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1 **Opportunities and Challenges for the Nasal Administration of Nanoemulsions**

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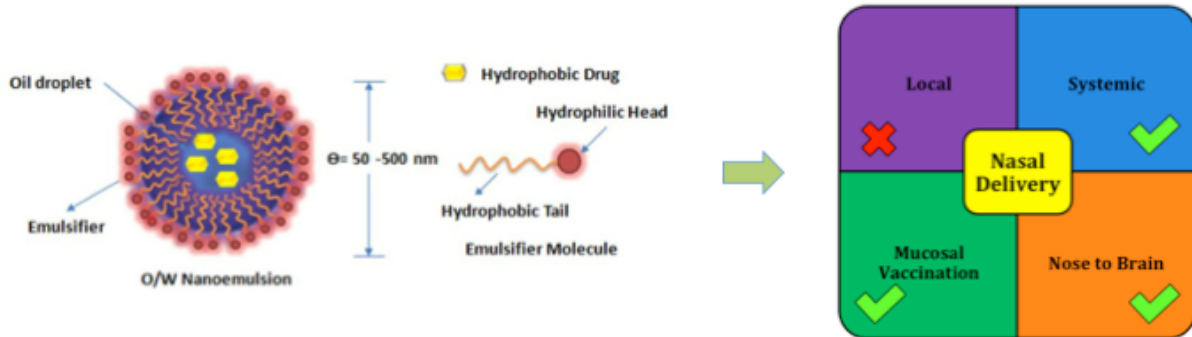
17 **Abstract**

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

33

34 **Graphical Abstract**

Nanoemulsions can improve efficacy by nasally delivered drugs



35

36

37 **Key words:** Drug Delivery, Mucosal Vaccine, Nanoemulsions, Nasal delivery, Nose to Brain, Pharmaceutical
38 nanotechnology

39 Running title: Nasal administration of nanoemulsions

40

41 **1. Introduction**

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

51

52 **1.1 Nasal delivery**

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

58

<Table 1>

59 Researchers have studied a number of different techniques by which many of the limitations posed by the nasal
60 mucosa can be reduced. The fundamental reasoning behind these techniques is to increase nasal residence time
61 and enhance nasal absorption or modify drug structure to produce more favourable physiochemical properties
62 for nasal absorption. The main techniques studied include nasal enzyme inhibition, permeation enhancing, drug
63 chemical structure modification and design of pro-drugs and particulate drug delivery systems such as
64 microparticles, nanoparticles and nanoemulsions [8].

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

67

68 **1.2 Nanoemulsions**

69 Emulsions are formed by the dispersion of one liquid, usually oil phase, into a second immiscible liquid, water
70 or aqueous phase [9]. Emulsions are typically distinguished by their particle size and stabilization into three
71 main categories namely macro-, nano- and microemulsions [10]. Table 2 outlines the different properties of
72 these three main emulsion categories. Nanoemulsions are a specific type of colloidal dispersion, which consist
73 of emulsions in which the dispersed phase droplets are in the nanometric scale [11]. They are also referred to in
74 different publications as miniemulsions, ultrafine emulsions, submicron emulsions, fine-dispersed emulsions,
75 parenteral emulsions and emulsoids [10-13]. In many ways nanoemulsions represent an intermediate between
76 the properties of macro- and microemulsions. Like microemulsions, nanoemulsions contains sub-micron size
77 droplets, appear transparent or translucent and possess stability against sedimentation or creaming. However,
78 microemulsions are thermodynamically stable and are formed spontaneously, while nanoemulsions are non-
79 equilibrium systems, in fact they are only kinetically stable and eventually subject to flocculation, coalescence
80 and Ostwald ripening. This partial overlap in properties, in conjunction with the fact that many authors do not
81 specify the nature of the submicron emulsion produced, has led to much confusion in the literature regarding
82 emulsion type definition and size range [14]. Moreover, it has been suggested that many microemulsion systems
83 studied in the literature are in fact misclassified nanoemulsion systems further adding to this confusion [15].

84 <Table 2>

85 Some physico-chemical aspects of nanoemulsion systems are essential to their superior stability when compared
86 to macroemulsions systems. The size of the dispersed phase droplets allows for the Brownian motions and
87 diffusion rate to overcome the effect gravitational force acting on the system leading to a significant reduction of
88 phenomena such as creaming, sedimentation and flocculation during storage. The system properties are also
89 preventing phase separation by coalescence, as droplets are not easily deformable and the significant surfactant
90 thickness on droplets surface impede the instability or disruption of the superficial film separating them [16, 21].

91 Nanoemulsions are non-equilibrium systems and thus, cannot be formed spontaneously. As a result, energy
92 input is required for their production. There are two main methods of production, namely low-energy and high-
93 energy methods [22, 23]. Low-energy methods utilize the intrinsic physicochemical properties the individual
94 components of the nanoemulsion to produce small droplets [24]. Techniques for the preparation of
95 nanoemulsions through low-energy methods include: self-emulsification (also referred to as titration method or

96 spontaneous emulsification method), emulsion phase inversion (EPI) and phase inversion temperature (PIT)
97 methods [25, 26].

98 Self-emulsification approaches exploit the diffusion of water miscible components, such as solvents, surfactants
99 and co-surfactants, from the organic phase into the continuous aqueous phase to produce a nanoemulsion. A
100 simple dilution process at constant temperature is sufficient to obtain the nanoemulsion without any phase
101 transition. The nanoemulsion formation can be obtained by dilution of homogeneous three-component solutions,
102 such as water, ethanol and oil, as in the Pastis/Ouzo effect, of an O/W microemulsion or of a cubic liquid
103 crystalline phase [24].

104 In the phase inversion processes, the emulsion system O/W reverse to W/O or vice versa. While the curvature of
105 the interface O/W gradually changes, the interfacial tension of the system decreases to minimum value and a
106 submicron emulsion can be obtained with minimal energy expenditure. Two types of phase inversion may
107 occur: (a) transitional inversion and (b) catastrophic inversion [27, 28]. The transitional inversion may occur
108 with changes in the affinity of the surfactants for aqueous and/or oil phases and may be induced by variations in
109 factors such as temperature, HLB values, salinity of the aqueous phase and polarity of the oily phase [29, 30].

110 In particular, changes in system temperature can promote modifications in the interactions (hydrogen bonding,
111 dipole-dipole interactions and induced dipoles) between the ethoxylated nonionic surfactants and the aqueous
112 phase. These surfactants have generally HLB values above 10, being amphiphilic molecules with a clear
113 predominance of hydrophilic aspect. However above the phase inversion temperature of the surfactant molecule
114 becomes predominantly lipophilic triggering the transitional inversion of the emulsion [31, 32].

