. Many potential drugs, despite being effective at the desirable site of action, have been discarded through clinical studies because of the limited entry to the brain parenchyma. Two physiological and biochemical barriers [i.e., the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB)] are responsible for the limited CNS access by the therapeutic agents [2]. The BBB and BCSFB are conceptualized by the brain microvessel endothelial cells (BMECs) and the epithelial cells of the choroid plexus, respectively. The BBB is, in fact, a specialized structure that controls the passage of materials from the systemic circulation into the very sensitive and vital CNS tissues. However, it also excludes various pharmacologically active agents from being available in an effective amount.

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Despite this worrying picture, significant evolution by introducing new drug delivery systems (DDSs), with the aim of effective drug transport to the CNS, have been reported with many promising results. Among others, various nanotechnology DDSs have been explored as carriers to bypass the BBB efficiently [3,4]. The present review will provide insights into the possible applications of nanoemulsion (NE)-based systems, one of the most promising types of nanocarriers, for the enhancement of CNS delivery of bioactive agents intended to find new solutions for therapy of hard-to-treat CNS disorders, particularly brain cancers and neurodegenerative disorders.

Biological aspects in CNS drug delivery

Blood–brain barrier

The BBB, the key barrier between the CNS and the peripheral circulation, is the homeostatic defense mechanism of the brain that regulates the traffic of immune surveillance cells (macrophages), xenobiotics and endogenous compounds in and out of the CNS. The functional complexity of the BBB is caused mostly by several biochemical-regulating mechanisms expressed in cerebral endothelial cells and the presence of the specialized tight junction structures between them. In particular, tight junction proteins (i.e., claudins, occludin) and adherent junction proteins (i.e., junctional adhesion molecule) create the high transendothelial electrical resistance ($>1500 \Omega \text{cm}^{-2}$ compared with $3\text{--}33 \Omega \text{cm}^{-2}$ of other organs) that limits the transport of hydrophilic molecules [5,6]. They are linked to the actin cytoskeleton, thereby forming the most intimate cell–cell connection. BMECs have several unique structural features such as the absence of fenestrations and very limited numbers of pinocytotic vesicles; but they possess more mitochondria. In addition, there are other obstacles against the passage of solutes across BMECs, including degrading enzymes: ecto- and endo-enzymes [7]. These morphological and functional features, collectively, result in limited transcytosis and endocytosis while supporting the low permeability of the BBB for many substances. There are other additional structures such as pericytes, astrocytes and a discontinuous basal membrane (or basal lamina), assisting BMECs in their specialized barrier action. The pericytes are adjacent to the brain capillaries along the basal lamina and play important parts in the regulation of CNS homeostasis, BBB integrity, macrophage activity and modulation of blood flow [8]. The basal lamina is situated between brain capillaries and the pericytes and consists of laminin, fibronectin, tenascin, collagens and proteoglycan. It serves as an extracellular matrix providing a scaffold for cell migration, mechanical support for cell attachment and separation of adjacent tissue. Another important supportive cell-type involved in the BBB function are astrocytes, which entirely envelop BMECs and pericytes with their end-feet. Recent studies have revealed that astrocytes are important in modulating the BBB function by regulation of water and ion homeostasis and secretion of chemokines as a sensor of pathologic changes.

Blood–cerebrospinal fluid barrier

The BCSFB barrier, known as the secondary barrier against drug penetration into the CNS, is composed of choroid plexus epithelial cells. The choroid plexus consists of a highly vascularized, ‘cauliflower-like’ structure with frequent villi that project into all

four cerebral ventricles [9]. In contrast to the BBB, the capillaries of the choroid plexus are fenestrated and noncontinuous with an obstruction surrounding them made by a monolayer of polarized epithelial cells linked together by tight junction proteins. These tight junctions are a major obstacle against passage of most macromolecules and ionized materials. The cerebrospinal fluid (CSF) secreted from the choroid plexus epithelial cells fills the ventricles of the brain, the spinal canal and subarachnoid space. CSF secretion is $350 \mu\text{l}/\text{min}$ in an adult male, which represents a turnover rate of $0.4\% \text{min}^{-1}$. The secretion of CSF maintains the concentration of substances that can penetrate the brain via passive diffusion in an extent lower than plasma – a phenomenon known as the CSF sink effect. The sink effect is greater for large molecular weight and hydrophilic compounds. In addition, the presence of ion channels, transporters and receptors has been reported at the BCSFB [10]. The penetration of a drug molecule across these two barriers depends on several physicochemical properties mainly including lipophilicity, size and degree of ionization.

Pathways across brain barriers

In general, penetration of materials into the brain can be classified into two main categories of paracellular and transcellular paths. Only small hydrophilic materials can diffuse through the tight junctions but not to a high degree. However, owing to the unique properties of the tight junctions, paracellular transport of hydrophilic drugs is virtually absent and transcellular transport by passive diffusion is only available to molecules that fulfill certain criteria such as: molecular weight $<500 \text{Da}$, $\log P \geq 2$, being non-ionized and cumulative number of H-bonds <10 [11]. Obviously, just a small proportion of therapeutics has these conditions. By contrast, it has been found that some of the nutrients such as blood glucose, proteins and peptides can easily cross the BBB [12].

A series of membrane transporter systems are expressed in the luminal and/or abluminal surfaces (i.e., blood-to-brain and brain-to-blood directions) of the BBB. These transporters are responsible for the different influx or efflux behaviors of the BBB for different substances exposed to this barrier. In addition, there are some carrier-mediated endocytosis and exocytosis mechanisms in the BBB and BCSFB. In carrier-mediated endocytosis the solutes bind to specific membrane protein carriers, then allow cell penetration via endocytosis. Mechanistically, a conformational transformation in the carrier protein takes place which provides the possibility for the solute to pass to the other side of the cellular membrane. The energy for movement of molecules against their concentration gradient is supplied by ATP. The main two classes of endocytosis are fluid-phase endocytosis (usually referred to as pinocytosis) and solid-phase endocytosis (known as phagocytosis) [13]. Fluid-phase endocytosis is a nonspecific uptake of extracellular fluids, driven by the concentration of the extracellular side. Although this type of endocytosis occurs at a very low level, this mechanism is assumed to be noncompetitive because there is no need for ligand binding. Receptor-mediated transcytosis (RMT) provides a means for selective brain uptake of small and large molecules such as hormones, growth factors, enzymes and plasma proteins. Because of the limited quantity of the receptors on the BMEC surface, RMT is, unfortunately, a saturable mechanism. RMT has been extensively studied for brain targeting. Adsorptive-mediated transport (AMT) is initiated by an electrostatic interaction between a

positive ingredient and the negatively charged plasma membrane surface. In contrast to RMT, AMT shows lower affinity and higher capacity. Macromolecules are concentrated before transportation by interaction with the BMEC surface, mainly via the clathrin-mediated mode. The advent of various CNS drug delivery technologies focuses on AMT, usually by exploiting the benefit of cationic proteins or cell-penetrating peptides. Moreover, cell-mediated transcytosis is an effective point of access for some pathogens for instance *Cryptococcus neoformans* and HIV into the brain, known as the 'Trojan horse' model. This mechanism depends on immune cells like monocytes or macrophages to reach the other side of the intact BBB [14]. Unlike the aforementioned transport paths, cell-mediated transcytosis can be applied virtually for any kind of molecules as well as particulate systems.

