Opportunities and Challenges for the Nasal Administration of Nanoemulsions

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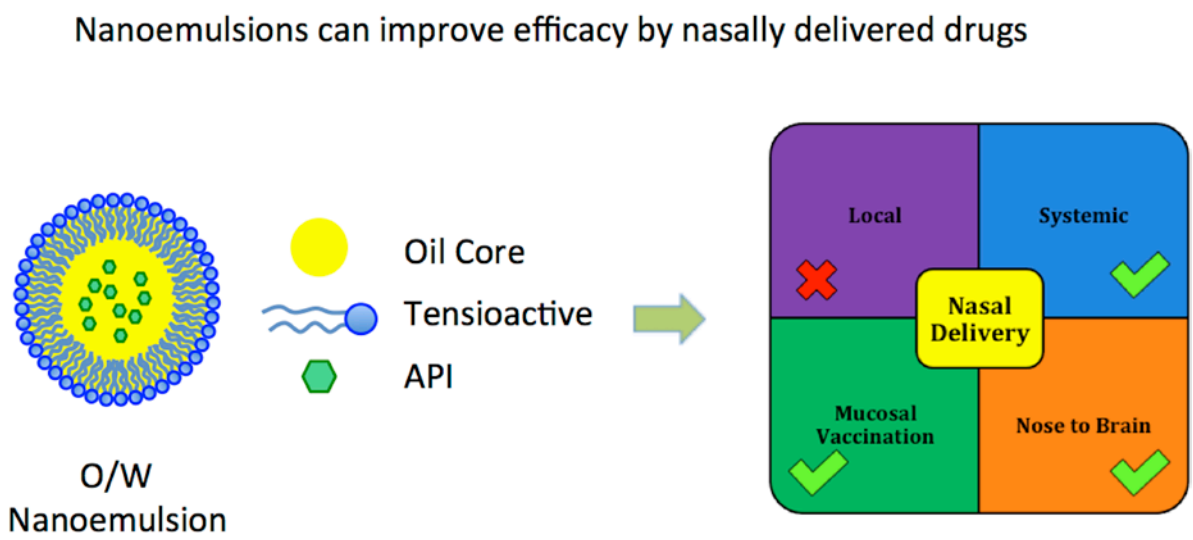
Abstract

Nasal delivery has become a growing area of interest for drug administration as a consequence of several practical advantages, such as ease of administration and non-invasiveness. Moreover, the avoidance of hepatic first-pass metabolism and rapid and efficient absorption across the permeable nasal mucosa offer a promising alternative to other traditional administration routes, such as oral or parenteral delivery. In fact, nasal delivery has been proposed for a number of applications, including local, systemic, direct nose-to-brain and mucosal vaccine delivery. Nanoemulsions, due to their stability, small droplet size and optimal solubilization properties, represent a versatile formulation approach suitable for several administration routes. Nanoemulsions demonstrated great potential in nasal drug delivery, increasing the absorption and the bioavailability of many drugs for systemic and nose-to-brain delivery. Furthermore, they act as an active component, i.e. an adjuvant, in nasal mucosal vaccinations, displaying the ability to induce robust mucosal immunity, high serum antibodies titres and a cellular immune response avoiding inflammatory response. Interestingly, nanoemulsions have not been proposed for the treatment of local ailments of the nose. Despite the promising results *in vitro* and *in vitro*, the application of nanoemulsions for nasal delivery in humans appears mainly hindered by the lack of detailed toxicology studies to determine the effect of these formulations on the nasal mucosa and cilia and the lack of extensive clinical trials.

Graphical Abstract



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Running title: Nasal administration of nanoemulsions

1. Introduction

Oral administration of drugs has long been the most desirable and convenient route of drug administration. However, limitations regarding low oral bioavailability of select compounds through this route of administration have led to research on alternate routes of drug delivery. Although there is no limitation to drug absorption via intravenous administration, and other parenteral routes such as intramuscular and subcutaneous delivery have shown promising delivery of most drugs, more convenient and non-invasive administration routes are desirable. Transdermal administration has been explored over the past few decades however, delivery by this route is hindered by inherently low skin permeability to many drugs. More recently nasal mucosa has become an interesting and growing area of research with the recognition of its therapeutic viability as an alternate route of administration [1].

1.1 Nasal delivery

The nose has long been recognized as a potential route of drug delivery with reports of its use in traditional Chinese medicine dating back as far as 403 BC [2]. Nasal administration is considered a viable route for delivering many drugs, particularly those that can't tolerate the harsh gastrointestinal environment following oral administration, such as proteins and peptides [3]. The fundamental features and limitations of nasal drug delivery are outlined in Table 1 [1, 4-7].

<Table 1>

The respiratory region of the nasal mucosa covers the largest area of the nasal cavity and is the main site for drug absorption into the systemic circulation [8]. Compounds are proposed to enter systemic circulation via a number of mechanisms including transcellular (through the interior of the epithelial cells), paracellular (through the tight junctions between cells), carrier-mediated (e.g. organic cation transporters and amino acids transporters) and transcytosis pathways [8-10]. The proportion of drug that successfully reaches systemic circulation is dependent on the physiological characteristics of the nasal mucosa, physicochemical/molecular properties of the drug, pharmaceutical properties of the formulation and factors related to the delivery device as shown in Figure 1 [4, 10].