115 The catastrophic phase inversion can occur when there is an increase in the volume of the dispersed phase or
116 variations in the ratio of the volumes of the aqueous and oil phase. This type of inversion is irreversible and can
117 occur over a wide range of volume fractions. The term catastrophic means a sudden change in behavior of a
118 system and occurs as a result of gradual changes in process conditions [33-35]. The phase inversion in this case
119 is triggered by the change of the water/oil ratio when the volume fraction of the dispersed phase increases. The
120 origin of the structural changes are related to the balance between droplet breakup and coalescence in the system
121 and the droplet size produced to the formation of the intermediate multiple emulsion dispersions (O/W/O for
122 O/W systems and W/O/W for W/O ones). The catastrophic phase inversion, although influenced by the
123 concentration of the surfactant is primarily dependent on the type and particle size distribution of the globules
124 formed, ie, the amount and morphology of the dispersed phase [34].

125 Emulsification by emulsion phase inversion (EPI) may be considered a type of catastrophic inversion, where the
126 point of phase inversion (PPI) is the composition at which the emulsion formed by the aqueous phase, oil and
127 surfactants reverses phases at constant temperature. The titration of water into an oily phase containing an
128 hydrophilic surfactant promotes the initial formation of an W/O dispersion. However, increasing the volume
129 fraction of water a change in the spontaneous curvature of the surfactant molecules occurs leading the inversion
130 to an O/W emulsion passing through an unstable multiple emulsion phase [35].

131 When using low-energy methods it is important to consider temperature control, especially when using the PIT
132 method, volumetric fraction of water and oil phases as well as surfactant and co-surfactant concentration and
133 weigh ratio [36, 37]. These factors are relatively easy to control on a small scale but may hinder the industrial
134 viability of these methods. Currently, there is less information regarding the industrial scale-up of
135 nanoemulsions produced by low-energy methods compared to high-energy ones.

136 In alternative to low-energy manufacturing methods, high-energy methods utilize mechanical devices to disrupt
137 the oil and water phases to form nano-sized droplets [22]. The main apparatuses utilized include rotor/stator
138 devices and, more recently, the high efficiency ultrasound generators and high-pressure homogenizers [11].
139 High-energy methods have the ability to produce submicron emulsions from a large variety of materials,
140 displaying homogenous flow and narrow droplet size distribution and thus have the potential to be utilized on an
141 industrial scale [9, 26]. However, there are a number of limitations to this method. Firstly it is not suitable for
142 heat sensitive drugs such as retinoids and macromolecules, including proteins, enzymes and nucleic acids [22].
143 Secondly, due to the high-energy requirements and inefficient use of energy (approximately 0.1% of the energy
144 produced is directly used for the emulsification process) this approach is also relatively expensive [23]. Thus,
145 low-energy methods are considered advantageous in regard to cost, energy efficiency, simplicity of
146 implementation and can be used for fragile or heat sensitive drugs [13]. However, low-energy methods generally
147 require higher surfactant concentrations than high-energy emulsification methods. A recent study by Ostertag
148 and colleagues compared the low-energy phase inversion technique to the high-energy microfluidisation
149 technique and found that small droplets could be produced by both methods, however much less surfactant was
150 needed for the high-energy method than the low-energy method, with a surfactant to oil ratio required to obtain
151 droplets with diameter smaller than 160 nm of ≥ 0.1 and ≥ 0.7 respectively [38].

152 Nanoemulsions have attracted much interest in recent years over a number of different fields including the
153 personal care, cosmetics, agrochemical, chemical, food and pharmaceutical industries [9, 13]. Within the

154 pharmaceutical industry, nanoemulsions are being investigated as a formulation approach suitable for a number
155 of different administration routes such as topical, transdermal, parenteral, ocular, pulmonary, nasal and oral [23,
156 25, 38]. Even though nanoemulsions are primarily regarded as a vehicle for drug formulation, they have
157 received increasing attention for a number of novel applications as delivery systems for the controlled release of
158 drugs, the targeted delivery of anti-cancer agents, and mucosal vaccination [23]. This interest can be largely
159 attributed to their many unique and favorable properties, providing a number of advantages over conventional
160 emulsions. Nanoemulsions are kinetically stable and are therefore not significantly affected by flocculation,
161 coalescence, creaming or sedimentation during storage time [39]. They can be formulated into foams, liquids,
162 creams and sprays and being transparent/translucent can be incorporated into these preparations without loss of
163 clarity [40, 41]. They can be used to deliver both hydrophilic and lipophilic drugs and are generally considered
164 non-toxic and non-irritant formulations. In fact, nanoemulsions are usually manufactured using reasonably low
165 concentrations of surfactants that are Generally Recognized As Safe (GRAS) for human consumption by the
166 FDA, rendering them safe for enteral and mucosal administration [24, 39, 40]. Furthermore, nanoemulsions
167 present large surface area and high free energy assuring faster and greater drug permeation of drug through
168 absorption barriers (intestinal epithelium, skin and mucosal surfaces); as a consequence enhanced bioavailability
169 is obtained, particularly of poorly water-soluble drugs, but also of peptide and proteins [41, 42]. One additional
170 advantage of nanoemulsions is the protection from hydrolysis and oxidation provided by the encapsulation of
171 the drug in the dispersed droplets, which also provides taste masking in regard to oral administration.

172 The effect of nanoemulsions on oral absorption of poorly soluble drugs is reported to be extremely significant.
173 Candesartan cilexetil (CC) is a drug used in the treatment of hypertension with low oral bioavailability due to
174 poor aqueous solubility. Gao *et al* proposed a CC loaded nanoemulsion for oral administration containing CC,
175 soybean oil, Solutol HS-15, Tween 80, dichloromethane and distilled water using the emulsification-solvent
176 evaporation technique, with a mean particle size of 35.5 ± 5.9 nm. This study found that CC loaded
177 nanoemulsions were associated with a peak concentration 27 times higher than control (CC dissolved in ethanol
178 and then diluted in Krebs-Ringer bicarbonate buffer) and a 10 fold increase in bioavailability [43].

179 Such effects are not limited to the oral administration route but can enable the transdermal delivery of many
180 drugs. The absorption of celecoxib through transdermally applied liquid nanoemulsions and nanoemulsion gels
181 was compared to the commercial oral capsule formulation. Nanoemulsions were prepared using the spontaneous
182 emulsification method and contained celecoxib (2% w/w), Sefsol-218 (7.5% w/w), Triacetin (7.5% w/w),

183 Cremophor-EL (17.5% w/w), Transcutol-P (17.5% w/w) and distilled water to 100 % w/w. The nanoemulsion
184 gel was prepared by dispersion and contained the same constituents used to prepare the previous nanoemulsion
185 with the addition of Carbopol-940 (1% w/w) and Triethanolamine (0.5% w/w). This study found that the
186 absorption of the drug through transdermally applied nanoemulsions and nanoemulsion gel resulted in a 3.30
187 and 2.97 fold increase in celecoxib bioavailability in comparison to the oral capsule formulation [44].

