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Review article

A review of intranasal formulations for the treatment of seizure emergencies

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ABSTRACT

Epileptic seizure emergencies are life-threatening conditions, which in their most severe form, status epilepticus, have a high mortality rate if not quickly terminated. Treatment requires rapid delivery of anti-epileptics such as benzodiazepines to the brain. The nasal route is attractive due to its non-invasiveness, potential for direct nose to brain delivery, high vascularity, relatively large absorptive surface area, and avoidance of intestinal/liver metabolism. However, the limited volume of the nasal cavity and poor water solubility of anti-epileptics restrict absorption, leading to insufficient therapeutic brain levels. This review covers various formulation approaches adopted to improve nasal delivery of drugs, especially benzodiazepines, used to treat seizure emergencies. Other general topics such as nasal anatomy, challenges to nasal delivery, and drug/formulation considerations for nose to brain delivery are also discussed.

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Contents

1. Introduction . . . 148
2. Nasal anatomy . . . 149
   2.1. Nasal vestibule . . . 149
   2.2. Main nasal cavity . . . 149
3. Challenges to nasal delivery . . . 150
   3.1. Nasal degradation enzymes . . . 150
   3.2. Limited nasal volume . . . 150
   3.3. Mucociliary clearance (MCC) . . . 150
   3.4. Nasal blood flow . . . 150
   3.5. PgP efflux transporters . . . 151
   3.6. Physical factors . . . 151
4. General drug and formulation considerations in nasal delivery . . . 151
5. Formulation approaches for improved intranasal delivery of drugs treating seizure emergencies . . . 151
   5.1. Low pH . . . 152
   5.2. Cyclodextrins . . . 153
   5.3. Non-aqueous solvents . . . 153
   5.4. Microemulsions . . . 153
   5.5. Drug particles . . . 154
      5.5.1. Microparticles/microspheres . . . 154
      5.5.2. Nanoparticles . . . 154
   5.6. In situ supersaturated solutions . . . 154
   5.7. Cosolvents . . . 154

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NEURELIS, INC., EX2024, page 1
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

5.8. Prodrug/enzyme systems . . . 155
6. Interspecies PK/PD differences for intranasal formulations . . . 155
7. Importance of the intranasal device in drug to brain delivery . . . 156
8. Summary and future prospects . . . 156
Acknowledgments . . . 157
References . . . 157

1. Introduction

Seizure clusters (also known as "acute repetitive seizures") and status epilepticus are medical emergencies that require immediate therapy. Status epilepticus, the most severe form, is a medical condition wherein an individual suffers from continuous or repetitive seizures in the brain, each lasting 5 min or more without regaining consciousness between seizures [1,2]. In the United States, 150,000 people each year experience one or more episodes of status epilepticus, with nearly 40,000 associated deaths [3].

Several drugs, listed in Table 1, have been successfully used to treat seizure emergencies. Of these, benzodiazepines (BZDs) such as lorazepam (LZP), diazepam (DZP) and midazolam (MDZ) have been the drugs of first choice for decades due to their potency and rapid onset of action. In practice, BZDs can be administered intravenously, but this route requires hospitalization or the presence of skilled medical personnel [4,5]. This can result in a delay in treatment, which can lead to brain damage. A relatively new approach to treating seizure emergencies involves out-of-hospital treatment, now known as "rescue therapy."

Intramuscular delivery of BZDs has been proposed for out-of-hospital treatment [6,7]. However, intramuscular drug delivery, even with the use of autoinjectors, is problematic. The technique requires training, risks improper injection and injury, and is difficult to apply to obese or emaciated individuals, or when several layers of clothing need to be removed.

Another out-of-hospital treatment is rectal DZP (Diastat®) which has been shown to be as effective as intravenous (IV) DZP in pediatric status epilepticus [8]. Diastat® is the only FDA-approved product for the treatment of cluster seizures outside the hospital setting. However, this route is socially objectionable, and many older children and adults decline rectal DZP despite its proven benefits. Buccal MDZ has been proposed as an alternative in children, with advantages of ease of use and social acceptability [9]. However, buccal dosing is challenging in patients with hypersalivation or convulsions, and aspiration and/or incomplete absorption are of concern [10]. Similar considerations apply to the sublingual route. The transdermal route has also been studied, and may be suitable for long term delivery of BZDs given their pronounced lipophilicity, but the time lags associated with initial crossing of the skin and appearance in the system circulation are too long for rescue therapy, even in the presence of permeation enhancers [11,12]. The intramuscular, oral mucosal and transdermal routes may be useful, however, for alternative applications such as sedation and protection against nerve agents [13,14].

Intranasal administration of BZDs is inherently attractive, as it requires little training, is easily performed, and carries little risk of injury. More importantly, it is more acceptable to older children and adults, compared to the rectal or IV routes, due to its noninvasive nature and improved patient comfort. Additionally, the relatively large surface area and high vascularization of the nasal cavity render it a favorable site for drug absorption.

There has been great interest in recent years in direct intranasal delivery to the brain, using access to the olfactory and trigeminal nerve pathways *via* the nasal cribriform plate [15–24]. This pathway seems most beneficial for active molecules that cannot access the brain by other routes, either due to low systemic bioavailability or due to the blood brain barrier. The latter problems, however, do not apply to BZDs, which readily enter the brain through the systemic circulation. (Otherwise they could not be delivered orally, rectally, or by IV.)

Table 1
Physicochemical and pharmacological properties of drugs used to treat seizure emergencies. Dashes indicate data provided earlier in table.

Drug	Log P[a]	pKa[a]	Aq. solubility (mg/L)[a] at 25 °C	MW[a]	Route of administration[b]	Adult dose (mg/kg)[b]	Mechanism of action[c]
First-line							
Lorazepam	2.4	13	80	321.2	Intravenous	0.1	GABA potentiation
Diazepam	2.8	3.4	50	284.7	Intravenous	0.15–0.2	GABA potentiation
Midazolam	4.3	3.5, 6.6	24	325.8	Intramuscular	0.25	GABA potentiation
If none of the above are available							
Phenobarbital	1.5	7.3	1110	232.2	Intravenous	15	GABA potentiation
Diazepam	–	–	–	–	Rectal	0.2–0.5	–
Midazolam	–	–	–	–	Intranasal, buccal	NA	–
Second-line							
Fosphenytoin	1.4	1.2	349	362.3	Intravenous	20 PE	Converts to phenytoin *in vivo* that blocks sodium channel
Valproic acid	2.8	4.8	1300	166.2	Intravenous	40	Not known clearly. Perhaps Inhibits GABA transaminase and NMDA receptors
Levetiracetam	−0.6	16.1	1,040,000	170.2	Intravenous	60	Stimulates synaptic vesicle protein 2A (SV2A), inhibiting neurotransmitter release
Phenobarbital	–	–	–	–	Intravenous	15	–
Third-line							
Repeat second line therapy or anesthetic doses of either thiopental, midazolam, pentobarbital, or propofol (all with continuous EEG monitoring)							

GABA - gamma amino butyric acid, PE – phenytoin equivalent, NA - not available
[a] Refer to citation [132].
[b] Refer to citation [81].
[c] Refer to citation [129–131].

NEURELIS, INC., EX2024, page 2
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

While the direct nose-to-brain pathway may contribute to intranasal delivery of BZDs, it is believed that the most important path is through initial absorption into the systemic circulation, followed by transport across capillaries in the brain.

The IN route for BZD delivery has demonstrated benefits compared to other routes. For example, IN MDZ is as effective as rectal DZP, but with relative ease of administration, rapid onset of action, and better social acceptance [25]. IN LZP was shown to be as effective as IV LZP in children aged 6–14 years, but the IN route was preferred due to improved patient compliance [26]. Due to the advantages of the IN route, several nasal formulations for seizure emergency treatment are in clinical trials (Table 2).