<Figure 1>

Researchers have studied a number of different techniques by which many of the limitations posed by the nasal mucosa can be reduced. The fundamental reasoning behind these techniques is to increase nasal residence time and enhance nasal absorption or modify drug structure to produce more favourable physiochemical properties for nasal absorption. The main techniques studied include nasal enzyme inhibition, permeation enhancing, drug chemical structure modification and design of pro-drugs and particulate drug delivery systems such as microparticles, nanoparticles and nanoemulsions [11].

The aim of this paper is to explore the opportunities and challenges associated with the intranasal delivery of nanoemulsions.

1.2 Nanoemulsions

Emulsions are formed by the dispersion of one liquid, usually oil phase, into a second immiscible liquid, water or aqueous phase [12]. Emulsions are typically distinguished by their particle size and stabilization into three main categories namely macro-, nano- and microemulsions [13]. Table 2 outlines the different properties of these three main emulsion categories. Nanoemulsions are a specific type of colloidal dispersion, which consist of emulsions in which the dispersed phase droplets are in the nanometric scale [14]. They are also referred to in different publications as miniemulsions, ultrafine emulsions, submicron emulsions, fine-dispersed emulsions, parenteral emulsions and emulsoids [13-16]. In many ways nanoemulsions represent an intermediate between the properties of macro- and microemulsions. Like microemusions, nanoemulsions contains sub-micron size droplets, appear transparent or translucent and possess stability against sedimentation or creaming. However, microemulsions are thermodynamically stable and are formed spontaneously, while nanoemulsions are non-equilibirium systems, in fact they are only kinetically stable and eventually subject to flocculation, coalescence and Ostwald ripening. This partial overlap in properties, in conjunction with the fact that many authors do not specify the nature of the submicron emulsion produced, has led to much confusion in the literature regarding emulsion type definition and size range [17]. Moreover, it has been suggested that many microemulsion systems studied in the literature are in fact misclassified nanoemulsion systems further adding to this confusion [18].

<Table 2>

Some physico-chemical aspects of nanoemulsion systems are essential to their superior stability when compared to macroemulsions systems. The size of the dispersed phase droplets allows for the Brownian motions and diffusion rate to overcome the effect gravitational force acting on the system leading to a significant reduction of

phenomena such as creaming, sedimentation and flocculation during storage. The system properties are also preventing phase separation by coalescence, as droplets are not easily deformable and the significant surfactant thickness on droplets surface impede the instability or disruption of the superficial film separating them [19-24].

Nanoemulsions are non-equilibrium systems and thus, cannot be formed spontaneously. As a result, energy input is required for their production. There are two main methods of production, namely low-energy and high-energy methods [25, 26]. Low-energy methods utilize the intrinsic physicochemical properties the individual components of the nanoemulsion to produce small droplets [27]. Techniques for the preparation of nanoemulsions through low-energy methods include: self-emulsification (also referred to as titration method or spontaneous emulsification method), emulsion phase inversion (EPI) and phase inversion temperature (PIT) methods [28, 29].

Self-emulsification approaches exploit the diffusion of water miscible components, such as solvents, surfactants and co-surfactants, from the organic phase into the continuous aqueous phase to produce a nanoemulsion. A simple dilution process at constant temperature is sufficient to obtain the nanoemulsion without any phase transition. The nanoemulsion formation can be obtained by dilution of homogeneous three-component solutions, such as water, ethanol and oil, as in the Pastis/Ouzo effect, of an O/W microemulsion or of a cubic liquid crystalline phase [27].

In the phase inversion processes, the emulsion system O/W reverse to W/O or vice versa. While the curvature of the interface O/W gradually changes, the interfacial tension of the system decreases to minimum value and a submicron emulsion can be obtained with minimal energy expenditure. Two types of phase inversion may occur: (a) transitional inversion and (b) catastrophic inversion [30, 31]. The transitional inversion may occur with changes in the affinity of the surfactants for aqueous and/or oil phases and may be induced by variations in factors such as temperature, HLB values, salinity of the aqueous phase and polarity of the oily phase [32, 33].

In particular, changes in system temperature can promote modifications in the interactions (hydrogen bonding, dipole-dipole interactions and induced dipoles) between the ethoxylated nonionic surfactants and the aqueous phase. These surfactants have generally HLB values above 10, being amphiphilic molecules with a clear predominance of hydrophilic aspect. However above the phase inversion temperature of the surfactant molecule becomes predominantly lipophilic triggering the transitional inversion of the emulsion [34, 35].

The catastrophic phase inversion can occur when there is an increase in the volume of the dispersed phase or variations in the ratio of the volumes of the aqueous and oil phase. This type of inversion is irreversible and can occur over a wide range of volume fractions. The term catastrophic means a sudden change in behavior of a system and occurs as a result of gradual changes in process conditions [36-38]. The phase inversion in this case is triggered by the change of the water/oil ratio when the volume fraction of the dispersed phase increases. The origin of the structural changes are related to the balance between droplet breakup and coalescence in the system and the droplet size produced to the formation of the intermediate multiple emulsion dispersions (O/W/O for O/W systems and W/O/W for W/O ones). The catastrophic phase inversion, although influenced by the concentration of the surfactant is primarily dependent on the type and particle size distribution of the globules formed, ie, the amount and morphology of the dispersed phase [37].

Emulsification by emulsion phase inversion (EPI) may be considered a type of catastrophic inversion, where the point of phase inversion (PPI) is the composition at which the emulsion formed by the aqueous phase, oil and surfactants reverses phases at constant temperature. The titration of water into an oily phase containing an hydrophilic surfactant promotes the initial formation of an W/O dispersion. However, increasing the volume fraction of water a change in the spontaneous curvature of the surfactant molecules occurs leading the inversion to an O/W emulsion passing through an unstable multiple emulsion phase [38].