188 Although nanoemulsions have good stability they are subject to droplet size increase over time and eventually
189 breakdown, via the Ostwald ripening process [13]. This process involves the movement of molecules of the
190 dispersed phase by passive or micelle-assisted diffusion leading to the increase in size of larger droplets at the
191 expense of smaller ones. The effect is more relevant for dispersed phases with high solubility in the dispersing
192 phase and for highly polydisperse systems [21]. Nanoemulsions can also be made unstable through changes in
193 environmental parameters such as temperature and pH, which can change upon delivery to patients [40, 45].
194 Moreover, nanoemulsions properties are formulation-dependent, meaning that a formulation that provides some
195 desired characteristics is not always suitable for obtaining other favourable properties [9]. For example, the
196 influence of co-solvent concentration on the initial mean droplet diameter, polydispersity index, turbidity and
197 storage stability of nanoemulsions formed using spontaneous emulsification was investigated by Saberi and co-
198 workers. One co-solvent investigated was propylene glycol (PG). This study found that transparent
199 nanoemulsions displaying smaller droplets and a narrower polydispersity index could be obtained by using a PG
200 concentration of approximately 30-40% however the same nanoemulsions were highly unstable during storage
201 showing significant droplet size growth [46]. Thus the characterization of nanoemulsions is an important
202 consideration in their production and storage stability. Formulations are typically characterised for particle size,
203 surface charge, drug content, morphology, stability and viscosity, all of which are important factors for their
204 efficacy.

205

206 **2. Nasal delivery of nanoemulsions**

207 **2.1 Local delivery**

208 Traditionally, nasal drug delivery has been exploited for the treatment of local ailments of the nose and
209 paranasal sinuses including allergic or infectious rhinitis, sinusitis, nasal polyposis, nasal infections and nasal
210 congestion [4, 47]. Commonly administered drugs for these ailments include decongestants (ephedrine,
211 oxymetazoline, phenylephrine, tramazolin, naphazoline and xylometaxolin), corticosteroids (beclamethasone,

212 budesonide, fluticasone, mometasone and triamcinolone), antihistamines (azelastine and levocabastine), mast
213 cell stabilisers (chromoglycate) and anticholinergics (ipratropium) [1, 48, 49]. However to the authors'
214 knowledge no nanoemulsion formulations have been proposed or developed for local delivery. One possible
215 reason for this is that nanoemulsions increase the permeability of drug across the nasal mucosa resulting in
216 increased systemic concentration, which is not desirable for local delivery where the goal is to attain therapeutic
217 concentrations of drug at the treatment site, avoiding systemic absorption [50].

218

219 **2.2 Systemic delivery**

220 It is well known that nasal drug administration is a viable means to obtain systemic drug delivery. This is
221 reflected in the number of nasal formulations currently marketed for systemically acting drugs such as those for
222 the treatment of migraine (butorphanol, ergotamine, sumatriptan and zolmitriptan), pain (fentanyl), diabetes
223 insipidus (desmopressin), opioid overdose (naloxone) prostate cancer (buserelin) and post-menopausal
224 osteoporosis (calcitonin) and the multitude currently under investigation including cardiovascular (propranolol,
225 carvedilol and nifedipine), antiviral (acyclovir and zanamivir) and anti-emetic drugs (metoclopramide,
226 ondansetron and scopolamine hydrobromide) [47, 51-53]. Nasal delivery offers the potential for rapid
227 absorption and fast onset of action, whilst avoiding hepatic first pass metabolism. For these reasons it has been
228 postulated for the delivery of proteins and peptides, which are difficult to administer by other routes, poorly
229 soluble drugs or those with low oral bioavailability, for the treatment of acute pain, nausea and vomiting and for
230 critical situations or circumstances where rapid onset of action is vital such as in the case of opioid overdose and
231 seizures [47, 54].

232 The respiratory region of the nasal mucosa covers the largest area of the nasal cavity and is the main site for
233 drug absorption into the systemic circulation [51]. Compounds are proposed to enter systemic circulation via a
234 number of mechanisms including transcellular (through the interior of the epithelial cells), paracellular (through
235 the tight junctions between cells), carrier-mediated (e.g. organic cation transporters and amino acids
236 transporters) and transcytosis pathways [51, 55, 56]. The proportion of drug that successfully reaches systemic
237 circulation is dependent on the physiological characteristics of the nasal mucosa, physicochemical/molecular
238 properties of the drug, pharmaceutical properties of the formulation and factors related to the delivery device as
239 shown in Figure 1 [4, 56].

240

<Figure 1>

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

245 Zolmitriptan (ZT) is a 5-HT_{1B/1D} receptor partial agonist used in the acute treatment of migraine and related
246 vascular headaches which undergoes first-pass metabolism resulting in poor oral bioavailability ($\leq 40\%$) [61].

247 Currently ZT is available on the market in both conventional and orodispersible oral formulations and as a nasal
248 spray. A study by Yu *et al* was conducted to compare the rate of absorption and efficacy of positively and
249 negatively charged nanoemulsions with a conventional ZT nasal solution [57]. Nanoemulsions were prepared
250 using high-pressure homogenisation and were composed of egg lecithin, ZT and medium chain triglycerides as
251 oil phase and egg lecithin, poloxamer 188, glycerol, disodium EDTA and benzalkonium bromide in water as the
252 aqueous phase. To create the two charged nanoemulsions oleic acid as a negative charge inducer was added to
253 the aqueous phase (ZTNE-1) or stearylamine as a positive charge inducer was added to the oil phase (ZTNE-2).

254 A simple ZT nasal solution (ZTS) was prepared by dissolving citric acid, hydrogen phosphate and ZT in water
255 and adjusting to a pH of about 5. ZTNE-1 exhibited creaming within 24 hours at pH of 6, considered the more
256 suitable for nasal administration, and was thus terminated from the study. On the contrary ZTNE-2 was found to
257 be stable and. increased the absolute bioavailability of ZT in beagle dogs by approximately 30% compared to
258 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
259 39.7 ng/ml [57]. These results indicate that the cationic nanoemulsion formulation was superior to the
260 conventional solution in terms of onset of action and bioavailability, appearing a promising approach for the
261 improvement of migraine therapy.