The IN route presents challenges such as a restricted access volume (max 150 μL/nostril), short residence time due to anterior leakage and posterior drainage, the presence of degrading enzymes, efflux transporters, and variable nasal absorption under physical (injury) or pathological conditions such as polyps, common cold, and allergy [27,28]. Accompanying the constraints presented by nasal anatomy and physiology, a drug's physicochemical properties such as size, water solubility and stability also play critical roles in determining rate and extent of absorption *via* the nasal route. For example, drugs with low water solubility such as BZDs (Table 1) exhibit poor nasal absorption due to the low aqueous dose accommodated by the nasal cavity. Small molecules (<300 Da) are generally absorbed better nasally than the larger ones [27].

Various formulation strategies have been adopted to overcome one or more of the aforementioned challenges associated with IN delivery to enhance direct or indirect brain delivery. For example, drug solubility has been increased using surfactants, polymers, pH change, *in situ* supersaturation, and organic solvents [29–33]. Drug residence time can be improved using mucoadhesives [34–36]. These components, when incorporated with seizure emergency drugs in IN solutions, suspensions, emulsions, nano- or microparticles, have led to improved performance of drug delivered *via* the nasal route [36–38].

Other than the molecule of choice and the formulation approach, selection of the nasal delivery device is critical for achieving desired formulation performance. For example, nasal solutions dispensed as drops are cleared much more rapidly (depending on head position) than those delivered as sprays [39]. Device characteristics also influence the amount and location of deposition [40].

Although there are several excellent reviews on intranasal drug delivery [18,19,24,41–44], a comprehensive review of intranasal formulations used for the treatment of seizure emergencies has not been published. (See [45] for a recent review of clinical trends in intranasal delivery of BZDs.) Here, we will discuss various examples of preclinical or clinical IN formulations for drugs used in seizure emergencies. Additionally, general topics such as nasal anatomy, barriers to nasal delivery, and drug and formulation properties required for nasal delivery, are covered. Finally, brief discussions of the effects of animal species and delivery device characteristics on drug disposition following intranasal dosing are presented.

2. Nasal anatomy

The human nasal cavity is responsible for the sense of smell, and humidification and filtering of inhaled air. The cavity has a total volume of 15–20 mL and surface area of 150–200 cm^2. The nasal cavity is divided into two symmetrical chambers (nostrils/nares) separated by the nasal septum. Each chamber consists of a nasal vestibule and main nasal cavity, the latter containing both the respiratory and olfactory regions, as illustrated in Fig. 1. The nasal sinuses are also potential sites of absorption, but generally these are difficult to reach by conventional methods.

2.1. Nasal vestibule

The anterior part of the nasal cavity (0.6 cm^2 area), the nasal vestibule, contains hairs, which filter inhaled particles. This region is non-ciliated and is covered by stratified squamous epithelia [46]. Blood perfusion is low in this region, so drug absorption is insignificant.

2.2. Main nasal cavity

The main nasal cavity is covered by mucus membranes and is highly vascularized. Mucus covering this region is responsible for trapping inhaled foreign particles and conveying them, by ciliary motion, through the nasopharynx into the esophagus, with ultimate elimination *via* the digestive system (Fig. 1). The main nasal cavity is subdivided into the respiratory and olfactory regions.

The respiratory region accounts for the largest portion of the human nasal cavity (around 80–90%, or 130 cm^2), owing to the presence of highly convoluted turbinates (conchae), which are designated superior, middle, and inferior. These convolutions are slit-like and are in intimate contact with air, enabling warming and humidification of inspired air by the perfusing blood from the underlying lamina propria [20]. As a useful consequence, drug solutions deposited on the turbinate mucosal surfaces will have a large surface-to-volume ratio, with potential for rapid drug absorption into the systemic circulation. Microvilli covering the respiratory region also contribute to the large surface area available for drug absorption. In addition to systemic absorption, direct drug delivery

Table 2
Seizure emergency clinical trials using the intranasal route for drug administration[a].

Drug	Product	Sponsor, collaborator	Phase	Study objective	Status[b]	Reference
Clonazepam	–	Jazz Pharmaceuticals	II	Proof-of-concept study to evaluate the efficacy, safety, and pharmacokinetics of intranasal clonazepam in adult subjects with epileptic seizures	Development suspended	[125]
Diazepam	NRL-1	Neurelis Inc.	I	Tolerability study in healthy volunteers	Complete	[118]
Diazepam	NRL-1	Neurelis Inc.	III	Repeat dose safety study of NRL-1 in epilepsy subjects	Recruiting	[126]
Diazepam	Plumiaz ™	Acorda/Neuronex	I	A pharmacokinetic study to assess dose proportionality, safety and tolerability of diazepam nasal spray in healthy adult volunteers	Ongoing	[126]
Diazepam	Plumiaz ™	Acorda/Neuronex	II	A pharmacokinetic study of a single-dose of diazepam nasal spray in adult epileptic patients experiencing a seizure episode	Complete	[126]
Diazepam	Plumiaz ™	Acorda/Neuronex	II	A study to determine the bioavailability, safety and tolerability of diazepam nasal spray *versus* diazepam rectal gel (Diastat®)	Recruiting	[126]
Diazepam	Plumiaz ™	Acorda/Neuronex	II	An open-label, safety and tolerability study of chronic intermittent use of diazepam nasal spray in adolescents and adults with cluster seizures	Recruiting	[126]
Diazepam	Plumiaz ™	Acorda/Neuronex	NA		Additional phase III safety studies underway	[126]
Midazolam	USL261	Upsher-Smith Laboratories	III	Study of the safety and efficacy of intranasal midazolam (USL261) in the outpatient treatment of subjects with seizure clusters	Recruiting	[127]

[a] (Commercial products only).
[b] Represents status of clinical trial or status of product development.

NEURELIS, INC., EX2024, page 3
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

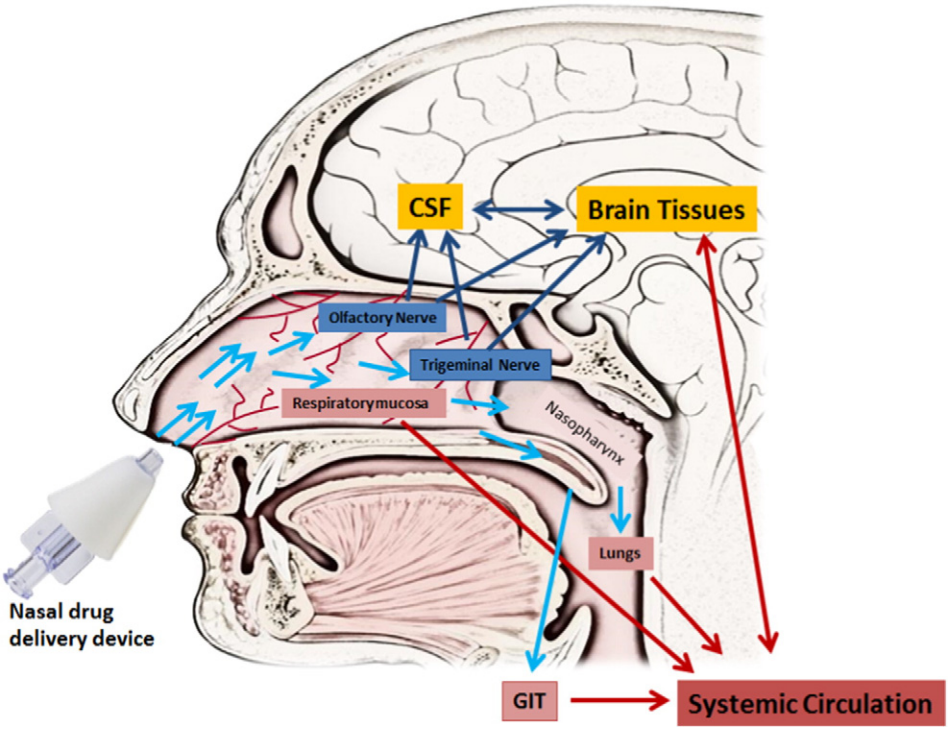


Fig. 1. Nasal drug delivery pathways. Nasally administered drug (light blue) can enter directly into the brain *via* the olfactory and/trigeminal neuronal pathways (dark blue), or indirectly following absorption into the systemic circulation (red). GIT is the gastrointestinal tract, CSF is cerebrospinal fluid.

to the brain can occur *via* the trigeminal nerve pathway [17], which connects the respiratory region to the brain *via* the cribriform plate [24]. However, for drugs exhibiting rapid systemic absorption and transport across the blood-brain barrier, this pathway is not likely to be significant.