Nanoemulsions

NEs are colloidal dispersions consisting of two immiscible liquids stabilized by surface-active agents, with dispersed droplets of diameters in the range of 20 to 500 nm (Figure 1) [15]. In some publications NEs are also referred to as mini-emulsions, ultrafine emulsions or submicron emulsions. Because of their small sizes, NEs are considered to be kinetically stable systems, because the Brownian motion effects dominate gravitational forces, thus leading to higher resistance against droplet aggregation than conventional emulsified systems. It is important to differentiate NEs from microemulsions, in that the second one, being an equilibrium structure, is highly sensitive to any variations in temperature and chemical composition.

Furthermore, ionic or nonionic surfactants are frequently used in the NE structure to prevent flocculations via electrostatic and steric stabilization [16]. The main mechanism of instability of NEs is reported to be the Ostwald ripening. Because of the differences in Laplace pressure, the smaller droplets of NEs become gradually smaller in size until being dissolved or disappearing in the external phase. The large ones continue to grow and finally separate out, affecting the long-term stability of the dispersion.

Depending on the dispersed phase diameter and according to the appearance, NEs are classified into two groups: transparent or translucent (50–200 nm); and milky (up to 500 nm). In addition, depending on ingredients, internal and external phases, there are two main types of NE, referred to as biphasic [i.e., oil-in-water (O/W) or water-in-oil (W/O)] and multiple NEs (W/O/W). The relative volumes of internal and external liquids, termed as phase:volume ratio (Φ), determine NE droplet number as well as system stability. Generally, the internal phase is the phase present in a lower volume in comparison with the continuous phase, where increasing $\Phi > 40\%$ can result in phase inversion of a W/O NE to an O/W one. A multiple NE contains a dispersed phase that, in turn, includes another internal droplet of a different immiscible liquid phase.

Some excellent properties of NEs such as the colloidal dispersion of the hydrophobic therapeutic agents, cellular transport either by paracellular or transcellular routes, preventing the entrapped substances from hydrolysis and/or enzymatic degradation and, finally, their remarkable biocompatibility make them highly promising carriers for advanced drug delivery [17,18]. Additionally, in contact with the continuous phase (water), once the drug diffuses out of the oil droplets it undergoes nanoprecipitation and, according to the Noye–Whitney equation, this transformation increases the surface area of the drug leading to the highly accelerated and enhanced drug dissolution. Furthermore, the rate and extent of drug release can be easily modulated by varying the composition of nanodispersion to obtain a sustained or controlled release system. More interestingly, the very large interfacial area provides opportunities for more-advanced engineering to develop targeted DDSs to specific sites. Some typical examples of drug-loaded NEs aimed at brain delivery, related to the scope of the current study, are summarized in Table 1.

Components of a nanoemulsion

A typical NE is composed of at least three essential components: oil, water and an emulsifier, at appropriate ratios [19]. However,

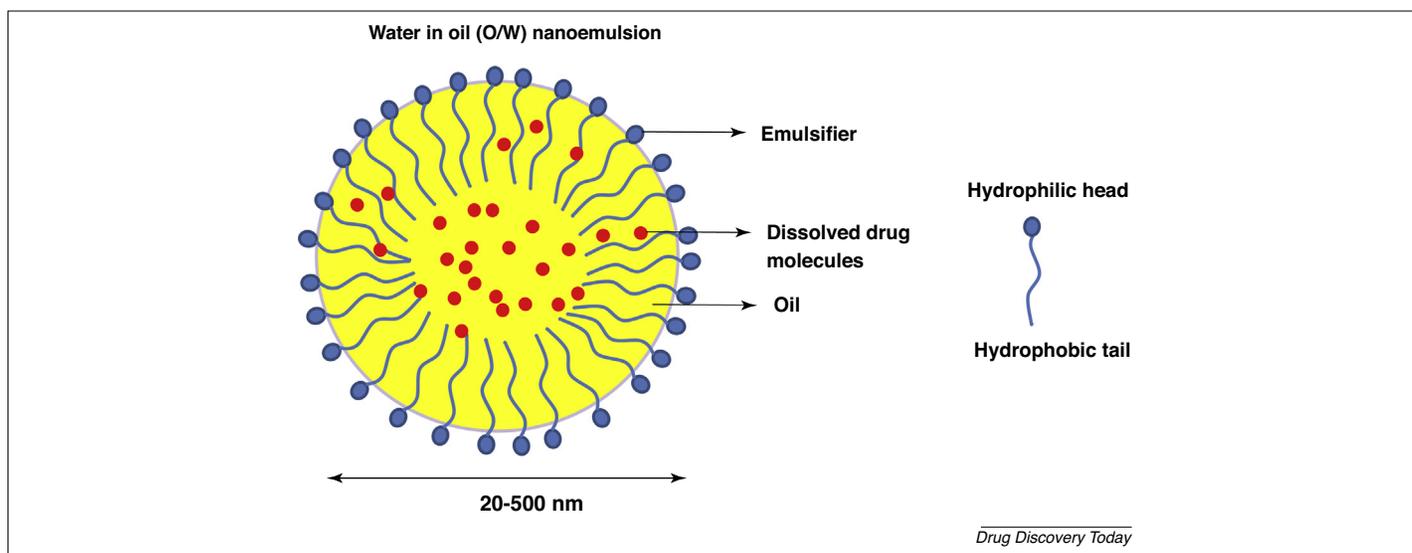


FIGURE 1

Schematic representation of an oil-in-water nanoemulsion system. Nanoemulsions are stable colloidal dispersions composed of two immiscible liquids, with the help of surface-active agents: 'emulsifiers'. Generally, the drug is dissolved in the internal phase of nanoemulsions.