262 Another example is that of nitrendipine (NDP), a potent antihypertensive drug which undergoes extensive first
263 past metabolism, resulting in a low oral bioavailability of only 10-20%. Jain and Patravale conducted a study to
264 enhance the bioavailability of NDP through a nanoemulsion formulation for nasal delivery. The NDP
265 nanoemulsion was composed of NDP solubilised in Caproyl 90, Tween 80, Transcutol P and Solutol HS-15.
266 NDP absorption from the nanoemulsion formulation provided rapid onset of action (t_{max} 1 hour vs. 3 hours for
267 the oral formulation) and a relative bioavailability of 60.44%, significantly higher than the oral formulation. The
268 daily administration of the formulation over four consecutive weeks had no effect on the histology of the nasal
269 mucosa [58].

270 A study by Mahajan and Dinger investigated the efficacy of an artemether nanoemulsion for nasal delivery and
271 found similar results [59]. Artemether is a low molecular weight, lipid soluble, methylether derivative of
272 artemisinin with low oral bioavailability (~40%). Artemether is an antimalarial drug and is highly effective
273 against the blood stages of plasmodium and multi drug-resistant plasmodium falciparum [62, 63]. In cases of
274 severe malaria oral medications are not well tolerated due to vomiting and convulsions, therefore fostering
275 research into alternative administration routes . In this study the artemether nanoemulsion was prepared using a
276 spontaneous emulsification method (titration method) and was comprised of ethyl oleate, Tween 20, Capmul PG
277 8 and artemether. The study, conducted on excised sheep nasal mucosa concluded that using the nanoemulsion
278 formulation resulted in a high amount of artemether permeating through the mucosa, with 93% of the drug
279 loaded crossing the membrane within 5 hours. However, it should be noted that this study lacked a control
280 formulation and the true relevance of the results may be somewhat skewed [59].

281 Interestingly, one study investigated the use of a nanoemulsion gel with the aim to increase nasal bioavailability
282 via increased residence time [60]. In this study Honsy and Banjar produced a zaleplon nanoemulsion composed
283 of 15% Miglyol, 30% Labrasol and 10% PEG 200 using the aqueous titration method. This nanoemulsion was
284 then gelled with 0.5% Carbopol to produce a pH dependent *in situ* gelling system containing dispersed droplets
285 between 35 to 73 nm. Zaleplon is a non-benzodiazepine sedative-hypnotic drug used in the short-term
286 management of insomnia [64, 65]. Following oral administration it undergoes extensive first pass metabolism,
287 resulting in only 30% bioavailability and shows a delayed onset of action due to poor aqueous solubility [60].
288 Compared to intranasal zaleplon aqueous suspension, the nanoemulsion gel increased permeation nine-fold with
289 the gel showing 75% permeation of the drug dose compared to only 8.5% obtained with the aqueous suspension.
290 Furthermore, in comparison to the marketed tablet the nanoemulsion gel increased bioavailability of zaleplon 8
291 times. This increase in absorption displayed by nanoemulsions was suggested to be a result of both reduced
292 particle size and presence of surfactants. This is highly plausible as surfactants are reported to increase
293 membrane permeation by altering the structural integrity of the nasal mucosa and allowing the opening of tight
294 junctions [50, 66].

295

296 **2.3 Mucosal Vaccination**

297 Vaccinations induce a long-lived protective immune response via the production of specific T and B cells as
298 well as readily circulating antibodies [67]. Nasal vaccination with live-attenuated viruses effectively induces
299 systemic and humoral immunities, however carries the inherent risk of viruses reverting back to their pathogenic

300 state and causing disease, particularly in immunocompromised as well as in young (< 2 years) and the elderly
301 patients. Alternative methods including the use of killed or purified antigen, or custom-made epitopes are safer,
302 however are poorly immunogenic and often require an adjuvant to produce a sufficient immune response.
303 Vaccine adjuvants including vaccine carriers are administered in conjunction with antigens and provide an
304 immunostimulatory and/or immunomodulatory effect [67-69]. However, well characterised, effective and safe
305 mucosal adjuvants are lacking [70].

306 The mucosal membranes provide a large surface area for the entry of many pathogens, with most infections of
307 the intestinal, respiratory and genital tract entering the body via this route [71]. In humans the respiratory tract is
308 the most common site of entry for many clinically significant pathogens including influenza, adeno-, corona-
309 and respiratory syncytial- viruses, mycobacteria tuberculosis and streptococcus pneumonia to name a few [72].
310 Furthermore the nasal mucosa is of particular interest in the pathogenesis of respiratory infection as it is the
311 body's first point of contact with inhaled pathogens [51, 71]. For this reason intranasal vaccination has been
312 recognized as a potential route of non-invasive immunisation, particularly for the prophylaxis of respiratory
313 diseases and extensively researched [71]. Currently there is one nasal vaccination product approved for human
314 use on the market, Flumist[®], a live-attenuated vaccine for influenza prophylaxis [51, 68, 69].

315 Nasal vaccination has been shown to have a number of advantages over traditional vaccination methods.
316 Perhaps the most important and significant of these is the induction of both humoral and cellular immunity
317 providing immunization at multiple mucosal sites, such as the lungs and genital tract in addition to the nasal
318 application site. Injected vaccines are generally poor inducers of mucosal immunity, on the contrary nasal
319 vaccination allows for enhanced disease protection based on an immune response at the site of infection [69, 73,
320 74]. Other advantages include non-invasiveness, reduced potential for injury and infection due to needle free
321 administration, improved patient compliance and ease possibility of self-administration. Moreover, trained
322 personnel for administration is not required, therefore reducing costs and maintaining suitability for use in mass
323 immunisation programs [69, 75]. In recognition of the potential for nasal vaccination the Centre for Disease
324 Control and Prevention, the World Health Organisation and Global Alliance for Vaccines and Immunization
325 have all expressed their support for the development of nasal immunisation delivery systems [69].

326 Nanoemulsions were originally developed for use in mucosal vaccines due to their broad antimicrobial activity.
327 In viruses this is thought to occur through inactivation via physical disruption of the viral envelope, potentially
328 allowing the development of preservative free vaccines. However, nanoemulsions were later recognised to

329 possess promising mucosal adjuvant properties [76-78]. Nanoemulsions are unique adjuvants in that they can
330 elicit a non-inflammatory immune response when mixed with protein antigens and are as a consequence much
331 more than inert vehicles for antigen delivery. In fact, they induce the production of robust mucosal immunity,
332 high serum titres and a cellular immune response through the activation of cytokine production by the epithelial
333 cells and the induction of dendritic cell trafficking (Figure 2) [69, 72, 73]. The mucosal immune response has
334 been attributed to the internalisation of the nanoemulsion droplets by the nasopharyngeal mucosa and
335 subsequent activation of Toll-Like-Receptors (TLR), specifically TLR-2 and TLR-4 [69, 70, 79]. In addition to
336 their potent adjuvant ability, nanoemulsions have a long shelf life at non-refrigerated temperatures (weeks to
337 months in some cases) and thus can be used in developing countries where the provision of reliable refrigerated
338 transport is lacking [68, 78]. The antigen stability at ambient temperature is believed to result from the antigen
339 becoming embedded in the oil droplets of the nanoemulsion thus preserving the immunostimulating epitopes
340 from degradation [80].