The olfactory region lies in the uppermost region of the nasal cavity and accounts for ~10% of the total nasal cavity area in humans. Olfactory epithelium is pseudostratified and is covered with non-beating mucus. This region contains olfactory receptors responsible for the sense of smell, plus serous glands [44]. Drug administered intranasally to this region can enter the central nervous system *via* olfactory nerves fibers of the olfactory bulb [47] and pass, by convection, through the olfactory neuroepithelium into the piriform cortex, amygdala and hypothalamus. Turnover of olfactory nerve fibers renders them leaky, promoting direct nose-to-brain transport [48]. As indicated previously, this pathway seems most useful for drugs with poor systemic bioavailability or inability to penetrate the blood-brain barrier.

3. Challenges to nasal delivery

Although the intranasal route is attractive for drug administration, it presents key challenges for drug absorption.

3.1. Nasal degradation enzymes

The nasal cavity expresses hydrolytic enzymes as a part of its defense mechanism against xenobiotics. Examples include cytochrome P450 isoenzymes, aminopeptidases, carboxylesterases, *carbonic anhydrase*, *aldehyde dehydrogenase*, *glucourolyl transferase* and *glutathione S-transferase* [43,44]. These enzymes can metabolize nasally administered drugs, thereby reducing their local or systemic bioavailability.

3.2. Limited nasal volume

The human nasal cavity retains <200 μL of fluid per nostril, and excess volume drains into the esophagus or leaks out. If the required dose of a nasally administered drug is high, then concentrated solutions are mandatory. This requirement is challenging for hydrophobic molecules, as these have a limited aqueous solubility. Suitable strategies, to be described below, must be adopted to enhance solubility.

3.3. Mucociliary clearance (MCC)

The mucosal membrane consists of two layers, namely the surface epithelium and embedded connective tissues known as the lamina propria. The surface epithelium is covered by watery, sticky mucus, which is produced by goblet cells in surface epithelium and epithelial glands in the lamina propria. Cilia in the surface epithelium layer propel inhaled material at a rate of 1000 beats/min from the nasal cavity towards the nasopharynx [49]. This process, known as mucociliary clearance (MCC) controls the contact time of inhaled drug with nasal epithelia, thereby influencing the extent of drug absorption. Forward motion is much faster in the respiratory region, with a turnover time of 15–20 min, [50] compared to the olfactory region, where the turnover time is several days [51]. Due to lower residence time in the respiratory region, drug absorption might be limited despite high vascularity. Accordingly, improved mucoadhesion in this region could increase residence time and hence drug absorption. Additionally, factors that impair ciliary movements and/or rheological properties of mucus in disease conditions, such as ciliary dyskinesia, common cold, cystic fibrosis, diabetes mellitus [46], vasomotor rhinitis [42], *etc.*, may affect drug absorption. Drug that is cleared from the nasal cavity by MCC enters the gastrointestinal tract *via* the nasopharynx and becomes essentially oral in character (Fig. 1). When this is significant, a second phase of oral absorption may occur, and a fraction may be excreted without absorption.

3.4. Nasal blood flow

As discussed previously, the lamina propria is perfused with a high density of blood vessels, which provide systemic availability of nasally

NEURELIS, INC., EX2024, page 4
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

administered drugs. Constriction/dilation of these vessels affects nasal blood flow and, therefore, nasal drug absorption. Vasoconstrictors such as phenylephrine, oxymetazoline, and ephedrine have been co-administered with other drugs to reduce their nasal absorption [44,52], while vasodilators such as clonidine and hydralazine have been added to increase absorption [53,54]. Nasal blood flow is also affected by environmental factors such as temperature, humidity, smoke, disease conditions (vasomotor rhinitis, common cold), and psychological factors such as stress, fear, and anxiety [46].

3.5. PgP efflux transporters

As a part of the physiological defense mechanism, epithelial plasma membranes contain transporters that pump drugs out of cells into the delivery lumen. Of these transporters, the most abundant is P-glycoprotein (PgP). While PgP is best known for its presence in the central nervous system, liver and gut, there are also reports of PgP in the nasal region [55–59], specifically in ciliated epithelia and the submucosa of the olfactory region [44]. These transporters may reduce drug absorption.

3.6. Physical factors

The nasal passage can be compromised due to trauma or injury, with deleterious effects on drug transport and absorption. Under certain atmospheric or pathological conditions, the nose may bleed or be too dry. The patient's condition, such as stuffy/runny nose or the presence of nasal polyps, can affect clearance from the nose and hence drug absorption. Holfti et al. observed improved intranasal absorption of MDZ from patients whose nasal passages were cleared of mucus before drug administration [60].

4. General drug and formulation considerations in nasal delivery

Several recent reviews cover this topic in detail [28,61,62] so we present only a summary of drug properties and formulation strategies required to improve nasal delivery.

Mucosal membranes mediate drug transport into the systemic circulation by passive transcellular or paracellular diffusion, *via* carrier-mediated transport or *via* endocytosis, depending on molecular properties [44]. Additionally, a small percentage of hydrophobic drugs are reported to undergo direct neuronal transport *via* the olfactory or trigeminal nerve pathways [17–22,47]. Besides lipophilicity of the molecule, molecular weight plays a critical role in determining rate and extent of drug absorption. For example, small (<300 Da) hydrophilic molecules can be absorbed through mucosal epithelia either paracellularly or by carrier mediated transport. Lipophilic molecules, both large (300 to 1000 Da) and small (<300 Da), are typically absorbed *via* passive diffusion [44]. The extent of absorption of hydrophilic molecules larger than 300 Da decreases with molecular weight [63]. Hydrophilic macromolecules such as proteins and peptides are known to have <1% bioavailability, and such agents are absorbed *via* endocytosis [44]. To potentiate absorption of hydrophilic molecules, absorption enhancers such *N*-tridecyl-beta-D-maltoside have been used [64]. These agents work by disrupting the epithelial membrane or by opening tight junctions.

Prior to absorption, a drug must dissolve in the aqueous peri-mucosal layer. Given the low volume of nasal cavity, the drug's solubility in water must be high enough to accommodate the necessary dose. This requirement poses a challenge for hydrophobic drugs with poor water solubility. This issue can be resolved by using a water-soluble prodrug that is metabolized intranasally to produce the parent drug (see below), or by solubilizing the drug using surfactants, pH adjustment, co-solvents, or nanocarriers such as polymeric nanoparticles, microemulsions or cyclodextrins. As will be discussed below, transient supersaturation may provide another means to enhance the deliverable dose of hydrophobic drugs. For hydrophobic but ionizable drugs, pKa also affects absorption in the nasal environment, where pH ranges from 5.5 to 6.5 [65]. As predicted by the pH-partition hypothesis, the unionized drug fraction permeates more readily than the ionized fraction.

For high bioavailability, a drug must be resistant to metabolizing enzymes in the nasal environment. Alternately, a drug's stability and hence its degree of absorption can be improved by derivatizing it into a prodrug that is cleaved after passage into or through the epithelium, by using enzyme inhibitors, or by encapsulating the drug in nanoparticles, microparticles, or liposomes.

Drug residence time in contact with the mucosal membrane is an important factor influencing drug absorption. As mentioned previously, drugs can be cleared from the nasal mucosa *via* MCC, thereby limiting nasal absorption. Mucoadhesives have been used to increase residence time. Some examples include sodium alginate, hydroxypropylcellulose, hydroxyethylcellulose and chitosan [66]. Alternately, formulation viscosity can be enhanced with gelling/viscosity building agents, the latter polymers being examples [27].

Particulate forms *e.g.* nanoparticles, microspheres, liposomes, *etc.*, can incorporate several components at once. For example, particles can include surfactants and mucoadhesives, together with the therapeutic moiety [67]. A particulate form can also protect drug from *in vivo* metabolism, thereby enhancing the drug's bioavailability [67–69].