When using low-energy methods it is important to consider temperature control, especially when using the PIT method, volumetric fraction of water and oil phases as well as surfactant and co-surfactant concentration and weigh ratio [39, 40]. These factors are relatively easy to control on a small scale but may hinder the industrial viability of these methods. Currently, there is less information regarding the industrial scale-up of nanoemulsions produced by low-energy methods compared to high-energy ones.

In alternative to low-energy manufacturing methods, high-energy methods utilize mechanical devices to disrupt the oil and water phases to form nano-sized droplets [25]. The main apparatuses utilized include rotor/stator devices and, more recently, the high efficiency ultrasound generators and high-pressure homogenizers [14]. High-energy methods have the ability to produce submicron emulsions from a large variety of materials, displaying homogenous flow and narrow droplet size distribution and thus have the potential to be utilized on an industrial scale [12, 29]. However, there are a number of limitations to this method. Firstly it is not suitable for heat sensitive drugs such as retinoids and macromolecules, including proteins, enzymes and nucleic acids [25]. Secondly, due to the high-energy requirements and inefficient use of energy (approximately 0.1% of the energy

produced is directly used for the emulsification process) this approach is also relatively expensive [26]. Thus, low-energy methods are considered advantageous in regard to cost, energy efficiency, simplicity of implementation and can be used for fragile or heat sensitive drugs [16]. However, low-energy methods generally require higher surfactant concentrations than high-energy emulsification methods. A recent study by Ostertag and colleagues compared the low-energy phase inversion technique to the high-energy microfluidisation technique and found that small droplets could be produced by both methods, however much less surfactant was needed for the high-energy method than the low-energy method, with a surfactant to oil ratio required to obtain droplets with diameter smaller than 160 nm of ≥ 0.1 and ≥ 0.7 respectively [41].

Nanoemulsions have attracted much interest in recent years over a number of different fields including the personal care, cosmetics, agrochemical, chemical, food and pharmaceutical industries [12, 15]. Within the pharmaceutical industry, nanoemulsions are being investigated as a formulation approach suitable for a number of different administration routes such as topical, transdermal, parenteral, ocular, pulmonary, nasal and oral [26, 28, 41]. Even though nanoemulsions are primarily regarded as a vehicle for drug formulation, they have received increasing attention for a number of novel applications as delivery systems for the controlled release of drugs, the targeted delivery of anti-cancer agents, and mucosal vaccination [26]. This interest can be largely attributed to their many unique and favorable properties, providing a number of advantages over conventional emulsions. Nanoemulsions are kinetically stable and are therefore not significantly affected by flocculation, coalescence, creaming or sedimentation during storage time [42]. They can be formulated into foams, liquids, creams and sprays and being transparent/translucent can be incorporated into these preparations without loss of clarity [43, 44]. They can be used to deliver both hydrophilic and lipophilic drugs and are generally considered non-toxic and non-irritant formulations. In fact, nanoemulsions are usually manufactured using reasonably low concentrations of surfactants that are Generally Recognized As Safe (GRAS) for human consumption by the FDA, rendering them safe for enteral and mucosal administration [27, 42, 43]. Furthermore, nanoemulsions present large surface area and high free energy assuring faster and greater drug permeation of drug through absorption barriers (intestinal epithelium, skin and mucosal surfaces); as a consequence enhanced bioavailability is obtatined, particularly of poorly water-soluble drugs, but also of peptide and proteins [44, 45]. One additional advantage of nanoemulsions is the protection from hydrolysis and oxidation provided by the encapsulation of the drug in the dispersed droplets, which also provides taste masking in regard to oral administration.

The effect of nanoemulsions on oral absorption of poorly soluble drugs is reported to be extremely significant. Candesartan cilexetil (CC) is a drug used in the treatment of hypertension with low oral bioavailability due to poor aqueous solubility. Gao *et* al proposed a CC loaded nanoemulsion for oral administration containing CC, soybean oil, Solutol HS-15, Tween 80, dichloromethane and distilled water using the emulsification-solvent evaporation technique, with a mean particle size of 35.5 ± 5.9 nm. This study found that CC loaded nanoemulsions were associated with a peak concentration 27 times higher than control (CC dissolved in ethanol and then diluted in Krebs-Ringer bicarbonate buffer) and a 10 fold increase in bioavailability [46].

Such effects are not limited to the oral administration rout but can enable the transdermal delivery of many drugs. The absorption of celecoxib through transdermally applied liquid nanoemulsions and nanoemulsion gels was compared to the commercial oral capsule formulation. Nanoemulsions were prepared using the spontaneous emulsification method and contained celecoxib (2% w/w), Sefsol-218 (7.5% w/w), Triacetin (7.5% w/w), Cremophor-EL (17.5% w/w), Transcutol-P (17.5% w/w) and distilled water to 100 % w/w. The nanoemulsion gel was prepared by dispersion and contained the same constituents used to prepare the previous nanoemulsion with the addition of Carbopol-940 (1% w/w) and Triethanolamine (0.5% w/w). This study found that the absorption of the drug through transdermally applied nanoemulsions and nanoemulsion gel resulted in a 3.30 and 2.97 fold increase in celecoxib bioavailability in comparison to the oral capsule formulation [47].