341 <Figure 2>

342 The W₈₀5EC nanoemulsion formulation is the most widely studied nanoemulsion adjuvant for nasal
343 administration with trials in several animal models (including mice, ferrets and guinea pigs) conducted using
344 ovalbumin [68, 72, 73, 77] respiratory syndical virus [78], anthrax [70], influenza [69, 76, 78], HIV [83] and
345 *Burkholderia cenocepacia*, an important infection cause for immunocompromised individuals and those with
346 cystic fibrosis [84]. The W₈₀5EC nanoemulsion is an optimised formulation manufactured by the NanoBio
347 Corporation (Ann Arbor, MI, USA) using high speed emulsification method to obtain an O/W emulsion with
348 droplets of 200 – 600 nm. It is composed of 64% soybean oil, 1% cetylpyridinium chloride (CDC), 5% Tween
349 80 and 8% ethanol in water. The W₈₀5EC formulation is a balance of both FDA-approved excipients and desired
350 characteristics such as potency and stability of the antigen/nanoemulsion formulation [79].

351 A study by Stanberry and co-workers was conducted to determine the safety and immunogenicity of W₈₀5EC
352 nanoemulsion as an adjuvant for the administration of seasonal influenza antigens [69]. In this Phase 1 human
353 clinical trial involving 199 healthy adult volunteers, W₈₀5EC nanoemulsion was administered with Fluzone[®]
354 (approved inactivated seasonal influenza antigen) without safety concerns, significant adverse effects or dose-
355 limiting toxicity observable at the highest concentration evaluated (20% W₈₀5EC) [69, 79]. Furthermore, the
356 novel formulation elicited both systemic and mucosal immunity following a single administration allowing the
357 production of an immune response at the site of infection, with particular benefit for populations at high risk of

358 contagion. This study concluded that the W₈₀5EC nanoemulsion mucosal vaccine elicited an immune response
359 to the inactivated influenza virus greater than a control vaccine not containing the nanoemulsion as an adjuvant
360 and comparable to that induced by the marketed formulation Flumist[®] [69].

361 Another study investigated if the accurate and reliable delivery of nanoemulsion based vaccines to the nasal
362 mucosa could face a significant challenge: antigens may undergo functional changes due to protein unfolding
363 caused as a consequence of the shear forces applied upon device actuation [68]. In this study W₈₀5EC
364 nanoemulsion was administered to mice in conjunction with a monomeric protein, ovalbumin (OVA), a
365 particulate antigen, hepatitis B surface antigen (HBsAg) or an enzyme, alkaline phosphatase (AlkP). Two
366 different commercially available nasal spray devices (Pfeiffer SAP-62602 multidose pump and the BD Hypak
367 SCF 0.5 ml unit dose Accuspray[™]) were used to evaluate the effect of dose administration on proteins sensitive
368 epitopes. This study concluded that despite significant differences in spray characteristics including droplet size,
369 spray angle, plume width and ovality ratios between the two devices, nanoemulsions were not physically or
370 chemically altered and retained the same potency following device actuation, suggesting that specially
371 engineered devices are not required for the delivery of nanoemulsion-based vaccines [68].

372

373 **2.4 Nose-to-brain delivery**

374 Drug delivery to the CNS, despite the relatively high blood flow to the area, is significantly hindered by the
375 presence of both the blood brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB) [55].
376 Although it is possible for systemically administered compounds with favourable characteristics such as low
377 molecular weight and high lipophilicity to penetrate the BBB and reach the brain parenchyma, their use is
378 limited as high doses are required to achieve therapeutic levels in the CNS, typically eliciting significant adverse
379 effects [85, 86]. Alternative CNS delivery methods include intracerebroventricular, intrathecal or
380 intraparenchymal injections. However these methods are not suitable for drugs requiring multiple doses as they
381 are invasive, risky, expensive and require surgical intervention [55, 85]. The delivery of drugs to the CNS via
382 nasal administration provides a promising and novel alternative to these invasive methods, enabling drugs to
383 circumvent the BBB thereby providing direct and rapid delivery to the brain [85].

384 There are three main pathways by which drugs can reach the CNS following nasal administration, namely: A)
385 the olfactory nerve pathway, which innervates the olfactory epithelium of the nasal mucosa and terminates in the
386 olfactory bulb, B) the trigeminal nerve pathway, which innervates both the respiratory and to a lesser degree the

387 olfactory epithelium of the nasal mucosa and terminates in the pons or olfactory bulb respectively and C) the
388 vascular pathway [4, 85]. Figure 3 outlines these three brain-targeting pathways for nose to brain delivery. Of
389 these, the olfactory and/or trigeminal nerve pathways are believed to predominate and provide a means of direct
390 drug delivery via axonal (slow) or perineural (fast) transport from the sub-mucosal space of the nose into the
391 cerebrospinal fluid (CSF) compartment of the brain (Figure 4) [4, 87]. In particular the olfactory
392 'neuroepithelium' is unique in the body and present exclusively in the nasal cavity as it is the only part of the
393 CNS that is in direct contact with the external environment [4]. The vascular pathway provides a secondary,
394 indirect mechanism of delivery, whereby the drug is firstly absorbed into systemic circulation and subsequently
395 transported across the BBB [4, 85].

396 <Figure 3>

397 Direct nose to CNS transport of nanoemulsions has been demonstrated using a number of different drugs
398 including risperidone [89, 90], olanzapine [91], ziprasidone [92], curcumin [93], saquinavir [94], rizatriptan
399 [95], carbamazepine [96], ropinirole [97], sumatriptan [98], clonazepam [99], tacrine [100] and zolmitriptan
400 [101]. Interestingly, the majority of these studies investigated the use of mucoadhesive formulations obtained by
401 either the addition of chitosan [90-93], polycarbophil [98, 99, 101] or by the preparation of a gel formulation
402 [95, 96] and found these to be superior to simple nanoemulsion formulations for CNS delivery.