Several reports have demonstrated that absorption into brain *via* nose is improved when drug is incorporated in particles sized at 5–10 μm, either due to an increased nasal residence time or to escape from deposition in the upper respiratory tract [40,70–72]. However, there is also evidence that efficient nose-to-brain delivery occurs when nano-sized particles, particularly below 100 nm, are used [73,74]. One can speculate that nasal residence time is enhanced with the larger particles, but the smaller particles are absorbed into the brain *via* the transcellular or paracellular (<15 nm [75]) routes followed by axonal or peri-axonal transport. The direct nose-to-brain route may be particularly attractive for CNS delivery of drugs with poor permeability through the blood brain barrier, especially if they can be formulated with suitable mucoadhesives, specific targeting ligands on the nanoparticle surfaces, and devices that preferentially spray into the olfactory region [34,76–78].

When using particulate forms, potential toxicities need to be considered in parallel with benefits. For example, microparticles smaller than 5 μm have been shown to cause pulmonary complications [40].

Because of the high degree of perfusion by blood vessels and their close proximity to flowing air, the temperature of the nasal mucosa lies between the ambient and body temperatures. At room temperature, the intranasal temperature cycles in the range 30–34 °C, with valleys and peaks occurring at the end of inspiration and expiration, respectively, and with some geographical variations [79]. Any formulation must be expected to perform well in this temperature range.

Nasal solutions with tonicities ranging from 0.6–1.8% NaCl equiv. are well tolerated, 0.9% NaCl equiv. being isotonic. Solutions of higher tonicities have been used as douching formulations, and it has been found that MCC is increased when tonicity reaches 5.0% NaCl equiv. [80]. Since it is usually desirable to retain a formulation in the nasal cavity long enough for absorption of active to occur, there is probably little incentive to use strongly hypertonic solutions.

5. Formulation approaches for improved intranasal delivery of drugs treating seizure emergencies

Table 1 lists physicochemical properties of drugs used for the treatment of seizure emergencies, administered either intravenously or rectally. First line intravenous/intramuscular therapies utilize highly potent BZDs such as LZP, DZP and MDZ. Intravenous phenobarbital, rectal DZP and intranasal/buccal MDZ can be used as alternate first-line therapies [81]. These drugs are followed by intravenously administered fosphenytoin, valproic acid, phenobarbital and, levetiracetam as

NEURELIS, INC., EX2024, page 5
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

second-line therapies (for complete dosage regimens refer to [81]). This sequence is based on efficiency in responding to seizure emergency, which is related to a) the rapidity by which a drug can be administered and diffuse into the brain, with an ideal t_{max} of 5–10 min, and b) the drug's pharmacodynamics [81]. The lower efficiency of second-line therapies can be due to slow drug absorption across the BBB and high drug efflux (in case of phenytoin (PHN), levetiracetam, phenobarbital) by membrane efflux transporters [82]. Due to advantages of the IN route over other routes, discussed previously, much attention is being paid towards developing formulations of seizure emergency drugs that are suitable for IN administration. Formulation approaches for these agents, summarized in Table 3, are now described in detail.

5.1. Low pH

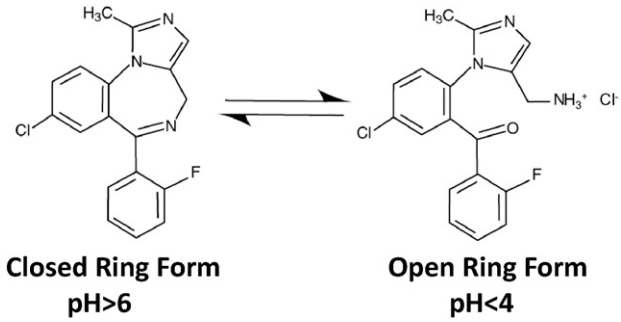
For drugs to be more membrane permeable, they need to be lipophilic, which usually implies poor water solubility. However, the nasal cavity can accommodate only a low solution volume, necessitating highly concentrated nasal drug solutions. For weakly basic drugs such as BZDs, solubility can be enhanced by lowering pH and increasing the ionized drug fraction. For example, the solubility of MDZ increases from μg/mL to mg/mL concentrations by reducing pH from near physiological values to pH 3.3. In these acidic conditions, the closed ring and open ring structures of MDZ, the latter being formed by the acid-catalyzed opening of the 4,5-double bond of the diazepine ring, are at equilibrium (Scheme 1). Once in the body at physiological pH, the open ring form reverts back to the physiologically active, lipophilic, closed ring form (MDZ), which is absorbed across the mucosal membrane. MDZ hydrochloride (5 mg/mL) is a commercially available formulation given IV and IM for sedation and anesthesia [83]. Unfortunately this concentration is too low for IN administration, due to the low volume of the nasal cavity. Furthermore, the low pH of this formulation likely causes nasal irritation, which may signal mild nasal toxicity [84]. Accordingly, concentrated formulations with higher pH values have been prepared (pH 4 to 5) which, although causing comparatively less nasal irritation, present other side effects such as watery eyes and unpleasant taste [29].

Table 3
Formulation strategies with seizure emergency drugs.

Formulation strategy	Drug	Formulation type	Formulation composition	Model	Dose (mg/kg)	PK/PD[a]	Ref.
Low pH	Midazolam (as hydrochloride)	Solution	Propylene glycol, water, pH 3.3	Human	0.2	t_{max} 14 min, 83% bioavailability, causes nasal irritation, teary eyes, bad taste	[128]
Cyclodextrins	Clonazepam	Suspension	Dimethyl-3-cyclodextrin, pH 3.0	Human	2	t_{max} 15–20 min	[87]
	Midazolam	Suspension	Sulfobutylether β-cyclodextrin, hydroxypropylmethylcellulose, pH 4.3	Human	0.06	t_{max} 15 min, 73% bioavailability, with mild to moderate nasal irritation	[29]
Non-aqueous solvents	Diazepam	Solution	Glycofurol, tetraethylene glycol	Rabbit	0.79	54% bioavailability, PD response in <5 min	[89]
	Diazepam	Solution	PEG300	Sheep	0.18	t_{max} 5 min, 15% bioavailability, with pain and patient discomfort	[30]
	Diazepam	Co-solvent	Propylene glycol, ethanol, sodium glycocholate, water	Rabbit	1	t_{max} 2 min, 77% bioavailability, PD response in 1.5 min	[91]
	Midazolam	Co-solvent	Propylene glycol, polyethylene glycol 400, pH 7.4	Human	0.08	t_{max} 10 min, 72.5% bioavailability, causes nasophyrngeal irritation, bad taste in patients	[37]
	Lorazepam	Co-solvent	Propylene glycol, PEG 400, saccharin, butylated hydroxytoluene and water	Human	0.03	t_{max} 30 min, 78% bioavailability	[92]
Microemulsions	Diazepam		Ethyl laurate, Tween 80, propylene glycol and ethanol	Rabbit	2	t_{max} 2–3 min, 50% bioavailability	[94]
	Diazepam		Ethyl laurate, Labrasol, Transcutol P and ethanol	Rat	2	t_{max} 5 min, 68.4% bioavailability, DTE% = 100	[38]
	Lorazepam		Cremophor EL, Transcutol P and Lauroglycol FCC, water	Rabbit	0.38	t_{max} 2 min, 80% bioavailability (compared to 0.16 mg/kg IM)	[95]
	Diazepam and Midazolam		Isopropyl palmitate, glyceryl oleate and Labrasol, propylene carbonate, water	Rabbit	0.4	t_{max} < 10 min, 34% bioavailability for both drugs	[96]
	Clobazam	With mucoadhesive	Glycerolmono- and dicaprate, mixture of polyoxyethylene-6-caprylic and capric glycerides, Tween 20, Carbopol 940P, water, pH 8.5	Mice	0.75	DTE%[b] = 70, DTP%[b] = 91 with mucoadhesive, DTE% = 65 without mucoadhesive	[36]
	Clonazepam	With mucoadhesive	Medium chain triglyceride, polyoxyethylene-35-ricinoleate, polysorbate 80, propylene glycol, polycarbophil	Rat	0.18	DTE% = 229, DTP% = 44 with mucoadhesive, DTE% = 131, DTP% = 20 without mucoadhesive, DTE% = 124, DTP% = 23 with clonazepam solution	[34]
Drug particles	Midazolam	Microspheres	Gelatin, chitosan	Rat	0.5	DTE% = 212, DTP% = 62.52	[69]
	Lorazepam	Microparticles	Polyvinylpyrrolidine (PVP), polyvinylalcohol (PVP)	Rabbit	5	t_{max} = 10 min, 1.6 times > pure lorazepam	[68]
	Valproic acid	Lipid nanoparticles	Cetyl palmitate, soy lecithin, octyldodecanol, poloxamer 188	Rat	4	Brain/plasma ratio 60 min after administration is 5-fold greater than intraperitonial. Equal effect compared to sodium salt solution but with 7 times lower dose. DTE% = 25 (lipid nanoparticles), 80 (solution)	[98]
In-situ supersaturated solutions	Diazepam	Co-solvent	Glycofurol, water	Human	0.07	t_{max} 20–30 min, 75% bioavailability, causes moderate pain and poor tolerance	[102]
	Diazepam	Prodrug-enzyme mixture	Avizafone, *Aspergillus oryzae protease* enzyme	MDCKII-wt cell monolayer	0.3 mg to 1.12 cm^2 cell monolayer	16-Fold faster absorption compared to saturated diazepam solution. Supersaturation levels reached in <5 min	[31]