Although nanoemulsions have good stability they are subject to droplet size increase over time and eventually breakdown, via the Ostwald ripening process [16]. This process involves the movement of molecules of the dispersed phase by passive or micelle-assisted diffusion leading to the increase in size of larger droplets at the expense of smaller ones. The effect is more relevant for dispersed phases with high solubility in the dispersing phase and for highly polydisperse systems [24]. Nanoemulsions can also be made unstable through changes in environmental parameters such as temperature and pH, which can change upon delivery to patients [43, 48]. Moreover, nanoemulsions properties are formulation-dependent, meaning that a formulation that provides some desired characteristics is not always suitable for obtaining other favourable properties [12]. For example, the influence of co-solvent concentration on the initial mean droplet diameter, polydispersity index, turbidity and storage stability of nanoemulsions formed using spontaneous emulsification was investigated by Saberi and co-workers. One co-solvent investigated was propylene glycol (PG). This study found that transparent nanoemulsions displaying smaller droplets and a narrower polydispersity index could be obtained by using a PG concentration of approximately 30-40% however the same nanoemulsions were highly unstable during storage

showing significant droplet size growth [49]. Thus the characterization of nanoemulsions is an important consideration in their production and storage stability. Formulations are typically characterised for particle size, surface charge, drug content, morphology, stability and viscosity, all of which are important factors for their efficacy.

2. Nasal delivery of nanoemulsions

2.1 Local delivery

Traditionally, nasal drug delivery has been exploited for the treatment of local ailments of the nose and paranasal sinuses including allergic or infectious rhinitis, sinusitis, nasal polyposis, nasal infections and nasal congestion [4, 50]. Commonly administered drugs for these ailments include decongestants (ephedrine, oxymetazoline, phenylephrine, tramazolin, naphazoline and xylometaxolin), corticosteroids (beclamethasone, budesonide, fluticasone, mometasone and triamcinolone), antihistamines (azelastine and levocabastine), mast cell stabilisers (chromoglycate) and anticholingergics (ipratropium) [1, 51, 52]. However to the authors' knowledge no nanoemulsion formulations have been proposed or developed for local delivery. One possible reason for this is that nanoemulsions increase the permeability of drug across the nasal mucosa resulting in increased systemic concentration, which is not desirable for local delivery where the goal is to attain therapeutic concentrations of drug at the treatment site, avoiding systemic absorption [53].

2.2 Systemic delivery

It is well known that nasal drug administration is a viable means to obtain systemic drug delivery. This is reflected in the number of nasal formulations currently marketed for systemically acting drugs such as those for the treatment of migraine (butorphanol, ergotamine, sumatriptan and zolmitriptan), pain (fentanyl), diabetes insipidus (desmopressin), opioid overdose (naloxone) prostate cancer (buserelin) and post-menopausal osteoporosis (calcitonin) and the multitude currently under investigation including cardiovascular (propranolol, carvedilol and nifedipine), antiviral (acyclovir and zanamivir) and anti-emetic drugs (metoclopramide, ondansetron and scopolamine hydrobromide) [8, 50, 54, 55]. Nasal delivery offers the potential for rapid absorption and fast onset of action, whilst avoiding hepatic first pass metabolism. For these reasons it has been postulated for the delivery of proteins and peptides, which are difficult to administer by other routes, poorly soluble drugs or those with low oral bioavailability, for the treatment of acute pain, nausea and vomiting and for

critical situations or circumstances where rapid onset of action is vital such as in the case of opioid overdose and seizures [50, 56].

Research has shown that nanoemulsion drug delivery systems can significantly improve the transport of drugs across the nasal mucosa resulting in higher bioavailability compared to conventional nasal solutions or suspensions. Furthermore drugs with low oral bioavailability have been shown to display increased systemic bioavailability following the nasal administration of nanoemulsions [57-60].

Zolmitriptan (ZT) is a 5-$HT_{1B/1D}$ receptor partial agonist used in the acute treatment of migraine and related vascular headaches which undergoes first-pass metabolism resulting in poor oral bioavailability (≤40%) [61]. Currently ZT is available on the market in both conventional and orodispersable oral formulations and as a nasal spray. A study by Yu *et al* was conducted to compare the rate of absorption and efficacy of positively and negatively charged nanoemulsions with a conventional ZT nasal solution [57]. Nanoemulsions were prepared using high-pressure homogenisation and were composed of egg lecithin, ZT and medium chain triglycerides as oil phase and egg lecithin, poloxamer 188, glycerol, disodium EDTA and benzalkonium bromide in water as the aqueous phase. To create the two charged nanoemulsions oleic acid as a negative charge inducer was added to the aqueous phase (ZTNE-1) or stearylamine as a positive charge inducer was added to the oil phase (ZTNE-2). A simple ZT nasal solution (ZTS) was prepared by dissolving citric acid, hydrogen phosphate and ZT in water and adjusting to a pH of about 5. ZTNE-1 exhibited creaming within 24 hours at pH of 6, considered the more suitable for nasal administration, and was thus terminated from the study. On the contrary ZTNE-2 was found to be stable and. increased the absolute bioavailability of ZT in beagle dogs by approximately 30% compared to ZTS, reduced the T_{max} from 1.3 hours in the ZTS to only 0.58 hours and increased the C_{max} from 16.3 ng/ml to 39.7 ng/ml [57]. These results indicate that the cationic nanoemulsion formulation was superior to the conventional solution in terms of onset of action and bioavailability, appearing a promising approach for the improvement of migraine therapy.