TABLE 1

Examples of nanoemulsions intended for brain delivery

Drug	Composition	Method	Size (nm)	Outcomes	Refs
Carbamazepine	Soybean oil/ Lecithin/ 1-O- alkylglycerol	High-pressure homogenization	207.3 ± 0.8	Increased the brain availability 2.37 times compared to free drug	[93]
Paclitaxel	Pine-nut oil/ Lipoid™	Sonication	212.5 ± 1.6	Increase in cytotoxicity correlated with enhancement in apoptotic activity in U-118 cells treated with nanoemulsion of combination of paclitaxel and ceramide	[94]
Atovaquone	Grape-seed oil / Polysorbate 80	Spontaneous emulsification	20 ± 3	NE-AT efficiency was proven by increasing survival time and decrease parasite load in infected mice with <i>T. gondii</i> , RH strain	[83]
Curcumin	Linseed oil/ Lipoid™/ Polyethylene glycol	High pressure homogenizer	103 ± 11	Improvement of total antioxidant activity in brain about six times in comparison to curcumin solution in linseed oil	[95]
Carbamazepine	Soya oil/ Tetrahydrofuran	Sonication	95	Significant anticonvulsant activity against seizures and relative bioavailability of 160% compared with unmodified substance More-effectively penetrated through BBB by 1.5 times relative to unmodified substance	[96]
Curcumin	Labrafac® lipophile WL1349/ Solutol® HS15/ Transcutol®	Pseudoternary phase diagram	67 ± 6	IC ₅₀ of 16.41 μM on U87 cells compared to 24.23 μM of curcumin solution	[97]
Ginkgolide B	Soybean oil/ Lecithin/ Ethyllactate	Magnetic stirring	80–100	Memory improvement of rats with cognition impaired was confirmed Significantly inhibited acetylcholinesterase activity and enhanced the activity of choline acetyltransferase in the hippocampus	[98]
Docetaxel	Soybean oil/ Lecithin/ Poloxamer 188/ Glycerol/ Cholic acid sodium	High-energy homogenization	72.3	<i>In vivo</i> toxicity significantly lower than Taxotere® Ability to target glioma sites and prolong the median survival time of mice with gliomas	[99]
Diazepam	Medium-chain triglycerides/ Soybean oil/ Lecithin/ Tween® 80	High-pressure homogenization	195–220	Fast and intense initial distribution into rat brain, suggesting their applicability in urgent situations	[100]
Resveratrol	Orange oil/ Grape- seed oil/ Tween® 80	Magnetic stirring	86.4 ± 12.7	Induced neuroprotective benefits Showed preventive potential for postoperative cognitive dysfunction via the SIRT1 signaling pathway	[101]

the internal and external phases can contain other multiple ingredients. The solubility of the drug in the internal phase (oil) is an essential criterion for selection of oils in O/W NEs. Edible oils and their re-esterified fractions, generally referred to as semisynthetic medium-chain derivatives [long chain triglycerides (LCT), medium-chain triglycerides (MCT) or short chain triglycerides (SCT)] are frequently used to prepare NEs. D- α -Tocopherol, oleic acid and ethyl oleate have also been used in a variety of pharmaceutical NEs.

Emulsifiers are amphiphilic molecules capable of stabilizing the NEs by decreasing the interfacial tension (the surface energy per unit area) to below 10 dynes/cm between the oily and aqueous phases of the dispersion. Besides, they play a vital part in stabilizing NEs through repulsive electrostatic interactions and steric hindrance [20]. Usually, a surfactant is used as an emulsifier, but amphiphilic proteins, polysaccharides and lipids can also be applied in the formulation of NEs as a stabilizer. An appropriate emulsifier should be adsorbed easily around the internal droplets to form a complete and coherent film, thereby being effective at a fairly low concentration for the construction of the desired size of droplets, and also to optimize the zeta potential and viscosity of the system. It is commonly believed that surfactants with a low hydrophilic-lipophilic balance (HLB) value of 3–6 are favored in

preparation of W/O NEs, whereas emulsifiers with high HLB values (8–18) are ideal for O/W types. Some common emulsifiers employed in the formulations of pharmaceutical NEs are lecithin (phosphatidylcholine derived from egg yolk or soybean), Cremophor® EL (polyoxyl-35 castor oil), Tweens® (polyoxyethylene sorbitan monolaurate), Spans® (sorbitan monolaurate), poloxamers, sodium deoxycholate (bile salt), casein, gums, starch derivatives and block copolymers [21–23].

Methods of preparation of nanoemulsions

NEs are thermodynamically unstable owing to a positive Gibbs free energy of their formation. Therefore, preparation of NEs is not a spontaneous process, thus needing energy along with applying emulsifiers to stabilize the formed nanodroplets [20]. There are typically two approaches for production of NEs, classified as high-energy emulsification and low-energy emulsification. High-energy procedures involve applying a mechanical device to create great disruptive forces to break down the internal phase, then dispersing it into the continuous phase. Some of the main methods of this group are: stirring, ultrasonication, high-pressure homogenization, microfluidization and membrane emulsification [24,25]. In low-energy methods oil nanoglobules start to grow via changing the condition of a mixed oil-water-surfactant system. The latter

method has definite phase inversion temperatures, emulsion inversion points and spontaneous emulsification conditions [26,27].

Main advantages of nanoemulsions in CNS drug delivery

Impact on drug solubility

Solubility in biological environments is one of the important factors in meeting the therapeutic role of active substances. According to the Biopharmaceutical Classification System (BCS), Class II and Class IV have a solubility limitation in aqueous media. For this reason, they are not able to fulfill their therapeutic activity in low concentrations, thus needing higher doses to achieve an appropriate pharmacological activity [28]. BCS Class II drugs do not have a particular problem in terms of membrane permeability with the only crucial issue being their aqueous solubility, which can be improved by using solubility-enhancement techniques – in particular the use of nanocarriers. BCS Class IV drugs, however, suffer from a limited permeability across biological membranes in addition to the aqueous solubility problem. Therefore, to improve the biological functions of this category, it is necessary to focus on increasing solubility and permeability at the same time. Given the complex structure of the BBB mentioned earlier, the chance of a substance entering the brain is limited by physicochemical as well as biochemical constraints, thereby favoring fat-loving substances with a molecular weight of 500 Da or lower. Therefore, it seems that the use of lipophilic nanocarriers, like NEs, could be a good option for loading lipophilic and amphiphilic drugs with BBB penetration problems to deliver them to the brain. By contrast, besides the high loading capacity, they can be used as targeted drug delivery carriers for the CNS active therapeutics owing to the possibility of particle engineering and surface modification. So far, several studies have demonstrated the efficacy of NEs in increasing the solubility of various insoluble drugs [29,30]. It can be concluded that, by increasing the solubility of the drug in the blood and the target tissue, its therapeutic function can be improved. Increasing the solubility of the poorly-water-soluble materials loaded in the NEs is done by the considerable fractional volume of the oily phase in the structure, the high surface area and the presence of an active film layer of surfactants at the interface of the internal and external phases.

