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On a highway to the brain: A review on nose-to-brain drug delivery using nanoparticles

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ABSTRACT

The administration of drugs to the central nervous system (CNS) is primarily controlled by the blood-brain barrier (BBB), a structure that prevents the passage of foreign compounds from the blood to the brain's extracellular fluid. Although currently available treatments for brain diseases that affect millions of people globally are partially effective, they are associated with severe side effects of systemic drug distribution. On the other hand, the ability of certain drugs to permeate through the BBB is impeded by their physicochemical properties, achieving sub-therapeutic concentrations in their target tissues. In this sense, the intranasal route with its unique anatomical features provides a promising passage for the delivery of drugs to the brain. Nanoparticle-based systems, in particular, have demonstrated an outstanding capacity to overcome the challenges presented by the intranasal route and produce drug accumulation in the brain while avoiding systemic distribution. This review covers recent developments in the use of polymer, lipid, and inorganic nanoparticles, as well as drug nanocrystals, to deliver drugs to the brain *via* intranasal administration. A general discussion including favourable aspects and limitations of this approach is also provided.

1. Introduction

The cerebrospinal fluid (CSF) and blood-brain barrier (BBB) isolate the central nervous system (CNS) from the systemic circulation, preserve its integrity, maintain homeostasis, and protect it against external injuries [1]. When healthy, the BBB ensures correct brain function by preventing external blood materials from entering the brain's extracellular fluid. The BBBs presence was initially demonstrated by Paul

Abbreviations: AUC, area under the curve; AuNC, gold nanoclusters; BBB, blood-brain barrier; BSA, bovine serum albumin; CD-CS, β-cyclodextrin-chitosan; CNS, central nervous system; CSF, cerebrospinal fluid; C_{max}, maximum drug concentration in the target tissue; DTE, drug targeting efficiency; DTP, drug targeting potential; FUSIN, focused ultrasound combined with microbubble-mediated intranasal delivery; GNP, gold nanoparticles; GNPr, gold nanoprisms; GNPr-D1, gold nanoprisms functionalized with D1 peptide; GNS, gold nanospheres; GNS-D1, gold nanospheres functionalized with D1 peptide; GO, graphene oxide; HSA, human serum albumin; IN, intranasal; IV, intravenous; LCN, hybrid lecithin/chitosan nanoparticles; Lf, lactoferrin; Lf-TMC, (Lf)-conjugated mucoadhesive N-trimethylated chitosan; LNC, lipid nanocapsules; Mw, molecular weight; MRI, magnetic resonance imaging; MSN, mesoporous silica nanoparticles; NAP, neuroprotective octapeptide; NC, drug nanocrystals; NLC, nanostructured lipid carriers; NP, nanoparticles; NS, nanosuspensions; OB, olfactory bulb; OL, odorranalectin; QD, quantum dots; QTPP, quality target product profile; PCL, poly(caprolactone); PCL_P80, polymer poly(caprolactone) nanocapsules stabilized with Tween® 80; PCL_SCH, polymer poly(caprolactone) nanocapsules coated with sodium caproyl hyaluronate; PEG, poly(ethylene glycol); PEG-PLGA, poly(ethylene glycol)-poly(lactic-co-glycolic acid); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PolyGION, theranostic polyfunctional gold-iron oxide nanoparticles; PSA, poly(sebacic anhydride); SLN, solid lipid nanoparticles; TAT, transactivator of transcription; T_{max}, time required to reach the maximum drug concentration in the target tissue; us-GO, ultrasmall graphene oxide-Sheets; WGA, wheat germ agglutinin.

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Ehrlich and subsequently supported by his student Edwin Goldman in 1885 and 1913, respectively [2]. They proved the existence of a barrier that impedes the passage of dyes from the blood to the CNS and *vice versa* using animal models [3]. Later investigations led to a better comprehension of the anatomical and physiological features of the BBB and its pivotal function regarding drug access to the CNS.