Another example is that of nitrendipine (NDP), a potent antihypertensive drug which undergoes extensive first past metabolism, resulting in a low oral bioavailability of only 10-20%. Jain and Patravale conducted a study to enhance the bioavailability of NDP through a nanoemulsion formulation for nasal delivery. The NDP nanoemulsion was composed of NDP solubilised in Caproyl 90, Tween 80, Transcutol P and Solutol HS-15. NDP absorption from the nanoemulsion formulation provided rapid onset of action (t_{max} 1 hour vs. 3 hours for the oral formulation) and a relative bioavailability of 60.44%, significantly higher than the oral formulation. The

daily administration of the formulation over four consecutive weeks had no effect on the histology of the nasal mucosa [58].

A study by Mahajan and Dinger investigated the efficacy of an artemether nanoemulsion for nasal delivery and found similar results [59]. Artemether is a low molecular weight, lipid soluble, methylether derivative of artemisinin with low oral bioavailability (~40%). Artemether is an antimalarial drug and is highly effective against the blood stages of plasmodium and multi drug-resistant plasmodium falciparum [62, 63]. In cases of severe malaria oral medications are not well tolerated due to vomiting and convulsions, therefore fostering research into alternative administration routes . In this study the artemether nanoemulsion was prepared using a spontaneous emulsification method (titration method) and was comprised of ethyl oleate, Tween 20, Capmul PG 8 and artemether. The study, conducted on excised sheep nasal mucosa concluded that using the nanoemulsion formulation resulted in a high amount of artermether permeating through the mucosa, with 93% of the drug loaded crossing the membrane within 5 hours. However, it should be noted that this study lacked a control formulation and the true relevance of the results may be somewhat skewed [59].

Interestingly, one study investigated the use of a nanoemulsion gel with the aim to increase nasal bioavailability via increased residence time [60]. In this study Honsy and Banjar produced a zaleplon nanoemulsion composed of 15% Miglyol, 30% Labrasol and 10% PEG 200 using the aqueous titration method. This nanoemulsion was then gelled with 0.5% Carbopol to produce a pH dependent *in situ* gelling system containing dispersed droplets between 35 to 73 nm. Zaleplon is a non-benzodiazepine sedative-hypnotic drug used in the short-term management of insomnia [64, 65]. Following oral administration it undergoes extensive first pass metabolism, resulting in only 30% bioavailability and shows a delayed onset of action due to poor aqueous solubility [60]. Compared to intranasal zaleplon aqueous suspension, the nanoemulsion gel increased permeation nine-fold with the gel showing 75% permeation of the drug dose compared to only 8.5% obtained with the aqueous suspension. Furthermore, in comparison to the marketed tablet the nanoemulsion gel increased bioavailability of zaleplon 8 times. This increase in absorption displayed by nanoemulsions was suggested to be a result of both reduced particle size and presence of surfactants. This is highly plausible as surfactants are reported to increase membrane permeation by altering the structural integrity of the nasal mucosa and allowing the opening of tight junctions [53, 66].

2.3 Mucosal Vaccination

Vaccinations induce a long-lived protective immune response via the production of specific T and B cells as well as readily circulating antibodies [67]. Nasal vaccination with live-attenuated viruses effectively induces systemic and humoral immunities, however carries the inherent risk of viruses reverting back to their pathogenic state and causing disease, particularly in immunocompromised as well as in young (< 2 years) and the elderly patients. Alternative methods including the use of killed or purified antigen, or custom-made epitopes are safer, however are poorly immunogenic and often require an adjuvant to produce a sufficient immune response. Vaccine adjuvants including vaccine carriers are administered in conjunction with antigens and provide an immunostimulatory and/or immunomodulatory effect [67-69]. However, well characterised, effective and safe mucosal adjuvants are lacking [70].

The mucosal membranes provide a large surface area for the entry of many pathogens, with most infections of the intestinal, respiratory and genital tract entering the body via this route [71]. In humans the respiratory tract is the most common site of entry for many clinically significant pathogens including influenza, adeno-, corona- and respiratory syncytial- viruses, mycobacteria tuberculosis and streptococcus pneumonia to name a few [72]. Furthermore the nasal mucosa is of particular interest in the pathogenesis of respiratory infection as it is the body's first point of contact with inhaled pathogens [8, 71]. For this reason intranasal vaccination has been recognized as a potential route of non-invasive immunisation, particularly for the prophylaxis of respiratory diseases and extensively researched [71]. Currently there is one nasal vaccination product approved for human use on the market, Flumist®, a live-attenuated vaccine for influenza prophylaxis [8, 68, 69].

Nasal vaccination has been shown to have a number of advantages over traditional vaccination methods. Perhaps the most important and significant of these is the induction of both humoral and cellular immunity providing immunization at multiple mucosal sites, such as the lungs and genital tract in addition to the nasal application site. Injected vaccines are generally poor inducers of mucosal immunity, on the contrary nasal vaccination allows for enhanced disease protection based on an immune response at the site of infection [69, 73, 74]. Other advantages include non-invasiveness, reduced potential for injury and infection due to needle free administration, improved patient compliance and ease possibility of self-administration. Moreover, trained personnel for administration is not required, therefore reducing costs and maintaining suitability for use in mass immunisation programs [69, 75]. In recognition of the potential for nasal vaccination the Centre for Disease

Control and Prevention, the World Health Organisation and Global Alliance for Vaccines and Immunization have all expressed their support for the development of nasal immunisation delivery systems [69].