Ferreira *et al.* developed ketoprofen-loaded NEs (KP-NEs) stabilized by pullulan as an antiangioma formulation [31]. The main ingredients used in preparation of KP-NEs were Span[®] 80 and pomegranate-seed oil. The *in vitro* release experiments revealed that nanoemulsion improved drug release relative to the free drug solution, where 95% of drug was released in 5 h. The authors proposed that enhancing the solubility of the drug could lead to an increase in its plasma concentration and, subsequently, could improve the net brain permeation. KP-NEs showed 40% inhibition of cell growth in incubation against a C6 cell line. Another NE-based drug delivery system was developed from castor oil, soybean lecithin and Polysorbate 80 (P80) for intravenous administration of thalidomide (THD) [32]. The *in vitro* dissolution profile showed a release profile similar to that of a THD acetonitrile solution, with 95% THD being dissolved within 4 h. Finally, the pharmacokinetic (PK) simulation of the intravenous (i.v.) infusion of developed NEs containing 0.01% THD has indicated that a dose as low as 25 mg can produce therapeutic plasma concentrations. In

another study, it was observed that, by reducing the mean diameter of a NE incorporated by Coenzyme Q10 (CoQ10), the cumulative release percentage of lipophilic drug was increased [33]. Also, the NE formulation of CoQ10 was more efficient in enhancement of drug release in phosphate buffer (pH 6.8) compared with free CoQ10 suspension. The PK study in albino rats showed 1.81-times improvement in bioavailability compared with CoQ10 suspension. Moreover, the haloperidol-challenged rats treated with CoQ10 NEs revealed improved behavioral activities compared with the control group, by reducing nigrostriatal dopamine depletion, therefore treating Parkinson's disease.

Curcumin (CUR), a lipophilic molecule with a logP value of 3.28, has limited solubility in aqueous media. Sood *et al.* developed CUR-loaded NEs (CUR-NEs) made of a mixture of Capmul[®] MCM and Captex[®] 500 as the oil phase, Cremophor[®] EL and P80 as surfactants, and PEG 400 and Transcutol[®] as co-surfactants [34]. The cumulative release of drug from CUR-NEs in simulated nasal fluid containing 1% SLS (sink conditions) was remarkably higher ($P < 0.001$) in comparison to the free CUR, where the total CUR release of free drug and CUR-NEs were $50.68 \pm 1.04\%$ and $74.34 \pm 1.3\%$, respectively.

Impact on membrane interactions

Different pathways are described for transferring drug by NEs into the cells within the target tissue (Figure 2). Because most drugs loaded into NEs are lipophilic, the main possibility for drug delivery seems to be through the fusion of the oily phase with the cell membrane phospholipids by lipid exchange. Therefore, NEs, because of the oil phase and the surfactant layer, seem to be a suitable option for transferring CNS active drugs via interaction with the membrane of the BMECs. In an interesting study, the brain delivery capability of some lipid-based nanostructures including solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and NEs was investigated as sulforhodamine B vehicles [35]. Evaluation of the dye accumulation in rats showed that the retention in the brain was most prolonged using NEs – from 20 to 50 min. It seems that, owing to the flexibility of NE oil droplets, compared with the more rigid nanostructured lipid-based carriers, the efficiency of overcoming the BBB via carrier uptake into the brain is improved using the NEs.

Moreover, some of the polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6 possess special transporters on the luminal membrane of the BBB. Using PUFA-rich oil NEs, Vyas *et al.* showed remarkable increased oral bioavailability and brain delivery of saquinavir (SQV) – an anti-HIV protease inhibitor [36]. By comparing NE formulation with drug suspension, the C_{max} and the $AUC_{0-\infty}$ were observed to be fivefold and threefold greater in the brain, respectively, suggestive of the enhanced rate and extent of drug absorption in a murine animal model. They also studied the role of oil type in NE efficacy in brain delivery of SQV in a parallel study. The brain concentration of SQV following administration of SQV-loaded flax-seed oil NEs led to significantly higher ($P < 0.05$) C_{max} and $AUC_{0-\infty}$ values as compared with the *Carthamus tinctorius* (safflower) oil NEs. This might be attributable to the higher level of omega-3 fatty acid in the flax-seed oil relative to safflower oil, where, flax-seed oil consists of 58% omega-3 and 14% omega-6, whereas the omega-6 fatty acid content of safflower-oil is 75%. These findings suggest great selectivity in the transport of PUFAs across the BBB.

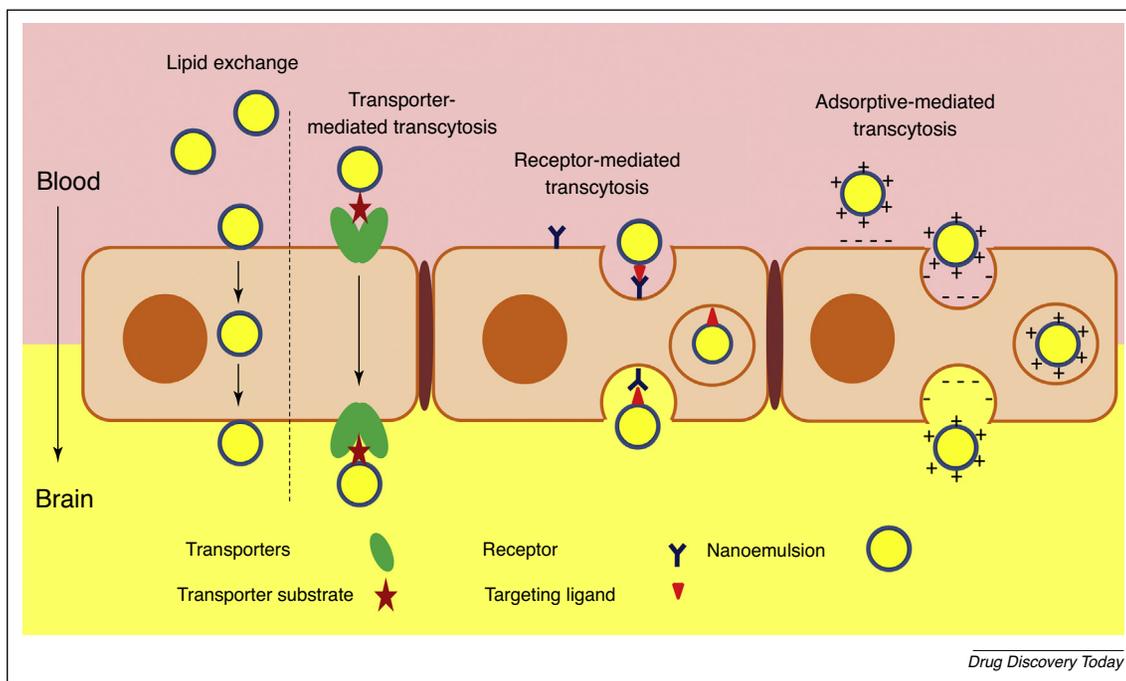


FIGURE 2

Proposed mechanisms of nanoemulsion (NE) transport across the blood–brain barrier (BBB). Lipid exchange: through the interaction of the oil phase and the surfactant layer of NEs with the membrane of the brain microvessel endothelial cells. Transporter-mediated transcytosis: occurs through the interaction of NEs with membrane transporters expressed in the luminal and/or abluminal surfaces. Receptor-mediated transcytosis: a selective brain uptake of NEs decorated with targeting ligands that bind BBB receptors. Adsorptive-mediated transcytosis: initiated by an electrostatic interaction between positively charged NEs and the negative surface of the BBB.