The BBB consists of a monolayer of tightly connected endothelial capillary cells that allow the selective entrance of nutrients and hormones and impairs the passage of pathogens, toxins, and other foreign substances, like drugs. After oral or systemic drug administration, the therapeutic agent has first to cross the BBB to reach the CNS. Entry of compounds to the brain mainly occurs through active transport and passive diffusion (paracellular or transcellular) across endothelial cells. Thus, tight junctions make the BBB impermeable to large molecules and most low molecular weight (Mw) molecules, allowing only the passage of smaller and highly lipophilic substances [4,5]. Furthermore, this structure has specific transporter proteins and receptors on the surface that allow the entry of essential substances such as glucose transporter GLUT1, insulin receptor, and transferrin receptor TfR, among others. In contrast, some efflux transporters on the endothelial cells can prevent the uptake of several molecules into the brain and force drugs to return to the systemic circulation, as in the cases of the P-glycoprotein system and multidrug resistance-related proteins [1,5,6].

An adequate dosage of the administered drug must reach the brain at the therapeutic levels necessary for the therapy of CNS disorders, such as Parkinson's disease, multiple sclerosis, Alzheimer's disease, meningitis, epilepsy, and neurocysticercosis, among others [7,8]. These disorders generally involve a wide range of pathological manifestations resulting in both modifications of neural function and gradual damage of neural structures. Although currently available treatments for these diseases are to some extent effective, the drugs' systemic distribution is often linked with a significant burden of side effects that negatively affect patients' quality of life. While significant strides have been made in comprehending the pathogenic process of these disorders, the development of more effective treatments with reduced side effects remains unmet.

Intranasal (IN) administration is an attractive way of attaining high drug levels in the brain, which has been suggested as an alternative approach to the traditional parenteral and oral routes for the direct delivery of drugs to the brain. The nasal cavity possesses a unique set of anatomical characteristics for drug delivery, resulting in a minimally invasive route, allowing a rapid onset of action, and avoiding hepatic first pass-effect [9]. With a surface area of around 160 cm^2 (96,000 cm^2 comprising the microvilli), the IN route has been extensively studied to deliver topical and systemic treatments [10,11]. Meanwhile, the olfactory region, providing direct access to the brain, has an area of only around 5 cm^2 (3,000 cm^2 comprising the microvilli) [11]. Furthermore, the IN cavity has a high-density microvasculature partly responsible for drug absorption and distribution. However, nose physiology poses some challenges that should be considered when developing drug formulations for this route. The limited volume of formulation that can be applied into the nose, mucociliary clearance, presence of a mucus layer, and local enzymes are some of the factors that can hamper drug absorption through IN route. In light of this, nanoparticle-based drug delivery systems have shown to be a valuable tool to promote drug accumulation in the CNS through an increased permeation across the olfactory region. In this article, we will critically review the recent progress on the development of nanoparticles (NP) for drug delivery to the brain *via* the IN route (Fig. 1). Special emphasis will be placed on the nanocarrier nature, incluidng polymer, lipid, and inorganic NP, as well as drug nanocrystals.

2. Advantages, challenges, and barriers in nose-to-brain drug delivery

The IN route is a non-invasive or minimally invasive route of administration to the CNS, more effective than intravenous (IV) and oral routes. This direct route to the CNS can avoid the BBB besides reducing systemic side effects. In the case of the other parenteral routes and oral administration, drugs must first cross several barriers to achieve systemic circulation and then cross the BBB to reach the CNS. Moreover, IN route avoids hepatic first-pass metabolism and drug degradation in the gastrointestinal tract, being an alternative route for parenteral administration, especially for biopharmaceuticals (like proteins and peptides). Thus, direct drug delivery to the brain can be achieved by IN administration, mainly through the sensory neuronal pathway or indirectly by the passage across the BBB from the systemic circulation.

The main areas of the nasal cavity include the vestibular region, the respiratory region, and the olfactory region (Fig. 1A). The first region is the outermost area of the nasal cavity covered by ciliated hairs and a mucous layer, constraining the access of external particles, antigens, and pathogens. Next, the respiratory region is provided with trigeminal sensory nerves and blood vessels. Finally, the olfactory region is located on the upper segment of the nasal cavity, presenting an epithelium formed by supporting cells, basal cells, and olfactory sensory neurons (Fig. 1B). This region is in close contact with the olfactory bulb (OB) of