PK - pharmacokinetic, PD - pharmacodynamic, DTE - drug targeting efficiency, DTP - drug targeting potential, MDCKII-wt - Madin-Darby canine kidney II-wild type.
[a] t_{max} is in plasma. Bioavailability is absolute unless otherwise indicated.
[b] Refer to citation [121].

NEURELIS, INC., EX2024, page 6
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414



Scheme 1. Predominant forms of midazolam (MDZ) at different pH values.

5.2. Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides containing six or more D-(+) glucopyranose units, connected by α-(1, 4) glucosidic bonds. CDs contain a central hydrophobic cavity and hydrophilic outer surface, and can be utilized to solubilize lipophilic drugs. There are three natural CDs, α-, β-, and γ-, which differ in ring size, molecular weight and solubility. Of these, β-CDs are well tolerated in humans [85]. The external hydroxyl groups on CDs participate in intermolecular hydrogen bonding, and are not available to interact with water, reducing the aqueous solubility of these molecules. Water solubility of CDs has been improved by modifying the terminal –OH group *via* methylation, hydroxypropylation, *etc.* A few pharmaceutically relevant modified CDs include hydroxypropyl derivatives of β- and γ-cyclodextrin, methylated β-cyclodextrin, sulfobutylether β-cyclodextrin, and glucosyl-β-cyclodextrin [86].

CDs have been utilized to solubilize BZDs. CDs have been complexed to the ring-open form of MDZ, resulting in improved solubility in acidic aqueous environments. For example, Schols-Hendriks and co-workers prepared clonazepam solutions with dimethyl-3-cyclodextrin (10 mg/mL) at pH 3.0. 100 μL clonazepam (1 mg dose) sprayed into each nostril of seven healthy male volunteers resulted in a serum t_{max} of 15–20 min. By contrast, the same drug absorbed through the buccal route displayed a t_{max} of 50 min. However, serum drug concentrations following nasal administration were much lower than the therapeutic serum clonazepam concentration threshold of 18 ng/mL (obtained *via* intravenous route), and so the formulation was considered to be ineffective for the treatment of status epilepticus [87]. Loftsson et al. developed a MDZ nasal spray using 14% sulfobutylether β-cyclodextrin (solubilizer) and 0.1% hydroxypropylmethylcellulose (stabilizer) at pH 4.3. This formulation could deliver an effective dose intranasally with 73% absolute bioavailability [29]. Patients reported mild to moderate irritation due to the slightly acidic pH of the nasal spray. Cyclodextrin formulation of LZP has also been attempted [88].

5.3. Non-aqueous solvents

A common approach to improve dissolved drug concentration is to use nonaqueous or organic solvents. Bechgaard used various glycols to improve the solubility of BZDs. Of these, polyethylene glycol 200 (PEG200) and glycofurol were most effective [33]. Intranasal administration of DZP solutions (30 mg/mL) prepared in PEG200, glycofurol or tetraethylene glycol exhibited 40–60% bioavailability in rabbits, with pharmacodynamic effect observed in <5 min [89]. Lindhardt et al. prepared a simple DZP formulation in PEG300 (47 mg/mL) with t_{max} values 5 min (7 mg IN dose), 9 min (3 mg IN dose) and 18 min (2 mg IN dose), and bioavailabilities 15%, 54% and 34% in sheep, rabbits and humans, respectively [30].

Organic solvents can improve the solubility of BZDs, but when used in nasal formulations they can lead to patient discomfort due to irritation, and local toxicity. Nasal histopathology coupled with innovative dosimetric models can be utilized to estimate dose/toxicity relationships and to elucidate the mechanisms of toxicity [51]. For example, Hjortkjer et al. studied nasal toxicity after single and repeated administration to rabbits of poly(ethylene glycol) 200, tetra(ethylene glycol), glycofurol, and mixtures of these vehicles both with and without benzodiazepines (DZP, LZP, clonazepam). Histopathological examination of nasal tissues 5 and 10 min after administration (single-dose and repeated-dose) indicated mild toxicities with these formulations (with or without drug) [90].

Water/organic (W/O) cosolvents may reduce irritation and toxicity but still maintain adequate drug solubility. For example, Li et al. prepared DZP solutions (21 mg/mL) using 60% propylene glycol, 30% ethanol, 1% sodium glycocholate and 10% water. Intranasal administration of this formulation in rabbits led to rapid *in vivo* absorption ($t_{max} = 2$ min) with 77% absolute bioavailability [91]. Using cosolvents such as propylene glycol and polyethylene glycol 400, Wermeling et al. prepared a concentrated (25 mg/mL) MDZ formulation and administered it in healthy volunteers *via* the IV, IN and IM routes (5 mg dose each). The results demonstrated a median plasma t_{max} of 10 min and plasma C_{max} of 80 ng/mL with the IN route: this C_{max} was 1.3-fold greater than the C_{max} obtained *via* the IM route. MDZ administration also resulted in psychomotor impairment and sedation in the subjects. Psychomotor recovery times were similar after IN, IV and IM administrations [37].

In another study, a LZP solution (0.03 mg/kg) was prepared using propylene glycol, PEG 400, saccharin, butylated hydroxytoluene, and water. IV, IM and IN administration of this solution in 11 human subjects showed rapid LZP absorption following IV and IN administration, and drug could be detected in the blood post-IN administration in as early as 5 min. The reported value of t_{max} was 6 min for the IV dose, 3 h for the IM dose and 30 min for the IN dose. Intranasal bioavailability was 78%, with plasma levels comparable to those obtained following IM dosing. Detailed nasal examination in subjects revealed no pathology of the nasopharynx following single administration of the nasal formulation [92].

5.4. Microemulsions

Microemulsions (MEs) are thermodynamically stable formulations comprising an oil phase, a water phase, a surfactant and a co-surfactant (usually a co-solvent). Compared to regular emulsions, microemulsions are relatively simple to prepare by mixing and do not require vigorous shearing or homogenization. MEs have gained popularity for nasal delivery owing to their ease of preparation, high solubilization potential, self-preservation, smaller droplet size, and long term physical stability [93]. When formulated along with a mucoadhesive polymer, MEs help improve the drug's contact time with nasal mucosa, thereby enhancing drug absorption. Despite the 'micro' label, droplets are usually in the nano-size range.

Several groups have been working on intranasal delivery of antiepileptics using MEs. For example, Li et al. prepared ethyl laurate ME for DZP using Tween 80, propylene glycol and ethanol. Intranasal administration of this formulation in rabbits (2 mg/kg) demonstrated rapid drug absorption (t_{max} of 2–3 min) with 50% absolute bioavailability [94]. Kim's group prepared another variant of ethyl laurate ME using Labrasol® (surfactant), Transcutol® P, and ethanol as co-surfactants. The optimal formulation gave rise to rapid nasal absorption of DZP in rats ($t_{max} = 5$ min) and rabbits ($t_{max} = 10$ min), with comparable (in rats) or slightly lower (in rabbits) brain-to-plasma ratios for IN *versus* IV administration. From IN administration of this formulation, it was observed that the drug transport into the brain occurred mostly *via* the blood-brain barrier, as opposed to direct absorption through the olfactory epithelium [38].