So far, various theories have been described to explain how fatty acids penetrate the brain. It has been suggested that PUFAs bypass the luminal and the transluminal leaflets of the BBB through a reversible flip-flop mechanism. When the PUFAs reach the neurons, acetyl-CoA synthetase traps them by creating acetyl-CoA that does not have the capability to exit the cell. Another theory is based on PUFA–albumin associations, where the PUFAs translocate from the extracellular to intracellular leaflet of the membrane via transporters [37]. Furthermore, PUFAs are explored as active absorption enhancers at the BBB by triggering membrane fluidification and modulation of the tight junctions; thus promoting paracellular and transcellular transportation [38].

Shah *et al.* showed that incorporation of DALDA peptide in fatty-acid-rich NEs significantly improves brain entry of peptide compared with the free peptide solution, ultimately leading to enhanced brain exposure (3.3-fold higher $AUC_{0-\infty}$) [39]. Based on the MRT values, DALDA-NEs provided higher residence of peptide in brain (i.e., 145.4 min vs 198.4 min) for free DALDA solution. In a similar study, the DALDA-C8 peptide-encapsulated omega-3-rich NE (DALDA-C8-NE) proved its ability in the capsaicin-pain-induced functional MRI model in rats [40]. A significant ($P < 0.05$) difference was detected in the positive blood-oxygen-level-dependent signal in several brain sections involved in the pain circuit, as characterized by the reduced extent of activation in animals receiving DALDA-C8-NEs before capsaicin shock.

Impact on modulation of drug efflux transporters

Despite the entry routes, the presence of a series of active efflux transporters (AETs) on the BBB, mainly ATP-binding cassette (ABC) transporters, limits the activity of many drugs by removing them

from the brain parenchyma back into the bloodstream [41]. P-glycoprotein (P-gp), multidrug-resistance protein (MRP) and breast-cancer-resistance protein (BCRP), the three important members of the ABC transporter group, are demonstrated to be responsible for the efflux of a wide variety of therapeutic agents such as methotrexate, vincristine, indinavir, camptothecin, anthracyclines, mitoxantrone, taxanes and cyclosporine A, among many others, from the brain parenchyma. It is obvious that inhibition of BBB efflux transporters can be assumed as a possible strategy for improving brain entry of their substrates. NEs can be generally considered as useful tools for bypassing the P-gp pump from two different points of view: (i) the general ‘masking’ concept of the active agents by the droplets from being discovered by the transporter while they enter the BBB; and (ii) the presence of surfactants such as P80 in NE droplets that have been identified as well-known P-gp inhibitors. Dorpevic *et al.* suggested that the inhibition of the P-gp efflux system at the BBB by P80 and Poloxamer188 (PL188) could play an important part among other features of P80- and PL188-containing NEs of risperidone to improve brain availability of the drug [42]. Indinavir, a protease inhibitor used in the treatment of HIV infection, has limited entry to the brain owing to efflux by P-gp expressed at the BBB [43]. The pegylated NE of indinavir caused significantly enhanced drug concentrations in brain in comparison to the free drug solution as well as the control blend [44]. The increase in indinavir levels in brain, despite the greater hydrophilic surface caused by PEG chains, could be explained by prolonged circulation time of stealth NEs, thereby leading to the BBB penetration to a greater level. Further, it has been reported that the PEGs and their derivatives inhibit intestinal P-gp; therefore, the other probable mechanism

would be the inhibition of P-gp at the BBB by PEG as a chemical entity.

Recent trends to optimize nanoemulsion application for CNS drug delivery

Intranasal administration

Intranasal administration has been considered as a noninvasive delivery option that bypasses the BBB and enables direct access of a variety of therapeutic agents to the CNS, avoiding drug first-pass elimination by the liver and gastrointestinal tract, or enzymatic degradation in plasma before reaching the CNS. However, poor absorption, enzymatic degradation and poor permeation and retention of drugs can be addressed as some drawbacks of this route. In recent years, NEs attracted a great deal of attention for intranasal drug delivery, which can be attributed to their advantages such as the ability to protect the drug from biological and/or chemical degradation, the potential to improve the drug absorption via inducing mucoadhesion or create a positive electrical surface for increasing the nasal residence time [45]. To visualize brain uptake following intranasal (i.n.) and i.v. administrations of technetium-labeled risperidone nanoemulsions (RP-NEs), a gamma scintigraphy study was used [46]. A remarkably higher radioactivity accumulation was seen in brain following i.n. administration of RP-NEs compared with i.v. administration. In a similar study, the olanzapine mucoadhesive NEs (OZ-MNEs) and olanzapine NEs (OZ-NEs) were prepared and their biodistributions in rats following i.n. and i.v. administration were examined [47]. From an *in vivo* study, the brain-to-blood uptake ratios of 0.45, 0.88, 0.80 and 0.04 were obtained for drug solution (i.n.), OZ-NEs (i.n.), OZ-MNEs (i.n.) and OZ-NEs (i.v.), respectively, at 0.5 h. Gamma scintigraphy imaging of the rat brain, by contrast, showed a rapid and greater degree of transport of drug by technetium-labeled OZ-MNEs (i.n.), compared with other groups. Ahmad et al. reported the successful preparation of an amiloride-NE consisting of oleic acid, Polysorbate 20 and Carbitol® with mean droplet diameter of 89.36 ± 11.18 nm [48]. From an *in vivo* study, the AUC_{0-t} in brain following i.n. versus i.v. administration were 7937.46 ± 101.19 and 1254.37 ± 25.94 ng min/ml/g, respectively, with a significant improvement in the seizure threshold in rodent models of epilepsy.

One of the alternative ways to increase the penetration of the drugs to the brain through the i.n. route can be to increase their concentration near the nasal mucus. It is known that mucus contains mucin, a complex glycoprotein compound with significant proportions of sialic acid. At physiological pH, sialic acid carries a net negative charge, so, if the drug-loaded nanocarrier has a positive zeta potential, through electrostatic interaction, the concentration and residence time of the drug near the absorption site would be increased, thereby enhancing the delivery of the drug into the brain parenchyma. Jeong and colleagues reported that the transfection efficiency of a cationic emulsion carrier was 3-times better than commonly used commercial liposome carriers [49]. The increased transfection efficiency using the emulsion carrier could be ascribed to the increased probability of delivering intact DNA to the nasal mucosae because the complex is strong and insensitive to mucosal destabilizers. A cationic NE encapsulating an anti-tumor necrosis factor (TNF) α siRNA (siRNA-NEs), which has indications in the prevention of neuroinflammation,

was developed using cationic lipid DOTAP [50]. Intracellular uptake studies by confocal imaging and flow cytometry showed higher cell entry of the developed siRNA-NEs relative to Lipofectamine®-transfected siRNA, where siRNA-NEs decreased TNF α levels in lipopolysaccharide (LPS)-stimulated cells to a significantly higher extent. Upon i.n. administration of siRNA-NEs, almost a fivefold higher level was detected in the rat brain compared with nonencapsulated siRNA. More importantly, i.n. delivery of siRNA-NEs decreased the unregulated levels of TNF α in an LPS-induced model of neuroinflammation.