403 <Figure 4>

404 A study conducted by Kumar *et al* [90] investigated the effectiveness of nanoemulsions for the delivery of
405 risperidone to the brain via the nose. Risperidone is an approved antipsychotic drug available in tablet, oral
406 liquid and orally disintegrating tablet formulations that exhibits low bioavailability due to both extensive first-
407 pass metabolism and relatively poor and non-specific brain delivery, resulting in numerous side-effects. This
408 particular study compared the uptake of risperidone solution (RS), risperidone nanoemulsion (RNE) and
409 risperidone mucoadhesive nanoemulsion (RMNE) following nasal administration (i.n) as well as RNE
410 administered intravenously (i.v). The drug solution (RS) was prepared by combining risperidone, ethanol,
411 propylene glycol and distilled water. The RNE was prepared using the titration method and was composed of
412 risperidone, Campul MCM, Tween 80, Tanscutol, propylene glycol and distilled water. Finally, chitosan was
413 added to the RNE formulation to produce the mucoadhesive RMNE formulation. This study found that the
414 concentration of risperidone in the brain of rats was significantly higher at all the time points following the
415 intranasal administration of the RME formulation. Furthermore after 0.5 hours the brain to blood ratios

416 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
417 0.054 respectively, demonstrating the superiority of the formulations administered intranasally over the
418 intravenous administration for drug delivery to the CNS. The results were explained by a direct nose-to-brain
419 transport and the bypass of the BBB [90]. Moreover, of the formulations tested the RMNE formulation was
420 found to have the highest percentage of drug targeting efficiency (%DTE) and nose-to-brain direct transport
421 percentage (%DTP) which was nearly two-fold higher compared to the RS and RNE formulations, further
422 illustrating the benefit of the mucoadhesive nanoemulsion formulation in CNS drug delivery (Figure 5) [90, 94].
423 The same authors obtained similar results with other antipsychotic drug, i.e. olanzapine and ziprasidone [91,
424 92].

425 <Figure 5>

426 Another study by Vyas *et al* [99] conducted using clonazepam found similar results. Clonazepam is a
427 benzodiazepine derivative used in the treatment of *status epilepticus*. This study compared a clonazepam
428 solution (CS), clonazepam microemulsion (CME) and clonazepam mucoadhesive microemulsion (CMME)
429 administered intranasally as well as CME administered intravenously for effectiveness of drug delivery to the
430 CNS in rats. The CS was prepared by the addition of clonazepam to distilled water and ethyl alcohol mixture.
431 The CME was composed of medium chain triglyceride, polyoxyethylene-35-ricinoleate, polysorbate 80 and
432 propylene glycol and prepared using the titration method with a droplet size of approximately 15.21 nm. The
433 CMME was prepared by the addition of polycarbophil to the CME formulation previously described and
434 contained droplets of about 11.27 nm. This study found that the time for the drug to reach maximum
435 concentration (T_{max}) was much faster following the nasal administration of drugs, with a T_{max} of 1-2 hours for
436 the brain compared to 2-4 hours for the blood. Furthermore the concentration of drug in the brain following
437 intranasal administration of CME and CMME was found to be significantly higher than intravenously
438 administered CME at all the time points. The systemic bioavailability (AUC) and maximum concentration
439 (C_{max}) of clonazepam after intravenous administration was significantly higher than that elicited from the
440 intranasal administration of the drug microemulsion (CME) and solution CS. The CMME formulation instead
441 produced an AUC and C_{max} comparable to that produced by the intravenous formulation probably due to the
442 increased retention time produced by the polycarbophil mucoadhesion. In the brain, the CME and CMME
443 produced significantly higher AUC and C_{max} compared to the CS following nasal administration, suggesting that
444 the microemulsion formulation was responsible for this improvement. Moreover, the CMME produced the

445 highest %DTE and %DTP followed by the CME, highlighting the great brain targeting potential of
446 nanoemulsion formulations [99].

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

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

461 Tacrine is a centrally acting, non-competitive, reversible, acetylcholinesterase inhibitor with an oral
462 bioavailability between 10 and 30%, used in the treatment of Alzheimer's disease [103]. A study by Jogani *et al*
463 investigated the effectiveness of tacrine microemulsion (TME) and mucoadhesive microemulsion (TMME) for
464 brain targeting and for memory improvement in scopolamine-induced amnesic mice. The TME was produced
465 using the titration method. Biodistribution studies of tacrine solution and microemulsion formulations following
466 intravenous and intranasal administration were evaluated. These studies found that the T_{max} was lower following
467 nasal administration (60 mins) compared to intravenous administration (120 mins) suggesting selective nose-to-
468 brain transport. Furthermore, the concentration of tacrine in the brain was 2-fold higher following the intranasal
469 administration of the TMME formulation compared to the tacrine solution. Those mice treated with the TMME
470 formulation were also the fastest to regain memory [100].

471

472 **3. Conclusion**

473 Nanoemulsions have a number of significant and unique advantages favourable for drug delivery via a several
474 administration routes. Of note is their ability to increase drugs absorption/permeation and bioavailability. In
475 particular, they have demonstrated great potential in nasal drug delivery systems, not only as drug carriers for
476 systemic and nose-to-brain delivery but also as an active component of mucosal vaccinations. Currently,
477 nanoemulsions have not been proposed for the treatment of local ailments of the nose, however in the future this
478 may become an area of interest. In any case further *in vitro* and toxicology studies to determine the effect of the
479 nanoemulsion formulation on the nasal mucosa and cilia, followed by clinical studies able to prove the
480 improvement over traditional formulations should be conducted before these formulations are to be available on
481 the market.