Yao and co-workers prepared LZP ME using Cremophor EL (surfactant), Transcutol® P (co-surfactant), Lauroglycol™ FCC (oil), and water [95]. IN administration of LZP in rabbits (0.38 mg/kg) led to rapid drug absorption ($t_{max} = 2$ min) with ~80% bioavailability

NEURELIS, INC., EX2024, page 7
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

(compared to 0.16 mg/kg IM). Botner et al. prepared MEs (25 mg/mL) for co-delivery of DZP and MDZ using isopropyl palmitate (oil), glyceryl oleate and Labrasol® (surfactants), propylene carbonate (co-surfactant) and water. Spraying this formulation into rabbit nostrils (0.4 mg/kg) yielded a t_{max} of <10 min with 34% absolute bioavailability for both drugs. In this study, it was also observed that MDZ was better absorbed from W/O ME (20% water) compared to O/W ME (50% water) [96].

Other BZDs such as clobazam and clonazepam have been formulated as MEs for intranasal delivery. Clobazam has been formulated as an O/W mucoadhesive ME (pH 8.5) using glycerolmono- and dicaprate (oil), a mixture of polyoxyethylene-6-caprylic and capric glycerides (surfactant), Tween 20 (cosurfactant), Carbopol 940P (mucoadhesive) and distilled water (aqueous phase) [36]. IN administration of clobazam ME in BALB/c mice (0.75 mg/kg) subjected to pentylenetetrazole (PTZ)-induced convulsions (0.75 mg/kg) demonstrated improved brain uptake compared to that observed following IV administration. Improved brain uptake from mucoadhesive clobazam MEs was confirmed in mice, where there was a delay in onset of PTZ-induced seizures compared to the positive control (saline treated) and placebo-treated groups. Brain targeting with mucoadhesive ME was greater than with ME prepared without the mucoadhesive. Vyas et al. prepared mucoadhesive MEs with clonazepam using medium chain triglyceride as an oil (10% w/w), polyoxyethylene-35-ricinoleate as a surfactant (26.67% w/w), polysorbate 80 as a cosurfactant (13.33% w/w), propylene glycol (50% w/w) as an anhydrous continuous phase, and polycarbophil (0.50% w/w) as the mucoadhesive. Upon IN administration these mucoadhesive MEs demonstrated improved brain uptake in rats (0.16–0.2 mg/kg) when compared to MEs without mucoadhesive and clonazepam solution [34].

From the above examples, one can conclude that improved product performance with intranasal MEs could be due to either improved aqueous solubility of drug in the ME form and/or increased retention time due to the mucoadhesive.

5.5. Drug particles

Due to the tolerability issues associated with pH modification and cosolvent approaches, some seizure medications have been manufactured as particulate formulations utilizing lipid particles, microparticles, or nanoparticles. This approach helps protect the molecule from enzymatic degradation, improves drug solubility, and permits incorporation of adjuvants such as mucoadhesives.

5.5.1. Microparticles/microspheres

We first consider micron sized particles with drug dispersed in a polymer matrix. Upon interaction with water/mucus, the polymer swells and releases drug by diffusion and/or polymer erosion. Usually the polymer, *e.g.* chitosan, acts as a mucoadhesive. Microspheres can therefore be utilized to improve delivery by enhancing drug solubility and residence time in the nasal mucosa. For example, gelatin-chitosan mucoadhesive MDZ microspheres were shown to exhibit efficient nose-to-brain delivery in male albino rats when presented intranasally (0.5 mg/kg). Mucoadhesion was due to chitosan electrostatically binding to sialic acid groups in mucus [69]. Zhao et al. prepared LZP microparticles at various polyvinylpyrrolidine (PVP)/polyvinylalcohol (PVA) ratios. Microparticles with greater proportions of PVP exhibited superior intranasal delivery compared to those with more PVA. Administration of intranasal PVP-PVA microparticles (1:0.6) into rabbits led to a t_{max} of around 10 min, and an increase in plasma AUC compared to pure LZP particles [68]. PVP is known to plasticize PVA, indirectly promoting interaction of PVA with nasal mucosa, with increased mucoadhesion and hence reduced drug clearance and improved drug absorption [97].

5.5.2. Nanoparticles

We turn now to colloidal particles made up of polymers or lipids with size in the nano range (<1000 nm). Like microparticles, nanoparticles can improve drug solubility and protect drug against metabolizing enzymes or efflux transporters, and also improve retention time when formulated with mucoadhesives.

For brain targeting, lipid nanoparticles are probably superior compared to conventional polymeric nanoparticles, due to rapid uptake by the brain, faster biodegradability, lower toxicity, absence of burst effect, and easier scale-up of lipid nanoparticles. Eskandari et al. prepared lipid nanoparticles of valproic acid (VPA) using cetyl palmitate, soy lecithin, octyldodecanol and poloxamer 188 (surfactant) [98]. The brain/plasma concentration ratio in rats with maximum electroshock seizures was 25-fold greater when the formulation was given IN compared to IP. Intranasally administered VPA nanoparticles with seven fold lower dose were as effective as a nasal VPA salt solution. High potency resulting from the nanoparticle form could be due to improved drug absorption and protection of drug from hydrolytic enzymes and/or efflux transporters. Additionally, the lipid coating could have improved direct brain uptake compared to the solution form [98].

5.6. In situ supersaturated solutions

In most cases, intranasal drug solutions are prepared and stored long before they are administered. Solutions containing organic solvents or co-solvents and microemulsions, while solubilizing the drug and preventing precipitation, must do so such that the drug's chemical potential (or thermodynamic activity referenced to pure aqueous solvent), never exceeds its saturation value. Otherwise, precipitation will occur during storage. The same is true when cyclodextrins are added to form complexes with the drug.

Supersaturated solutions contain more dissolved drug than the solvent can hold under normal conditions. They are by nature transient, and ultimately precipitates will form, again reducing the dissolved drug concentration relative to its saturation concentration. However, precipitation need not be immediate, and if supersaturated solutions can be prepared just before, during, or immediately after instillation of drug and solvent(s) into the nasal cavity, then rapid absorption may occur before precipitation starts. During the period of rapid absorption the drug's concentration in the nasal cavity decreases, reducing the driving force for precipitation. Transient supersaturation might therefore lead to an increased rate and extent of drug absorption compared to formulation approaches in which the drug's activity is limited to its saturation value.

There has been extensive interest in enhancing the oral bioavailability of poorly soluble drugs by formulating them in the solid state either as metastable polymorphs or as amorphous forms [99–101]. Such drugs could be transiently supersaturated upon dissolution. To our knowledge, this approach has not been tried in IN formulations. Maintenance of the metastable state and preventing conversion to the stable crystal form of the drug over long periods of time in storage is challenging, and rapid conversion to the stable form can occur upon exposure to moisture.

An alternative means to induce transient supersaturation is to create this condition at the point of administration, by rapid production of supersaturated drug just before or during administration of drug to the nasal cavity. We now discuss two approaches along this line.

5.7. Cosolvents

In situ supersaturation can be achieved by first solubilizing the drug in a 'good' solvent and then adding a 'poor' solvent just prior to drug administration at the desired site. Using this method, supersaturated DZP solutions in glycofurol/water mixtures were prepared by adding water to saturated DZP-in-glycofurol solutions [32]. Permeability studies using silicone membranes, modeling nasal mucosa, revealed several

NEURELIS, INC., EX2024, page 8
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

fold greater transport flux with supersaturated solutions compared to saturated DZP. The diffusion time lag was unaffected by both degree of supersaturation and the cosolvent composition, indicating that the model membrane's barrier function was not compromised by the cosolvent. Clinical pharmacokinetic studies with an intranasally administered formulation containing 5 mg or 10 mg of DZP dissolved in 60/40 (v/v) glycofurol/water cosolvent exhibited a t_{max} of 20–30 min with 75% absolute bioavailability. However, the subjects reported moderate pain and poor tolerance, probably due to the glycofurol [102].