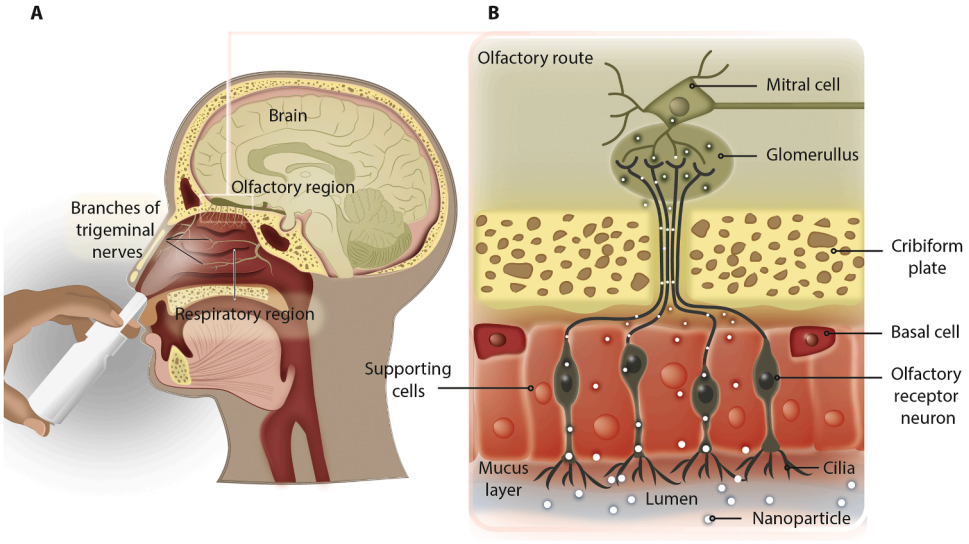

Fig. 1. Anatomical features of the intranasal route (**A**), including key structures involved in nose-to-brain drug delivery (**B**).

the brain through the olfactory nerves, which are placed below the cribriform plate of the skull. In turn, trigeminal nerves are present in this area [1,12,13].

Once a therapeutic formulation is deposited inside the nose, it must overcome mucociliary clearance in the vestibular region. Upon reaching the internal part of the nasal cavity, the drug can attain the CNS through the olfactory nerves, trigeminal nerves, or indirectly *via* the systemic circulation [7]. The trigeminal and olfactory pathways in the nasal cavity allow direct delivery to the brain, positively impacting the pharmacokinetic/pharmacodynamic (PK/PD) profiles of CNS drugs. In the case of the olfactory pathway, the drug can reach the OB, upon passing the olfactory mucosa, or the CSF, mixing later with interstitial fluid in the brain. The therapeutic agents must pass through the cribriform plate and the olfactory nerve after interacting with olfactory receptors on olfactory neurons. Direct delivery of drugs to the brain by different olfactory nerve transport mechanisms comprises intraneuronal and extraneuronal pathways, in which transport occurs along axons and through perineural channels, respectively [9,13].

The trigeminal pathway involves branches of the trigeminal nerves that supply both the respiratory and olfactory mucosa, transporting therapeutically active molecules towards the brain stem and coupled tissues. These branches enter the brainstem at the level of the pons and are extended to the hindbrain and forebrain. This pathway enables intracellular transport across axons and extracellular transport *via* diffusion and bulk flow through perineuronal channels, perivascular spaces, or lymphatic channels connected to the CSF and brain tissues. The physicochemical properties of the drug will determine if transport can occur intracellularly or extracellularly [14].

On the other hand, the respiratory region is substantially vascularized, favouring systemic drug absorption. Small lipophilic molecules can access the blood and cross the BBB more readily than hydrophilic and high Mw molecules. Once the drug enters the nasal blood vessels, it can reach the carotid artery and then the brain and spinal cord. This route is less preferred since the limitations offered by the BBB for the access of drugs to the CNS and the undesired peripheral effects that can be caused by systemic distribution [12,15]. Despite the benefits and potential of the nose-to-brain administration route, there are significant challenges to overcome for drugs reaching the CNS, including the target site's anatomical, physiological, and biochemical characteristics.

One of the main challenges are related to the residence of mucus in the nasal mucosa coupled with ciliary movement due to they are the first barriers to overcome when drugs are administered *via* the IN route since both factors can limit the retention time of the drug dosage form in the nasal cavity and molecule movement towards the CNS. In addition, the small volume available for formulation delivery in each nostril may prevent an efficient brain drug delivery [7,16]. The anatomical location of the olfactory epithelium is another main limitation of this route since the dosage form must first be able to reach this site. Metabolic enzymes present in the olfactory mucosa must also be considered when designing a formulation for the nose-to-brain route. Consequently, IN formulations must be composed of biocompatible and odourless excipients and avoid rapid elimination due to mucociliary clearance and/or enzymatic degradation.