482

483

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487

488

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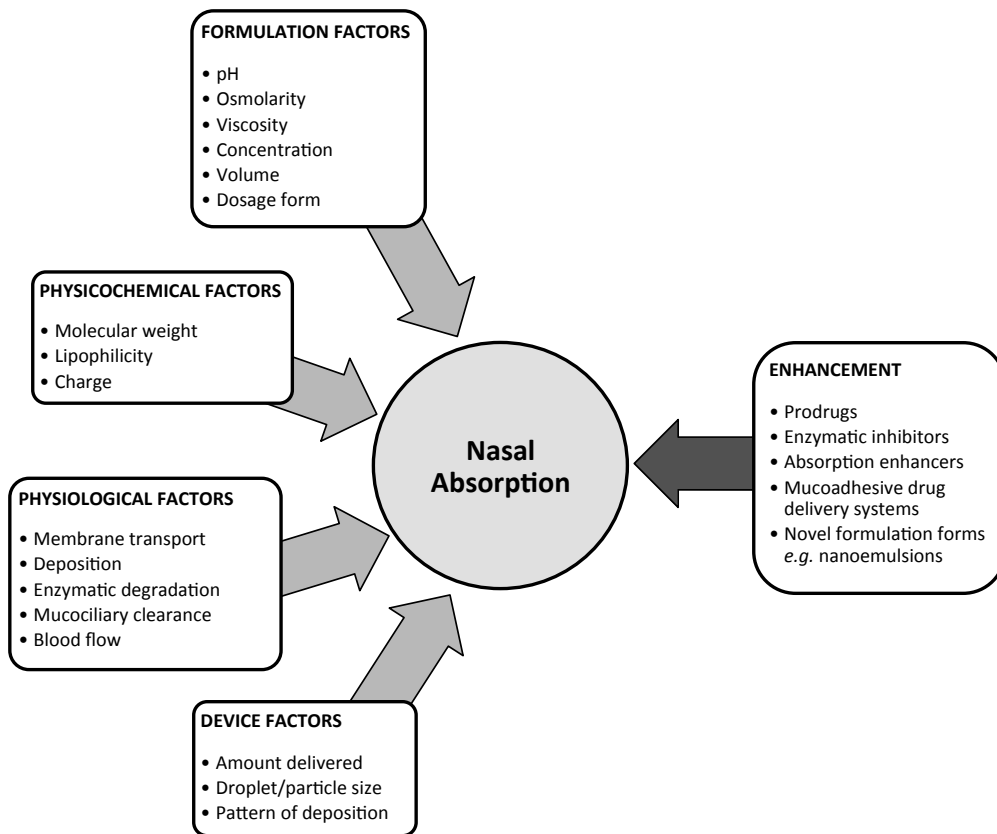
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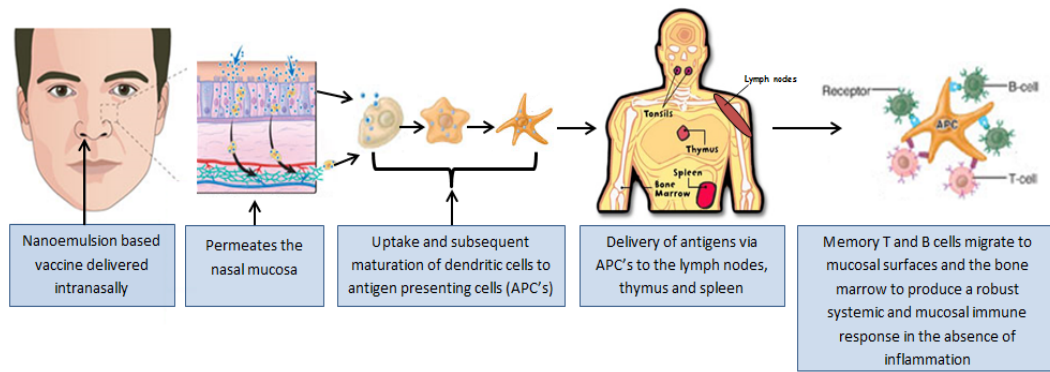
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731 **Figure 1:** Physiological, physicochemical, formulation factors and device factors influencing nasal absorption

732 and methods to increase nasal absorption (modified from [4, 51, 53]).

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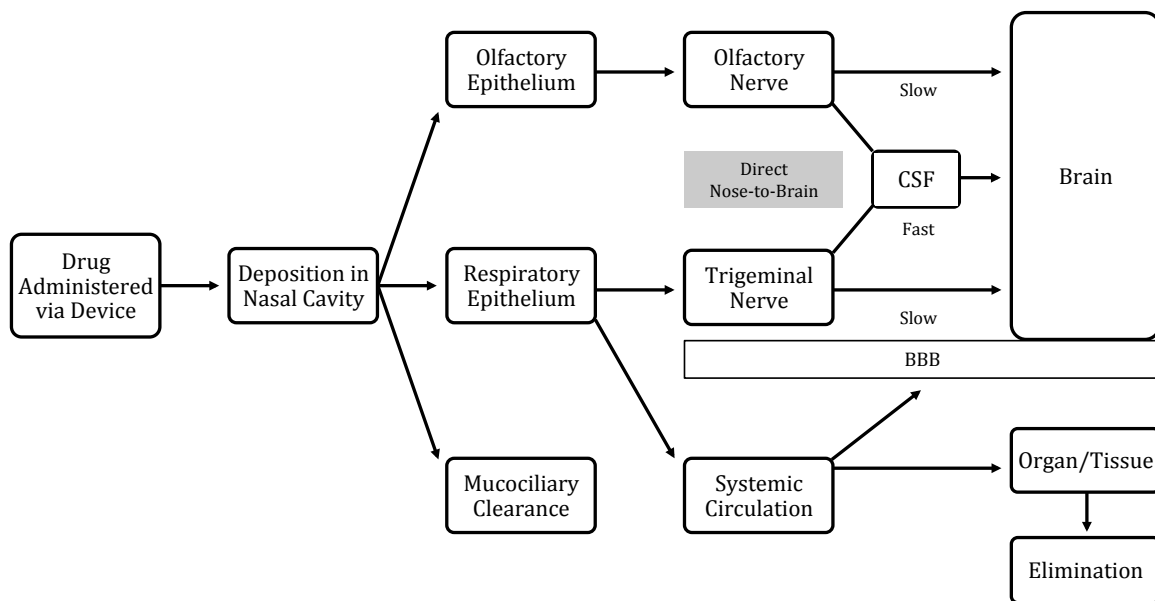


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736 **Figure 2:** Mechanism of action of nasal vaccination (modified from [81, 82]).