5.8. Prodrug/enzyme systems

A prodrug is a pharmacologically inactive moiety that undergoes chemical transformation to form the active drug, with transformation typically mediated by an enzyme. The nasal mucosal environment expresses hydrolytic enzymes, but less abundantly than other organs and tissues. Co-administration of an activating hydrolase with prodrug present at high concentrations can result in a supersaturated solution of active drug in the nasal cavity which, as indicated above, may be stable long enough for complete drug absorption to occur before precipitation ensues.

As proof of concept, we prepared *in situ* supersaturated phenytoin (PHN) solutions using fosphenytoin (FOS: phosphate prodrug) and *alkaline phosphatase* (enzyme) mixed on the apical side of Transwell-plated Madin-Darby canine kidney II-wild type (MDCKII-wt) monolayers, the latter being an established nasal epithelium model [103]. Supersaturated PHN produced in this manner, exhibited several-fold faster permeation compared to saturated PHN, without drug precipitation. Fosphenytoin exhibited negligible permeability through the monolayers [103]. More recently, Viegas et al. [104] mounted porcine mucosa in an Ussing chamber, exposed it to FOS solution on the apical side, and noted the appearance of both FOS and PHN on the basal side, suggesting both paracellular transport of FOS and bioconversion of FOS to PHN, either intracellularly or in the basal solution. They also noted that *alkaline phosphatase* is present in human mucus, and suggested that FOS might be administered intranasally without added enzyme.

Since PHN, which requires grams as a loading dose for seizure emergencies, is not sufficiently potent for intranasal administration; we prepared a prodrug/enzyme system for a more potent drug, DZP. Avizafone (AVF) (Scheme 2) is a lysyl, ring-opened derivative of DZP, which was developed by the French military for intramuscular administration to treat battlefield convulsions resulting from exposure to nerve agents [105]. Following hydrolytic removal of the lysine moiety, the ring rapidly closes, regenerating DZP. While previous pharmacokinetic studies confirmed conversion of AVF to DZP in the human body [106], the identity of the converting enzyme(s) was not reported. Following a screen of commercially available hydrolases, *A.O. protease*, a rice fungus enzyme mixture used by the food industry [107], was found to convert AVF to DZP (Scheme 2). As shown in Fig. 2, supersaturated aqueous DZP, produced by enzymatic conversion of AVF, could permeate across MDCKII-wt monolayer membranes, at rates proportional to the "supersaturation potential," *SP*, defined as the ratio of initial (molar) AVF concentration to the concentration of saturated DZP [31]. (*SP* can be thought of as the degree of supersaturation of DZP that would be obtained if it were produced all at once from AVF by the enzyme.) High levels of supersaturation of DZP were achieved, without precipitation, in the apical side of the Transwell in the process of enzymatic conversion followed by drug permeation across the membrane. In the absence of enzyme, transport of the prodrug (AVF) across the monolayer was negligibly slow.

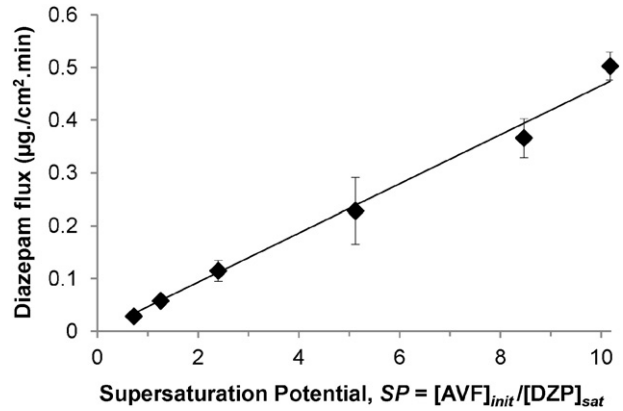
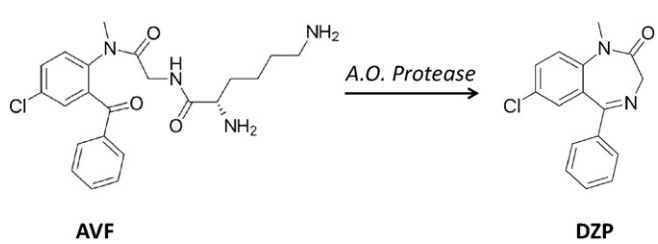


Fig. 2. Diazepam (DZP) flux across Madin-Darby canine kidney II-wild-type (MDCKII-wt) monolayer when Avizafone (AVF)-*A.O. protease* mixtures, prepared with different initial prodrug concentrations, $[AVF]_{init}$ (M), were incubated on the apical side, in pH 7.4 PBS at 32 °C. Rapid enzymatic conversion of AVF to DZP led to a high degree of supersaturation and enhanced transmembrane flux of DZP. $[DZP]_{sat}$ is the concentration (M) of saturated DZP. Adapted with permission from reference [31].



Scheme 2. Diazepam (DZP) and its prodrug, avizafone (AVF). AVF is converted to DZP by *Aspergillus oryzae* (*A.O.*) *protease*.

Encouraging results from these *in vitro* studies justify *in vivo* investigations to assess the true benefits of such a prodrug/enzyme system in humans. This aqueous system is expected to be better tolerated by patients than the glycofurol/water cosolvent system described above. However, the safety of the combined prodrug and enzyme, when administered intranasally, needs to be determined. Allergenicity/immunogenicity concerns regarding the fungal enzyme should be alleviated if a human converting enzyme such as human *aminopeptidase B*, which is active nasal mucosa cells and was recently demonstrated to convert AVF to DZP (D. Rautiola, private communication), is used.

Recently, an analogous prodrug for MDZ has been synthesized, and it was shown that it is also a substrate for conversion to MDZ by *A.O. protease*. An up to 30-fold increase in transport rate across MDCKII-wt membranes compared to saturated MDZ solutions was achieved following enzymatic conversion of the prodrug to supersaturated MDZ, again without precipitation [108,109].

6. Interspecies PK/PD differences for intranasal formulations

As with other vehicles for drug delivery, nasal formulations are tested in animals before evaluating them in humans. When extrapolating results from animal studies to humans, interspecies differences in nasal anatomy and physiology that may affect formulation performance are often ignored. Table 4 lists anatomical differences between rats, mice, rabbits, dogs, monkeys and humans with respect to nasal cavity length, volume, surface area, geometry and histology. Because of these differences, a nasally administered formulation may lead to variable rates and extents of drug absorption in different species. For example, the human nose volume is 25 mL, accommodating a maximum dose volume of 300–400 µL, including both nostrils. By contrast, only 100 µL nasal solutions can be given to rats due to a smaller nose. However rats have very high intranasal surface/volume ratio compared to humans, so drug absorption can be faster and more complete in rats compared to humans [48]. Further, the number of turbinates and their geometry differs across species, potentially affecting drug absorption from respiratory epithelium [51,110]. The olfactory epithelium area in humans (8 area%) is much smaller than in rats (50 area%) so the relative importance of the two absorptive pathways may differ [16]. Other

NEURELIS, INC., EX2024, page 9
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

Table 4
Interspecies differences in nasal anatomy and physiology [16,44,48,51,110].

Parameter	Mouse	Rat	Rabbit	Dog	Monkey	Human
Length	NF	2.3	5.2	10.0	5.3	7.5
Nasal cavity vol (cm^3)	0.03	0.40	6.00	20.00	8.00	25.00
Surface area (cm^2)	NF	14	61	221	62	150
Olfactory epithelium (area%)	NF	50	NF	NF	10–15	8
Formulation vol (μL)	24	40–100	NF	NF	NF	300–400
CSF replacement (h)	1.5	NF	NF	NF	NF	5.0
Turbinates	NF	3, t-shaped with elaborated scrolls	Folded scroll- like bones	NF	3, comma shaped	3, comma shaped
Breathing type	Nasal	Nasal	Nasal	Oronasal	Oronasal	Oronasal

NF - data not found.

interspecies differences are listed in Table 4. It can be concluded that the same formulation can result in different absorption profiles from species to species. We also note of course that the intrinsic pharmacokinetics and pharmacodynamics vary across species.