Nanoemulsions were originally developed for use in mucosal vaccines due to their broad antimicrobial activity. In viruses this is thought to occur through inactivation via physical disruption of the viral envelope, potentially allowing the development of preservative free vaccines. However, nanoemulsions were later recognised to possess promising mucosal adjuvant properties [76-78]. Nanoemulsions are unique adjuvants in that they can elicit a non-inflammatory immune response when mixed with protein antigens and are as a consequence much more than inert vehicles for antigen delivery. In fact, they induce the production of robust mucosal immunity, high serum titres and a cellular immune response through the activation of cytokine production by the epithelial cells and the induction of dendritic cell trafficking (Figure 2) [69, 72, 73]. The mucosal immune response has been attributed to the internalisation of the nanoemulsion droplets by the nasopharyngeal mucosa and subsequent activation of Toll-Like-Receptors (TLR), specifically TLR-2 and TLR-4 [69, 70, 79]. In addition to their potent adjuvant ability, nanoemulsions have a long shelf life at non-refrigerated temperatures (weeks to months in some cases) and thus can be used in developing countries where the provision of reliable refrigerated transport is lacking [68, 78]. The antigen stability at ambient temperature is believed to result from the antigen becoming embedded in the oil droplets of the nanoemulsion thus preserving the immunostimulating epitopes from degradation [80].

<Figure 2>

The W_{80}5EC nanoemulsion formulation is the most widely studied nanoemulsion adjuvant for nasal administration [81, 82] with trials in several animal models (including mice, ferrets and guinea pigs) conducted using ovalbumin [68, 72, 73, 77] respiratory syndical virus [78], anthrax [70], influenza [69, 76, 78], HIV [83] and *Burkholderia cenocepacia*, an important infection cause for immunocompromised individuals and those with cystic fibrosis [84]. The W_{80}5EC nanoemulsion is an optimised formulation manufactured by the NanoBio Corporation (Ann Arbor, MI, USA) using high speed emulsification method to obtain an O/W emulsion with droplets of 200 – 600 nm. It is composed of 64% soybean oil, 1% cetylpyridinium chloride (CDC), 5% Tween 80 and 8% ethanol in water. The W_{80}5EC formulation is a balance of both FDA-approved excipients and desired characteristics such as potency and stability of the antigen/nanoemulsion formulation [79].

A study by Stanberry and co-workers was conducted to determine the safety and immunogenicity of W_{80}5EC nanoemulsion as an adjuvant for the administration of seasonal influenza antigens [69]. In this Phase 1 human

clinical trial involving 199 healthy adult volunteers, W_{80}5EC nanoemulsion was administered with Fluzone® (approved inactivated seasonal influenza antigen) without safety concerns, significant adverse effects or dose-limiting toxicity observable at the highest concentration evaluated (20% W_{80}5EC) [69, 79]. Furthermore, the novel formulation elicited both systemic and mucosal immunity following a single administration allowing the production of an immune response at the site of infection, with particular benefit for populations at high risk of contagion. This study concluded that the W_{80}5EC nanoemulsion mucosal vaccine elicited an immune response to the inactivated influenza virus greater than a control vaccine not containing the nanoemulsion as an adjuvant and comparable to that induced by the marketed formulation Flumist® [69].

Another study investigated if the accurate and reliable delivery of nanoemulsion based vaccines to the nasal mucosa could face a significant challenge: antigens may undergo functional changes due to protein unfolding caused as a consequence of the shear forces applied upon device actuation [68]. In this study W_{80}5EC nanoemulsion was administered to mice in conjunction with a monomeric protein, ovalbumin (OVA), a particulate antigen, hepatitis B surface antigen (HBsAg) or an enzyme, alkaline phosphatase (AlkP). Two different commercially available nasal spray devices (Pfeiffer SAP-62602 multidose pump and the BD Hypak SCF 0.5 ml unit dose Accuspray™) were used to evaluate the effect of dose administration on proteins sensitive epitopes. This study concluded that despite significant differences in spray characteristics including droplet size, spray angle, plume width and ovality ratios between the two devices, nanoemulsions were not physically or chemically altered and retained the same potency following device actuation, suggesting that specially engineered devices are not required for the delivery of nanoemulsion-based vaccines [68].

2.4 Nose-to-brain delivery

Drug delivery to the CNS, despite the relatively high blood flow to the area, is significantly hindered by the presence of both the blood brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB) [9]. Although it is possible for systemically administered compounds with favourable characteristics such as low molecular weight and high lipophilicity to penetrate the BBB and reach the brain parenchyma, their use is limited as high doses are required to achieve therapeutic levels in the CNS, typically eliciting significant adverse effects [85, 86]. Alternative CNS delivery methods include intracerebroventricular, intrathecal or intraparenchymal injections. However these methods are not suitable for drugs requiring multiple doses as they are invasive, risky, expensive and require surgical intervention [9, 85]. The delivery of drugs to the CNS via nasal administration

provides a promising and novel alternative to these invasive methods, enabling drugs to circumvent the BBB thereby providing direct and rapid delivery to the brain [85].