The effectiveness of cyclosporine-A (CsA) loaded in positively charged NEs made of stearylamine (CsA-NEs, zeta potential of $+57 \pm 10$ mV) was evaluated following i.n. and i.v. administration in rats [51]. The brain-to-blood exposure ratios of 4.49, 0.01, 0.33 and 0.03 were found for CsA-NE (i.n.), CsA-NE (i.v.), CsA-solution (i.n.) and CsA-solution (i.v.), respectively, indicating that CsA-NE has been successful in the direct nose-to-brain transport of CsA, bypassing the BBB. The most likely explanation is that the positive charges on the surface of CsA-NE can bind strongly to negatively charged mucus, thus retaining the drug at the site of absorption for remarkably longer times.

Chitosan, a polycation derived from naturally occurring chitin, has attracted a great deal of interest for improvement of the nasal delivery of pharmaceutical actives owing to its bioadhesion and permeation-enhancing properties, and also decreasing mucociliary clearance, which, collectively, lead to increased permeation across the nasal membrane [52]. Besides, the positively charged chitosan could interact with the negatively charged nasal mucosa [53,54], thus providing a temporary opening of tight junctions [55] to further help the passage of the active ingredients through the nasal mucosa [56]. The *in vivo* studies revealed that the mucoadhesive NE of zolmitriptan, prepared by chitosan, provided a higher AUC_{0-8} and a faster onset of action than the i.v. or nasal solution [57]. In the same way, Fachel et al. reported using chitosan-coated NEs for rosmarinic acid, with notable permeation and retention of the drug in porcine nasal mucosa [58]. Some examples of nanoemulsion that have been developed for intranasal drug delivery to the brain are enlisted in Table 2.

Active targeting

Another way to enhance transport of actives through the BBB is via receptor-mediated transcytosis [59]. Applications of some surfactants such as P80 in the structure of NEs have shown promising results in crossing the BBB. It has been frequently reported that P80 can enhance the adsorption of apolipoprotein E on the surface of different nanocarriers with a subsequent increase in the brain uptake of P80-coated nanocarriers through the low-density lipoprotein (LDL) receptors on the BBB. The parenterally administered cefuroxime-loaded NE (CFX-NE) exhibited a significant C_{max} and AUC_{0-t} , prolonged half-life and reduced clearance out of the brain of rats compared with the free drug in solution [60]. This result could be explained by the use of P80 in CFX-NEs, promoting the receptor-mediated endocytosis of drug molecules across the BBB. This process was not a concentration-dependent process; hence, a higher drug concentration in plasma would not affect the rate of brain uptake.

Using the overexpressed receptor of transferrin (Tf) on BBB cells, Prabhakar et al. showed improved brain delivery of indinavir (IDV) through transferrin-coupled NEs (IDV-Tf-NEs) [61]. The average

TABLE 2
Nanoemulsions employed in intranasal drug delivery

Drug	Composition	Method	Size (nm)	Outcomes	Refs
Paroxetine	Capmul [®] MCM/ Solutol [®] HS 15/ Propylene glycol	Spontaneous emulsification	58.47 ± 3.02	A 2.57-fold enhancement in permeation as compared to the paroxetine suspension Effective in enhancing the depressed levels of glutathione and decreasing the elevated levels of TBARS	[102]
Paliperidone	Labrafil [®] / M1944CS/ Polysorbate 80/ Tween [®] 20	Spontaneous emulsification	38.25	Significant difference in the locomotor activity when compared with plain drug	[103]
Resveratrol Curcumin	Hyaluronic acid	Spontaneous emulsification	115.2 ± 0.15	Managed to increase the amounts of the two polyphenols in the brain (about 7- and 9-fold increase in AUC _{0–7 h} for resveratrol and curcumin, respectively)	[104]
Tramadol	Isopropyl myristate/ Tween [®] 20/ Labrasol [®]	Magnetic stirring	136.3 ± 4.3	Displayed significantly higher antinociceptive effect and AUC _{0–24h} as compared with tramadol solution	[105]
Pretomanid	Octadecylamine/ Tween [®] 80/ Asolectin	Sonication	186.46 ± 0.38	Peak concentrations (C _{max}) in brain of 12 062.3 ng/g that is significantly higher than the required therapeutic level	[106]
Selegiline	Grape-seed oil/ Solutol [®] / Labrasol [®]	High energy emulsification	61.43 ± 4.10	Significant improvement in behavioral activities in Parkinson's disease in rats	[107]
Resveratrol	Vitamin E/ Sefsol [®] / Polysorbate 80/ Transcutol [®] P	Spontaneous emulsification, High-pressure homogenization	102 ± 1.46	The levels of GSH and SOD were significantly higher and the level of MDA was significantly lower in the resveratrol-nanoemulsion-treated Parkinson's disease group	[108]
Safranal	Tween [®] 20/ Labrasol [®] / Cremophore [®] EL/ Carbitol [®]	Titration method	89.64 ± 9.12	Induced cerebral ischemia rats exhibited significant improvement in neurobehavioral (locomotor and grip strength) and antioxidant activity	[48]
Zaleplon	Cremophor [®] / RH40/ Transcutol [®] HP/ Miglyol [®]	Spontaneous emulsification	44.57 ± 3.4	Significant 3-fold increase in plasma and brain GABA levels compared with the marketed product sleep aid	[109]

number of transferrin molecules attached to the surface of each NE droplet was 552.53 ± 17.33 . The brain therapeutic availability of indinavir from IDV-Tf-NEs was 4.69- and 1.7-times higher than the free drug solution and unmodified NE, respectively, at 6 h after i.v. injection in mice, indicating the preferential uptake of IDV-Tf-NEs by receptor-mediated transcytosis.

L-amino acid transporter 1 is a major route for transportation of branched or aromatic amino acids through the BBB. The entry route of lipidic α -amino acids is through carrier-mediated endocytosis, via binding to the receptor and then internalization by endocytosis. Bollam et al. studied the effect of two lipo-amino-acids with different lipid chain length, tetradecyl aspartic acid (A14) and decyl glutamic acid (G10), on the concentration of indinavir in brain, where A14-NEs and G10-NEs increased the drug level 4.27- and 2.94-times, respectively, compared with free drug solution [62]. Moreover, in comparison with that of unmodified NE, the drug concentration in brain from G10-NEs and A14-NEs improved between 2.5- and 3.38-fold.

Liu and Hye developed an intravenous NE of brain-penetrating peptide K16ApoE (average droplet diameter <200 nm) to target brain tumors [63]. K16ApoE peptide consisted of 16 lysine residues and 20 amino acids corresponding to the LDL receptor binding sequence from apolipoprotein E in healthy and tumor-bearing mice. When 20 nmol of K16ApoE peptide NE was injected intravenously, the peptide transiently opened the BBB and allowed even higher amounts of NE accumulation at the brain tumor.