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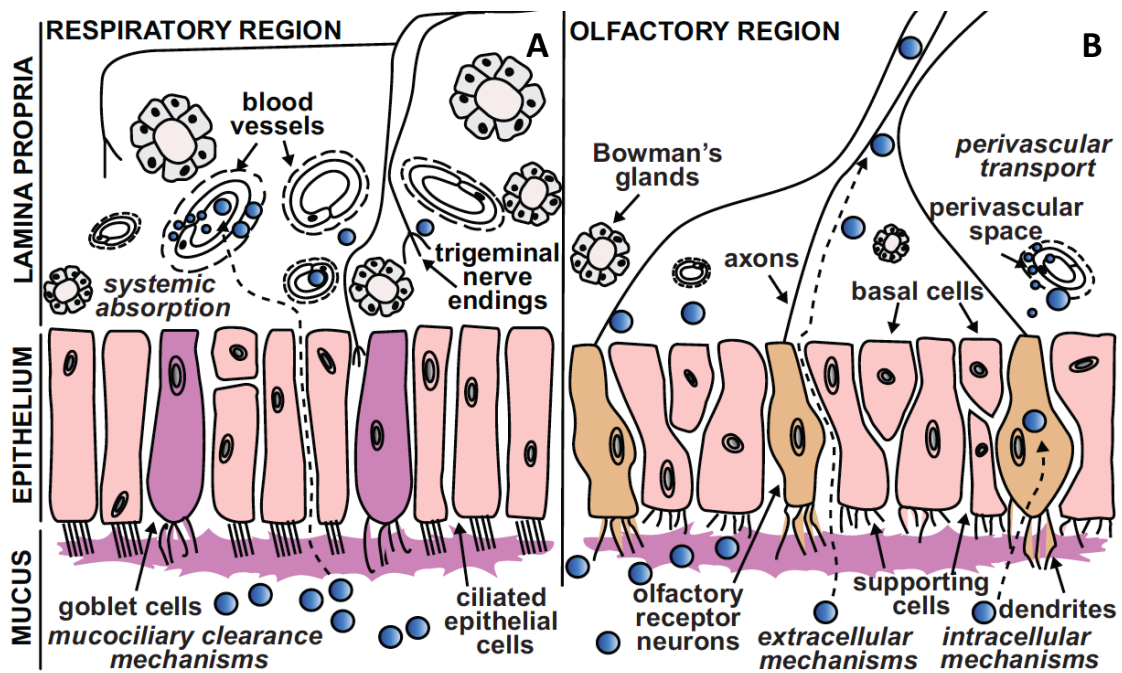
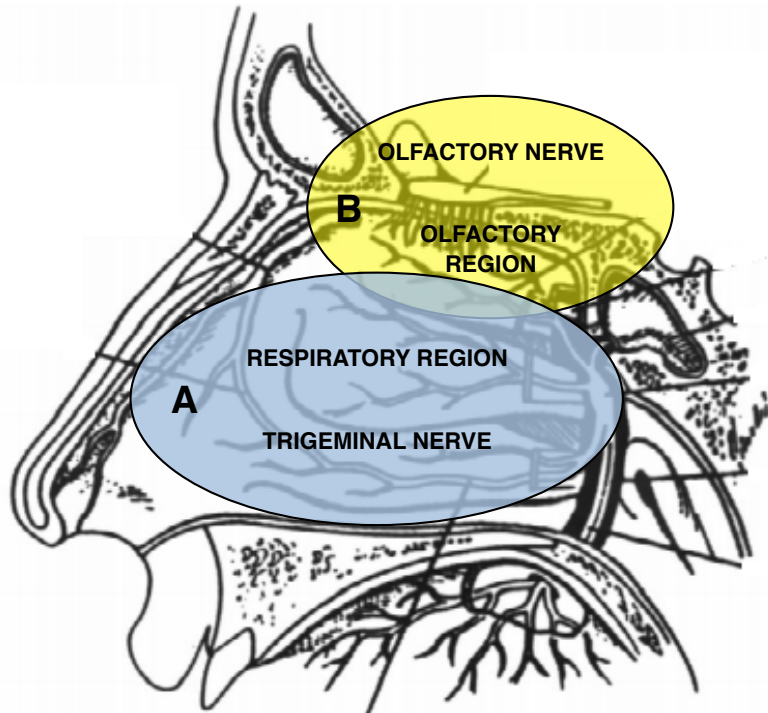
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740 **Figure 3:** Brain targeting pathways following nasal administration [4, 88].

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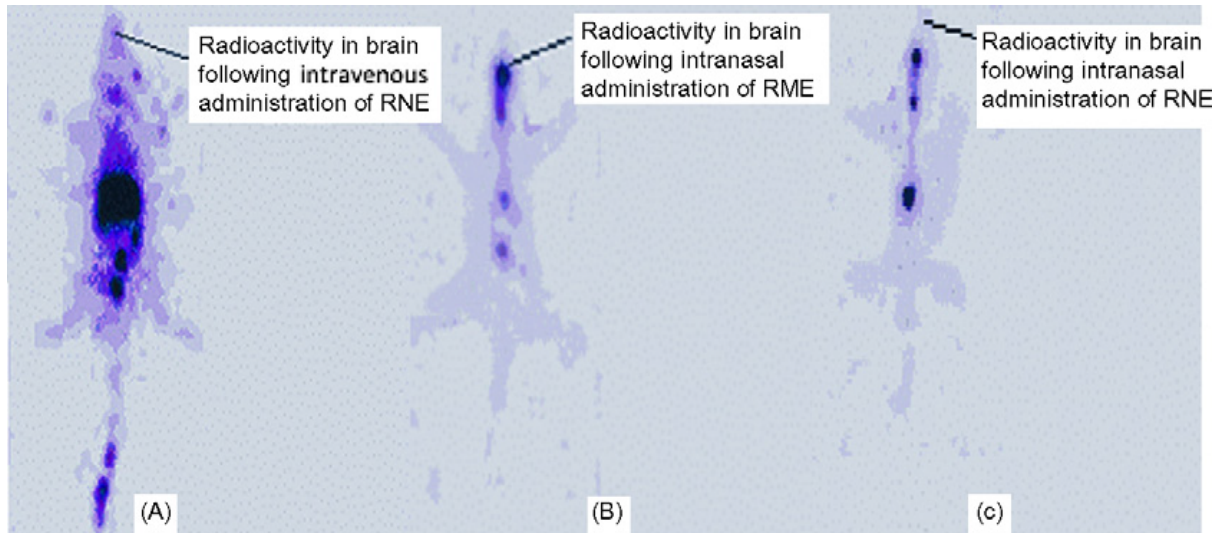
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743 **Figure 4:** Direct nose to brain pathways (modified from [85, 87]).

744 A shows the olfactory nerve pathway whereby the nerves penetrate the epithelial layer of the nasal
 745 mucosa providing both axonal (slow) and perineural (fast) absorption pathways.

746 B shows the trigeminal nerve pathway. The nerves do not penetrate the epithelial layer in this case and
 747 terminate in the lamina propria, only allowing absorption via axonal (slow) transport.

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

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

ADVANTAGES	LIMITATIONS
<ul style="list-style-type: none"> • Highly vascularized • Highly permeable • Increased bioavailability of many drugs • Reliable, safe, non-invasive and convenient • Avoidance of first-pass metabolism 	<ul style="list-style-type: none"> ○ Small dosage volume of only 25-200 μL ○ Mucociliary clearance (MCC) mechanism ○ Impaired drug absorption in case of nasal congestion ○ Improper administration technique could cause inefficient deposition
OPPORTUNITIES	UNIQUENESS
<ul style="list-style-type: none"> ⊕ 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 ($>1\text{kDa}$) ⊕ Potential differences in absorption and permeability potential between the different regions of the nasal cavity 	<ul style="list-style-type: none"> ✓ 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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757 **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	[10, 16, 17]	[10, 17-19]	[10, 16-18, 20]

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