Furthermore, they must present appropriate viscosity, physiological tonicity, and a pH compatible with the nasal mucosa [9,14]. Thus, different strategies have been explored to overcome the challenges of this route of administration. Most of these approaches aimed to enhance molecule absorption and permeability by increasing the time in which the dosage form remains in the nasal mucosa and promoting drug concentration in the CNS [17]. These strategies include the employment of permeation and absorption enhancers, cell-penetrating molecules, mucoadhesive and mucopenetrating agents, enzyme inhibitors, hydrogel systems, and nanoparticulate drug delivery systems or a combination of different strategies.

In particular, nanoparticulate-based systems have demonstrated a remarkable capacity to overcome the challenges presented by the IN route and produce drug accumulation in the brain while avoiding systemic distribution. The last advances in this field are outlined below.

3. Nanoparticles for nose-to-brain drug delivery

Nanoparticle-based systems have provided significant progress in seeking strategies for therapeutic agents to reach the CNS due to functional properties related to the nanometer scale and material composition. Thus, they can cross the BBB helped by their surface area, reactivity, strength, sensitivity, and solubility, among other properties. In order to deliver nanoparticle-based systems to the CNS, non-invasive methods, invasive methods, and alternative routes can be used [18]. The non-invasive methods involve endogenous cellular mechanisms that facilitate the transport of nanoparticulate-based systems across BBB through the transcellular pathway, according to colloidal, chemical, or biological properties. The invasive techniques involve the administration of the nanoparticulate-based system directly into the brain tissue by intraventricular, intrathecal, or interstitial injections, among others, as well as the methods based on the disruption of the BBB employing osmotic, ultrasound, chemical, or magnetic strategies.

Despite all the challenges faced, the IN administration of nanoparticulate-based systems is still the most promising alternative for delivering therapeutic agents to the CNS. Altogether, the advantages associated with the administration route and nanoparticle properties can facilitate addressing drugs to the CNS. In this way, the size of nanoparticulate-based systems is one of the main factors that must be controlled in the design of IN formulations. In addition, particle size can impact the drug loading, release, and stability, determining the *in vivo* distribution, toxicity, and targeting ability towards the CNS. Particle size distribution can also influence the pharmacokinetics of nanocarriers, including the circulation time, absorption, and biodistribution [19]. Thus, small particle sizes and greater surface areas can lead to an increased drug solubility, stronger interaction with mucosa, or better permeation than a drug solution which even can be favoured by the type of nanoparticulate system composition. The surface charge of nanocarriers is another important factor contributing to better drug performance after administration. Positive zeta potentials can allow better interaction with the negatively charged mucin residues, favouring the retention of the formulation in the nasal mucosa for a longer time.

IN nanoparticle-based systems have been demonstrated to improve the permeability and absorption of drugs, their uptake in the olfactory region, and their access and accumulation into the CNS. At the same time, they can protect therapeutic agents from degradation and prevent their extracellular transport by outgoing transporters [9,19,20]. Moreover, the combination of nanotechnology with other strategies has permitted to favour the accumulation of IN nanoparticulate-based systems into the CNS. Several surfactants are used to facilitate drug permeation, such as pegylated molecules [21], while mucoadhesive polymers able to interact with mucin, such as chitosan, are used to prolong its residence time [22]. On the other hand, incorporating cell-penetrating peptides (CPP) that can interact with biological membranes promotes cellular uptake [23,24]. In the same direction, many studies have investigated the use of biorecognition ligands to boost the nose-to-brain transport of nanocarriers. For instance, proteins with receptors in the olfactory region, like lectins, are the gold standard for active brain targeting.