Nanoemulsion application in CNS diseases

Brain tumors

Brain tumors are hard-to-treat neoplastic manifestations, mostly owing to the highly limited access of anticancer agents to the brain [64]. Mucoadhesive NEs loaded by kaempferol (KPF-MNEs) exhibited significantly increased concentrations of the drug in rat brain after i.n. administration (fivefold higher than the free drug) [65]. In addition, KPF-MNEs decreased C6 glioma cell viability via induction of apoptosis to a superior degree as compared with the free drug. The cytotoxicity of KPF-MNEs on the C6 glioma cell line was 20-fold better than the free drug because of the higher level of cellular uptake when the drug was incorporated into mucoadhesive NEs. Castilho-Fernandes et al. showed that chloroaluminum phthalocyanine NEs (AlClPc-NEs) induce cell death in U87-MG cells with a dose-dependent mechanism and, possibly, could serve as an appropriate adjuvant treatment for malignant glioma [66]. Their study revealed that caspase-9 activity was higher than that of caspase-3; so, AlClPc-NEs prompted apoptosis in U87-MG cells mostly through the mitochondrial intrinsic route. In this way, a fairly low dose of fisetin NEs reduced 53% of tumor volume in Lewis lung-carcinoma-bearing mice, whereas a sixfold higher dose was required to obtain a similar tumor growth inhibition in the case of free fisetin [67]. Meanwhile, the improved bioavailability of the fisetin NEs is possibly due to the unique lymphatic distribution following intraperitoneal (i.p.) administration, which can be considered as a favorable characteristic, particularly in the case of anticancer actives that must reach lymph nodes in metastases.

The use of ClinOleic, a commercially available parenteral nutrition NE for the delivery of paclitaxel (PX), was reported by Najlah et al. [68]. PX-loaded ClinOleic decreased the viability to 34.57% for SVG-P12 cells (normal glial cell lines) and to 6.4% for U87-MG cells (grade IV glioma cell lines). Meanwhile, PX-loaded ClinOleic also showed a significantly lower ($P < 0.05$) cytotoxicity toward SVG-P12 than that of free drug, indicating a better selectivity against the malignant cells. Cancerous cells are known to divide faster than normal cells; hence their intake of nutrients is faster; this might explain the reason behind the selectivity of the PX-loaded ClinOleic toward the cancerous cells – the presence of olive oil and/or sodium oleate in ClinOleic composition might, additionally, have a further enhancement effect on the targeting properties of this formulation toward the U87-MG cells.

Simultaneous studies on NE and solution forms of an anticancer substance isolated from the traditional Chinese herb magnolia: honokiol (HK), after i.v. administration to tumor-burdened mice was carried out [69]. The NEs with a mean diameter of 186.6 ± 1.7 nm were prepared from soybean lecithin, Synperonic® F68 and soybean oil. The half-life and targeting properties to tumor tissues of HK-NEs were remarkably improved compared with those seen with the free drug solution. *in vivo* studies indicated that HK-NE treatment caused significant inhibition of mouse sarcoma S180 tumor growth compared with the solution of HK. In a similar study, DHA-SBT-1214 was incorporated in a fish-oil-based NE, with an average diameter of 200 nm [70]. The NE formulation displayed an improved PK profile along with higher exposure of the drug to the tumor and almost doubled the tumor-targeting efficiency compared with the free drug solution. It was reported that the IC_{50} value decreased when the brain cancer cells were treated with a flaxseed-oil-NE formulation of chemotherapeutic agents, modified by a μ -opioid-receptor-targeting ligand (US patent no. WO2016090092A1).

Neurodegenerative disorders

Neurodegenerative disorders encompass a range of conditions characterized by the persistent and progressive loss of neuronal subtypes. One of the most common forms of dementia in the elderly is Alzheimer's disease, affecting >35 million people worldwide, and the number of people with Alzheimer's disease increases annually [71]. Similar to other CNS diseases, the BBB is the formidable obstacle for drugs accessing the brain and some aspects of the BBB in Alzheimer's disease patients can even differ from the normal BBB, such as P-gp function, cerebral blood flow and CSF reabsorption.

In an interesting study, following oridonin-NE (OD-NE) injection, numbers of amyloid plaques in the cortex of APP/PS1 mice as well as area percentages of β -amyloid ($A\beta$) deposition were significantly reduced compared with the control group [72]. Furthermore, the impaired nest construction behavior was significantly restored by OD-NE treatment. It was reported that the enhanced drug availability in brain, after i.n. administration of rivastigmine NEs (RVN-NEs) compared with free drug solution, was probably the result of small globule size, high drug release and longer residence time of the RVN-NEs in the nasal cavity [73]. Small globule size potentially allows RVN-NEs to be moved transcellularly through olfactory neurons to the brain by the several endocytic trails of sustentacular or neuronal cells in the olfactory

membrane. In addition, use of P80 and Transcutol® P in the NEs significantly improved the nasal absorption of the drug, possibly as a result of P-gp inhibition.

Thymoquinone-rich fraction NEs (TQRF-NEs) reduced soluble $A\beta_{40}$ and $A\beta_{42}$, partially via modulation of $A\beta$ precursor protein processing which stops: $A\beta$ generation; the upregulation of insulin-degrading enzyme, an enzyme that degrades $A\beta$; the upregulation of LRP1, a protein that transports $A\beta$ brain-to-blood; the downregulation of BACE1, an enzyme that initiates $A\beta$ production; and the downregulation of RAGE, a receptor that transports $A\beta$ from blood into the brain [74]. Thus, TQRF-NEs employ several mechanisms for decreasing $A\beta$ accumulation in the brain, by inducing reduction of $A\beta$ generation as well as increasing its degradation and clearance from the brain. Ismail et al. reported that the protective effects of TQRF-NEs against a high-fat-cholesterol-diet-induced hypercholesterolemia were related to a reduction in cholesterol and $A\beta$ levels, and growth of antioxidant levels [75]. The cholesterol- and fat-enriched diet used in that study exhibited pro-oxidant activity that could trigger Alzheimer's disease biomarkers such as $A\beta$. Therefore, TQRF-NEs, possibly through their antioxidant properties, were able to shield against this earlier destructive damage toward Alzheimer's disease pathology.

Selegiline NEs (SG-NEs) were developed from grape-seed oil, Sefsol 218®, P80 and Lauroglycol® 90 by high-pressure homogenization [76]. The SG-NEs showed droplet size and zeta potential of 61.43 ± 4.10 nm and -34 mV, respectively. Biochemical evaluation revealed an increase in the extent of antioxidant enzymes, whereas the thiobarbituric-acid-reactive substances were decreased in i.n. SG-NE-treated animals as compared with haloperidol-induced Parkinson's disease rats (control). Additionally, the brain dopamine concentrations of SG-NE-treated and control groups were 16.61 ± 3.06 and 8.59 ± 1.00 ng/ml, respectively. In conclusion, SG-NEs were successful in reducing the dopamine loss, which suggests they could be a potential technique for selegiline delivery to reduce the damage caused by free radicals, as well as avoiding subsequent biochemical changes that arise during Parkinson's disease. Similarly, the increase in the content of glutathione and decrease in the extent of lipid peroxidation showed the beneficial effects of the transdermal ropinirole NE in Parkinsonism rats [77]. The depleted level was restored significantly (35.09%) in the NE-receiving group in contrast to the free drug suspension group, where the restoration rate was only 13.3%.