There are three main pathways by which drugs can reach the CNS following nasal administration, namely: A) the olfactory nerve pathway, which innervates the olfactory epithelium of the nasal mucosa and terminates in the olfactory bulb, B) the trigeminal nerve pathway, which innervates both the respiratory and to a lesser degree the olfactory epithelium of the nasal mucosa and terminates in the pons or olfactory bulb respectively and C) the vascular pathway [4, 85]. Figure 3 outlines these three brain-targeting pathways for nose to brain delivery. Of these, the olfactory and/or trigeminal nerve pathways are believed to predominate and provide a means of direct drug delivery via axonal (slow) or perineural (fast) transport from the sub-mucosal space of the nose into the cerebrospinal fluid (CSF) compartment of the brain (Figure 4) [4, 87]. In particular the olfactory 'neuroepithelium' is unique in the body and present exclusively in the nasal cavity as it is the only part of the CNS that is in direct contact with the external environment [4]. The vascular pathway provides a secondary, indirect mechanism of delivery, whereby the drug is firstly absorbed into systemic circulation and subsequently transported across the BBB [4, 85].

<Figure 3>

Direct nose to CNS transport of nanoemulsions has been demonstrated using a number of different drugs including risperidone [89, 90], olanzapine [91], ziprasidone [92], curcumin [93], saquinavir [94], rizatriptan [95], carbamazepine [96], ropinirole [97], sumatriptan [98], clonazepam [99], tacrine [100] and zolmitriptan [101]. Interestingly, the majority of these studies investigated the use of mucoadhesive formulations obtained by either the addition of chitosan [90-93], polycarbophil [98, 99, 101] or by the preparation of a gel formulation [95, 96] and found these to be superior to simple nanoemulsion formulations for CNS delivery.

<Figure 4>

A study conducted by Kumar *et al* [90] investigated the effectiveness of nanoemulsions for the delivery of risperidone to the brain via the nose. Risperidone is an approved antipsychotic drug available in tablet, oral liquid and orally disintegrating tablet formulations that exhibits low bioavailability due to both extensive first-pass metabolism and relatively poor and non-specific brain delivery, resulting in numerous side-effects. This particular study compared the uptake of risperidone solution (RS), risperidone nanoemulsion (RNE) and risperidone mucoadhesive nanoemulsion (RMNE) following nasal administration (i.n) as well as RNE administered intravenously (i.v). The drug solution (RS) was prepared by combining risperidone, ethanol,

propylene glycol and distilled water. The RNE was prepared using the titration method and was composed of risperidone, Campul MCM, Tween 80, Tanscutol, propylene glycol and distilled water. Finally, chitosan was added to the RNE formulation to produce the mucoadhesive RMNE formulation. This study found that the concentration of risperidone in the brain of rats was significantly higher at all the time points following the intranasal administration of the RME formulation. Furthermore after 0.5 hours the brain to blood ratios following the administration of RS (i.n), RNE (i.n) and RMNE (i.n) and RNE (i.v) were 0.617, 0.754, 0.948 and 0.054 respectively, demonstrating the superiority of the formulations administered intranasally over the intravenous administration for drug delivery to the CNS. The results were explained by a direct nose-to-brain transport and the bypass of the BBB [90]. Moreover, of the formulations tested the RMNE formulation was found to have the highest percentage of drug targeting efficiency (%DTE) and nose-to-brain direct transport percentage (%DTP) which was nearly two-fold higher compared to the RS and RNE formulations, further illustrating the benefit of the mucoadhesive nanoemulsion formulation in CNS drug delivery (Figure 5) [90, 94]. The same authors obtained similar results with other antipsychotic drug, i.e. olanzapine and ziprasidone [91, 92].

<Figure 5>

Another study by Vyas *et al* [99] conducted using clonazepam found similar results. Clonazepam is a benzodiazepine derivative used in the treatment of *status epilepticus*. This study compared a clonazepam solution (CS), clonazepam microemulsion (CME) and clonazepam mucoadhesive microemulsion (CMME) administered intranasally as well as CME administered intravenously for effectiveness of drug delivery to the CNS in rats. The CS was prepared by the addition of clonazepam to distilled water and ethyl alcohol mixture. The CME was composed of medium chain triglyceride, polyoxyethylene-35-ricinoleate, polysorbate 80 and propylene glycol and prepared using the titration method with a droplet size of approximately 15.21 nm. The CMME was prepared by the addition of polycarbophil to the CME formulation previously described and contained droplets of about 11.27 nm. This study found that the time for the drug to reach maximum concentration (T_{max}) was much faster following the nasal administration of drugs, with a T_{max} of 1-2 hours for the brain compared to 2-4 hours for the blood. Furthermore the concentration of drug in the brain following intranasal administration of CME and CMME was found to be significantly higher than intravenously administered CME at all the time points. The systemic bioavailability (AUC) and maximum concentration (C_{max}) of clonazepam after intravenous administration was significantly higher than that elicited from the

intranasal administration of the drug microemulsion (CME) and solution CS. The CMME formulation instead produced an AUC and C_{max} comparable to that produced by the intravenous formulation probably due to the increased retention time produced by the polycarbophil mucoadhesion. In the brain, the CME and CMME produced significantly higher AUC and C_{max} compared to the CS following nasal administration, suggesting that the microemulsion formulation was responsible for this improvement. Moreover, the CMME produced the highest %DTE and %DTP followed by the CME, highlighting the great brain targeting potential of nanoemulsion formulations [99].

In a study by Samia *et al* [96] carbamazepine (CBZ) was loaded into a mucoadhesive nanoemugel (MNEG) and compared to intravenously administered CBZ solution in propylene glycol or propylene glycol alone. CBZ is an orally administered anti-epileptic drug with low solubility in water and slow and irregular gastrointestinal absorption leading to delayed brain uptake and a number of peripheral side effects. The nanoemulsion was prepared using the titration method containing oleic acid, labrasol and distilled water, the MNEG was then prepared by the addition of xanthan gum to the nanoemulsion previously prepared. Although no specific quantitative results were published, qualitative data indicates that the CBZ-MNEG is superior with those mice treated with CBZ-MNEG displaying a significantly delayed onset of convulsion and an increased protection from electric shocks.