Thus, the use of nanoparticulate-based systems in combination with other strategies such as incorporating permeation enhancers and/or mucoadhesive agents, among others, has enabled to achieve a higher degree of selective drug delivery to the brain. Different nanoparticulate-based systems have shown promising *in vivo* results related to pharmacokinetic parameters in the brain after an IN administration. Important brain pharmacokinetic parameters were enhanced, increasing C_{max} (maximum drug concentration in the target tissue), area under the curve (AUC, corresponding to the integral of the plasma concentration —or tissue concentration— of a drug against a defined time interval), and

mean retention time, while reducing T_{max} (time needed to achieve the C_{max} in the target tissue). Importantly, mathematical formulas are used to calculate brain targeting, for example, drug targeting efficiency (DTE) and direct transport percentage (DTP), which are commonly defined by Eq. (1) and Eq. (2), respectively [9,19,25,26]:

$$\mathrm{DTE}\ (\%) = \frac{\left(\frac{\mathrm{AUCbrain}}{\mathrm{AUCblood}}\right)\mathrm{IN}}{\left(\frac{\mathrm{AUCbrain}}{\mathrm{AUCblood}}\right)\mathrm{IV}} \mathrm{x}100 \quad (1)$$

$$\mathrm{DTP}\ (\%) = \frac{(\mathrm{AUCbrain})\mathrm{IN} - \left(\frac{(\mathrm{AUCbrain})\mathrm{IV}}{(\mathrm{AUCblood})\mathrm{IV}}\mathrm{x}(\mathrm{AUCblood})\mathrm{IN}\right)}{(\mathrm{AUCbrain})\mathrm{IN}} \mathrm{x}100 \quad (2)$$

where the (AUCbrain)IN and (AUCbrain)IV are the drug concentration variation over time in the brain within the experimental period after IN administration and IV administration, respectively; while the (AUCblood)IN and (AUCblood)IV are the drug concentration variation over time in the blood within the experimental period following IN administration and IV administration, respectively.

DTE represents the accumulation of the drug in the brain after IN administration compared to IV administration, while DTP determines the amount of drug that achieves the brain *via* olfactory pathway and/or trigeminal pathways. Values of DTE highest to 100% show brain targeting by IN administration to be more effective than IV administration, while values below 100% represent the opposite. A brain targeting through direct passages (the olfactory or trigeminal pathways) is evidenced when DTP values are greater than zero [19]. A DTP equal to 100 is only possible if the drug cannot cross the BBB at all (AUCbrain IV = 0), or if it cannot pass to the systemic circulation after IN administration (AUCblood IN = 0)[19].

The following sections describe and critically review different nanoparticle-based formulations developed mainly in the last two years as new therapeutic approaches for IN administration and accumulation of pharmacologically active agents into the brain.

3.1. Polymer nanoparticles

Polymer-based NP are perhaps the most popular nanocarriers used in nose-to-brain delivery and, thus, are currently at the forefront of novel neuropharmacological treatments [27]. Their chemical versatility, high drug loading capacity, and ease of surface functionalization with targeting ligands have placed this kind of nanocarriers on the focus of clinical research [28]. Different types of structures could be obtained using polymers as building blocks, encompassing polymer NP [29], micelles [30], nanocapsules [31], and dendrimers [32]. Furthermore, synthetic polymers and biopolymers could be employed to design IN drug delivery systems. For instance, biodegradable and biocompatible