Amyotrophic lateral sclerosis (ALS), a motor neuron disease, is a progressive neurodegenerative disorder characterized by the deterioration of upper and lower motor neurons. To date, only one drug (i.e., riluzole) has been approved for the treatment of ALS. Parikh and Patel developed riluzole-loaded NEs (RZ-NEs) by phase titration from Sefsol® 218, P80 and Carbitol®, with a droplet size of 23.92 ± 0.52 nm [78]. Brain uptake of riluzole post i.n. administration of RZ-NEs was significantly higher compared with oral administration of RZ-NEs.

HIV-associated CNS disorders

The abnormal immune responses caused by HIV infection can lead to neurological illnesses [79]. It is very important for the treatment regimens of HIV-associated neurological problems to be able to pass through the BBB with a proper concentration. It is well-known that the brain penetration of the majority of anti-HIV drugs is limited

mainly because these compounds are the typical substrates of the ABC transporters located at the BBB and BCSFB [80].

The developed saquinavir mesylate NEs (SQVM-NEs) from Capmul[®] MCM, P80 and PEG400 enhanced the drug permeation into the brain compared with the free drug suspension [81]. Gamma scintigraphy imaging confirmed the transport of SQVM into rat brain at greater levels after i.n. administration of SQVM-NEs. Moreover, in PK studies, the brain level of indinavir subsequent to administration of P80-containing NE was significantly ($P < 0.05$) higher than that produced by the administration of a free drug solution (2.44-fold) or a P80-lacking indinavir-loaded NE (1.48-fold) [82]. The increased brain-specific accumulation of indinavir by modified NE is possibly attributable to the enhanced LDL-mediated endocytosis as well as P-gp inhibition by P80 at the BBB. Treatment of toxoplasmosis is regularly required in immune-compromised patients. Atovaquone NEs (AQ-NEs) were prepared from grape-seed oil by spontaneous emulsification [83]. After oral administration, AQ-NEs showed improved oral bioavailability and survival time in mice along with decreased parasitemia and number and size of brain cysts.

Ischemic stroke

Ischemic brain stroke is one of the challenging causes of death and disability in major industrialized countries. The fatality of stroke is because of a narrow time window for therapeutic treatment, a fast progressing pathology (3 h) and lack of viable strategies to overcome the disease pathology [84]. Thymoquinone mucoadhesive NEs (TQ-MNEs) showed remarkable brain-targeting efficiency ($628.5786 \pm 44.79\%$) and brain-targeting index ($89.97 \pm 2.94\%$) following i.n. administration [85]. Improved neurobehavioral function (locomotor and grip strength) was discovered in middle-cerebral-artery-occlusion-induced cerebral ischemic rats following the administration of TQ-MNEs. A study by Gorain et al. showed significantly higher brain concentrations of olmesartan (0.290 ± 0.089 mg/ml, 0.333 ± 0.071 mg/ml and 0.217 ± 0.062 mg/ml at 0.5, 2.0 and 8.0 h post dosing, respectively) when administered orally as a NE formulation, compared with the aqueous drug suspension [86].

Quercetin is a polyphenol with an antioxidant effect *in vitro*, but it has limited bioavailability *in vivo* owing to its high lipophilicity. Quercetin mucoadhesive NEs (Qu-MNEs) were developed through ionic gelation from oleic acid, PEG 400, P80, Labrasol[®] and Transcutol[®] HP with mean droplet size of 91.63 ± 4.36 nm [87]. Some important findings such as improved neurobehavioral function (locomotor and grip strength), histopathology and reduced infarction volume effects were detected in middle-cerebral-artery-occlusion-induced cerebral ischemic rats after i.n. administration of Qu-MNEs. Galho et al. evaluated the effect of a quercetin-loaded NE (Qu-NE) prepared from castor oil, lecithin and PEG-660 stearate with a mean droplet size of 20 nm in a collagenase-induced intracerebral hemorrhage rat model [88]. Animals treated with Qu-NEs showed a significant improvement in the beam-walking and open-field tests. Similarly, Qu-NEs

decreased the size of the hematoma, while preserving the activity of glutathione-S-transferase, with increased glutathione (GSH) level, and the total antioxidant capacity. The authors concluded that the administration of the NE formulation of quercetin increased its antioxidant effect, which was reflected in the improvement of motor skills as well as the hematoma size decrease.

Schizophrenia

Schizophrenia is a disabling psychiatric disorder affecting many people worldwide. It manifests in a variety of symptoms ranging from misinterpretation of reality and illusions to disorganization of thinking and behavior. It is associated with progressive altered brain functions during the course of the illness [89,90]. Quetiapine (QTP), an antipsychotic drug, gets extensively metabolized by the liver. To increase bioavailability of QTP via bypassing its hepatic first-pass effect, a QTP-loaded NE (QTP-NE) was developed from Capmul[®] MCM, P80, Transcutol[®] P and propylene glycol, then administered i.n. in Wistar rats [91]. QTP concentration in the brain upon administration of the NE was observed to be higher at all time points than free drug solution, indicating the efficiency of brain targeting of QTP-NEs after i.n. delivery.

in vivo experiments of risperidone NEs stabilized with P80 (RSP-NEs) revealed 1.2–1.5-fold enhanced relative bioavailability, 1.1–1.8-fold reduced liver accumulation and nearly 1.3-fold higher brain entry of drug post i.p. administration of RSP-NEs compared with free drug solution in a rat model [92]. Moreover, from a behavioral study, RSP-NEs exhibited a decrease in basal and amphetamine-induced locomotor activity in rats. RSP-NE-receiving animals also showed an early onset of antipsychotic action which lasted for 90 min post injection.

Concluding remarks

The present review has attempted to demonstrate the potential of NEs, in the field of drug delivery to the brain, as efficient tools challenging the limited brain entry of a wide variety of therapeutic agents. NEs are kinetically stable dispersions of two immiscible liquids with intervention of emulsifier(s) and energy, with a mean droplet diameter of 20 to 500 nm. Their unique structures affect the solubility and stability of the incorporated drugs, along with the modulation of their interaction with the brain endothelial cell membrane. It is well-documented by several *in vivo* studies that NEs can be considered as one of the most promising delivery systems for the CNS-active agents used as part of the efficient regimens against the hard-to-treat CNS diseases like brain tumors, AIDS-related dementia, Alzheimer's disease, Parkinson's disease, stroke and schizophrenia. However, owing to the different aspects to be considered seriously before the widespread clinical application of this delivery system, extensive studies are still needed, especially in the field of toxicity, cost and efficacy of the protocols for this novel drug delivery system in the clinical setting.

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