7. Importance of the intranasal device in drug to brain delivery

Integration of drug, formulation and delivery device determines product performance [40,72]. Choice of device depends on the type of formulation (liquid, powder), frequency of use (single unit, multiple units), target site (local, systemic), particle size, desired particle velocity, and cost.

For liquid formulations, drops are the oldest form of nasal delivery. Drug solutions can be delivered as drops from squeeze bottles, pipettes, or a Hamilton syringe attached to a catheter, sometimes producing a liquid jet [72]. Drops deliver drugs mostly to the anterior nasal cavity, unless the head is placed in the supine, head back position before instillation. This maneuver is often difficult for infants, children and patients with rhinosinusitis [72], and it is generally uncomfortable. For this reason, nasal sprays are preferred over drops. Additionally, drops may run out of the nose or drain faster than sprays into the nasopharynx, followed by swallowing.

Gas pressurized sprays, *i.e.* atomizers, are even more efficient due to their greater surface area coverage in the nasal environment [111]. For atomizers, particle deposition efficiency is determined by nozzle characteristics such as cone angle and orifice size, which determine spray length, spray drop size, deposition area, *etc.* For example, a cone angle of 30 degrees is reported to cause superior drug absorption compared to a 60 degree angle [112]. Other than nozzle characteristics, dose volume also affects nasal drug bioavailability. A smaller volume applied to both nostrils (2 × 50 μL) was shown to be mechanically cleared from the nasal cavity more slowly compared to larger volume applied to one nostril (1 × 100 μL), providing more time for absorption [113]. The 100 μL dose covered a larger area in the posterior regions where clearance is faster due to a more ciliated epithelium. Atomizers must be used carefully since they may cause pulmonary complications, since smaller particles (<5 μm) may be carried by inspired air into the lung [40].

Powder formulations are advantageous over liquid formulations in terms of physical/chemical stability, but the performance of powder devices is influenced by particle properties such as particle size, hygroscopicity, powder flow, polymorphic nature and aqueous solubility. Powders, when deposited in the nasal cavity, dissolve in the nasal secretions and the dissolved drug is then absorbed. Some examples of powder devices include powder metered dose inhalers (pMDI), insufflators, and breath actuated inhalers. For further details on these devices are provided in a review by Djupesland et al. [72].

Several companies are developing device technologies for improved nasal drug absorption. The Mucosal Atomization Device (MAD) developed by LMA North America (LMA MAD Nasal™) is an easy to use, inexpensive device for delivery of nasal solutions, although it has not been approved by the FDA. MAD can be attached to a syringe filled with nasal solution [114]. The syringe is held between the second and third fingers and the plunger is pushed using the thumb. This action presents the nasal drug in the form of spray plume, which successfully deposits the particles in posterior regions of the nasal cavity, improving drug absorption.

Another popular device used for delivery of clinical trial liquid products is OptiNose®. This is a breath-actuated device that has a nosepiece and a mouthpiece. Drug is propelled to the posterior of the nasal cavity upon exhalation by the patient into the mouthpiece. This action closes the soft palate and prevents drug from reaching the throat or lung, maximizing its concentration in the nasal area. OptiNose® is used for both liquid and powder formulations. Recently OptiNose® based delivery of AVP-825 (the combination) has received FDA approval for acute treatment of migraine [115].

Newer technologies include VersiDoser® (liquid) and VRx2™ (powder) precision metered dose intranasal delivery systems (Mystic Pharmaceuticals) [116], and ViaNase™ atomizer (Kurve Technology) [117]. A proprietary drug delivery device combination for intranasal DZP (NRL-1) has been developed by Particle Sciences in association with Neurelis. Phase I pharmacokinetic studies have shown results with this drug delivery device to be comparable to those obtained with rectal DZP (Diastat®) [118,119].

8. Summary and future prospects

The intranasal route of drug administration for seizure emergency drugs is of growing interest, with an increasing number of nasal products in clinical trials (Table 2). The popularity of the intranasal route is due to its ready accessibility, ease of administration when oral/intravenous route is not available/appropriate, its non-invasive nature, potential for direct brain delivery, and improved drug bioavailability resulting from high vascularized tissue, large surface area for absorption, and substantial avoidance of first-pass metabolism in the nasal environment. IN delivery circumvents practical problems associated with the IV, rectal, buccal, sublingual, and intramuscular routes, which are either already used or are under investigation for rescue therapy. Better understanding of nasal anatomy and formulation strategies has resulted in improved bioavailability of intranasal products, as is evident from various examples covered in this review.

Of the various agents used to treat seizure emergencies, BZDs are the drugs of choice due to their efficacy against a broad range of seizure types, rapid onset of effect, and relatively limited adverse effects. However, the BZDs target GABA receptors, and have tolerance issues. Potent molecules binding to other targets, with efficacy comparable to BZDs, but not incurring tolerance issues, need to be explored. Recently, new targets for seizure emergencies such as SV2A receptors (drug: brivaracetam), AMPA receptors (drugs: propofol and perampanel), and serotonin receptors (drug PRX-00023) have been identified [120].

The precise pathways used by BZDs to reach active sites in the brain have not been well characterized, and may be species dependent. As discussed above, the respiratory component of the nasal epithelium dominates the olfactory component in humans, in contrast to rats and dogs (Table 4); we therefore believe that most of an intranasally administered BZD dose will reach the brain *via* absorption into the system

NEURELIS, INC., EX2024, page 10
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

circulation and crossing the BBB, which is very permeable to BZDs. However, the possibility of some direct absorption through the olfactory and trigeminal nerves, the latter enervating the respiratory epithelium, cannot be ruled out and the site of absorption may also depend on the deposition pattern of the drug formulation. While elucidation of this issue may be possible in animals by comparing blood and brain concentration profiles following IV and IN administration and calculating relevant targeting indices [121], such experiments are precluded in humans. Moreover, the "geographical" patterns of drug concentration in the brain may differ significantly following direct nose-to-brain absorption, compared to transport *via* the BBB. Since the exchange of BZDs across the BBB is rapid, these differences may only be important in the early stages of a dosing event. Precise elucidation of differences between direct nose-to-brain and BBB mediated first appearance in the brain may require sophisticated electrophysiological or time-resolved behavioral measurements.

In addition to therapeutic metrics, the toxic potential of intranasal formulations must be evaluated. While necrosis and inflammation of nasal tissues are of acute concern, allergenicity, immunogenicity, and carcinogenicity represent potential delayed manifestations. Solvents and excipients are obvious potential contributors to toxicity, but it should also be noted that the high concentrations of drug that are applied intranasally may cause local toxic effects. The toxicities of prodrug/enzyme combinations will need to be carefully evaluated. Finally, the toxic potential of a formulation may depend on whether it is targeted towards systemic drug absorption or direct nose-to-brain absorption.

Some patients with epilepsy report an "aura" just preceding a seizure, and it seems possible that seizures could be prevented or attenuated by nasal administration before onset. Of course, such an opportunity for prophylaxis must be weighed against the possibility of false alarms. Efforts are underway towards physiological monitoring and seizure prediction. An obvious marker would be EEG, and there are several reports showing success in using it predictively [122,123]. Electromyography (EMG) based devices have demonstrated effectiveness in detecting generalized tonic-clonic seizures, quickly sensing the tonic phase and sending signals to caregivers [124]. EMG based devices are more efficient than accelerometers, which detect repetitive shaking movements and therefore must wait for the clonic phase. In patients with non-convulsive seizures, devices that detect electrodermal activity have been useful in predicting seizures [124].

The prospect of noninvasive prevention or rapid amelioration of seizure emergencies using automatic seizure prediction and a portable nasal formulation of antiepileptic drugs such as BZDs will rely on convenience, reliability, and safety of the sensing elements, the predictive algorithms, the dosing device, and the nasal formulation. False positive and false negative rates for seizure detection must be minimized. The drug delivery system must be easy to use, and it should produce a very rapid onset of effect with minimal toxic side effects and irritation. Work is underway on all facets of the "control loop," with promising early results. If further progress is made, followed by translation into approved therapies, such therapies will represent a milestone in improving the quality of life of those suffering from epilepsy.

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