Another antiepileptic drug, amiloride, was investigated using a mucoadhesive nanoemulsion for nose-to-brain delivery [102]. The optimized formulations presented mean droplet size around 10 nm and pH just below 6. The nasal administration of the nanoemulsion did not produce irritation or toxicity on nasal goat mucosa. However the scanty preliminary data were not followed by further publications about the antiepllileptic effects of the formulation.

Tacrine is a centrally acting, non-competitive, reversible, acetylcholinesterase inhibitor with an oral bioavailability between 10 and 30%, used in the treatment of Alzheimer's disease [103]. A study by Jogani *et al* investigated the effectiveness of tacrine microemulsion (TME) and mucoadhesive microemulsion (TMME) for brain targeting and for memory improvement in scopolamine-induced amnesic mice. The TME was produced using the titration method. Biodistribution studies of tacrine solution and microemulsion formulations following intravenous and intranasal administration were evaluated. These studies found that the T_{max} was lower following nasal administration (60 mins) compared to intravenous administration (120 mins) suggesting selective nose-to-brain transport. Furthermore, the concentration of tacrine in the brain was 2-fold higher following the intranasal

administration of the TMME formulation compared to the tacrine solution. Those mice treated with the TMME formulation were also the fastest to regain memory [100].

3. Conclusions and perspectives

Nanoemulsions have a number of significant and unique advantages favourable for drug delivery via a several administration routes. Of note is their ability to increase drugs absorption/permeation and bioavailability. In particular, they have demonstrated great potential in nasal drug delivery, not only as drug carriers for systemic and nose-to-brain delivery but also as an active component of mucosal vaccinations. Currently, nanoemulsions have not been proposed for the treatment of local ailments of the nose, however in the future this may become an area of interest. Another interesting application would be the delivery of peptides and proteins to the CNS using nanoemulsions. However, a better understanding of the mechanisms related to the nanoemulsion absorption enhancement through the nasal mucosa and molecule transport to the brain is required to further advance this formulation approach. Concerning the safety of nanoemulsions, additional *in vitro* and toxicology studies appears to be necessary to determine the effect of these formulations on the nasal mucosa and cilia. Finally, clinical studies should be conducted in order to confirm the superiority of nanoemulsion formulations over traditional one before nanoemulsion-based nasal products will be available on the market.

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734 **Figure Captions**

735 **Figure 1:** Physiological, physicochemical, formulation factors and device factors influencing nasal absorption
736 and methods to increase nasal absorption (modified from [4, 8, 55]).

737 **Figure 2:** Mechanism of action of nasal vaccination (modified from [81, 82]).

738 **Figure 3:** Brain targeting pathways following nasal administration [4, 88].

739 **Figure 4:** Direct nose to brain pathways (modified from [85, 87])**.**

740 **A** shows the olfactory nerve pathway whereby the nerves penetrate the epithelial layer of the nasal

741 mucosa providing both axonal (slow) and perineural (fast) absorption pathways.

742 **B** shows the trigeminal nerve pathway. The nerves do not penetrate the epithelial layer in this case and

743 terminate in the lamina propria, only allowing absorption via axonal (slow) transport.

744 **Figure 5:** Gamma scintigraphy image showing the distribution of the radioactivity in rats after the
745 administration of (A) risperidone nanoemulsion intravenously (RNE), (B) riepseridone mucoadhesive
746 nanoemulsion intranasally (RME), (C) risperidone nanoemulsion intranasally (RNE) (reproduced with
747 permission from [90]).

748 **Table 1. Advantages and limitations of nasal drug delivery (adapted from [1, 4-7])**.

ADVANTAGES	LIMITATIONS
• Highly vascularized • Highly permeable • Increased bioavailability of many drugs • Reliable, safe, non-invasive and convenient • Avoidance of first-pass metabolism	o Small dosage volume of only 25-200 µL o Mucociliary clearance (MCC) mechanism o Impaired drug absorption in case of nasal congestion o Improper administration technique could cause inefficient deposition
OPPORTUNITIES	**UNIQUENESS**
⌖ Large surface area increased by the presence of microvilli ⌖ Fast onset of action ⌖ Wide range of options for the delivery of hydrophobic, hydrophilic and/or high molecular weight compounds (>1kDa) ⌖ Potential differences in absorption and permeability potential between the different regions of the nasal cavity	✓ Lower enzyme levels compared to the gastrointestinal tract and liver ✓ Direct transport from the nose to the central nervous system (CNS) is possible bypassing the Blood Brain Barrier ✓ Nasal lavage to remove unabsorbed excess drug if needed

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751 **Table 2. Discriminating properties of macro-, nano- and microemulsions.**

	Macroemulsion	Nanoemulsion	Microemulsion
Droplet size	>1000 nm	<500 nm	<100 nm
Polydispersity	Large	Small	Small
Stability	Kinetic	High Kinetic	Thermodynamic
Ostwald ripening	Yes	Yes	No
Coalescence	Yes	No	No
Sedimentation/Creaming	Yes	No	No
Surfactant Concentration	1-3 wt %	4-8 wt %	10-30 wt %
Appearance	White	Translucent	Translucent
Production	High energy methods	High or low energy methods	Spontaneous
References	[13, 19, 20]	[13, 20-22]	[13, 19-21, 23]

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