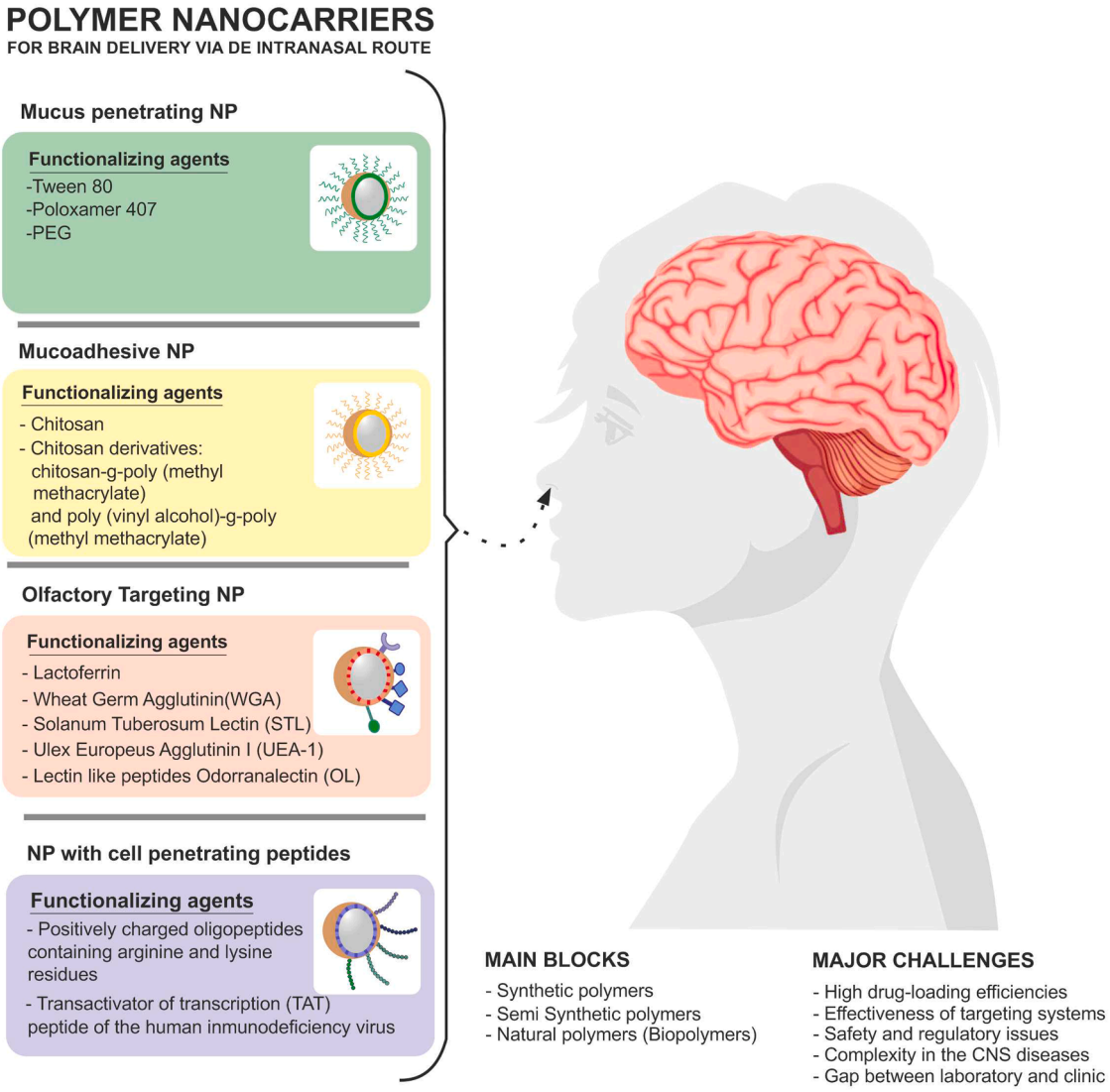

Fig. 2. Different explored strategies to ameliorate the nose-to-brain drug delivery using polymer NP.

poly(caprolactone) (PCL), poly(lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), and poly(ethylene glycol)-poly (lactic-co-glycolic acid) (PEG-PLGA) are routinely used as synthetic building blocks [21]. On the other hand, a plethora of biopolymers like gelatine, pullulan, alginate, sodium hyaluronate, and human serum albumin (HSA) have been explored. However, chitosan and chitosan derivatives, with valuable mucoadhesive and cell permeating properties, are by far the most exploited polysaccharides in IN drug delivery systems [33].

Indeed, functional NP have previously been shown to boost the nose-to-brain delivery by incorporating mucus penetrating polymer surfactants, coatings with mucoadhesive polymers, covalent linking of bio-recognition ligands targeting the olfactory region, and surface modifications with cell-penetrating peptides (Fig. 2) [34,35]. The following sections summarize these classes of functional polymer NP, detailing the latest relevant advances reported in the literature (Table 1).

3.1.1. *Mucus penetrating polymer nanoparticles*

Pegylated surfactants like Polysorbate 80 (Tween® 80) and Poloxamer 407 (Pluronic® F127) have traditionally been used to coat polymer NP in IN formulations owing to their permeation and muco-diffusive properties [21]. Puglisi et al. prepared rhodamine-loaded PLGA (132 nm, -15.8 mV) and PLA (152 nm, -30 mV) NP using Tween® 80 as a stabilizer, and their uptakes in olfactory ensheathing cells were compared with that for chitosan NP (no surfactant, 181 nm, +34 mV) [36]. The results showed that Tween® 80 was responsible for promoting cellular uptake of the polymer NP, obtaining the best performance for PLGA nanospheres with the lowest absolute surface charge.

Very recently, Del Favero et al. reported a comparative study of three nanoformulations designed to deliver simvastatin nasally: polymer PCL nanocapsules stabilized with Tween® 80 (PCL_P80), polymer PCL nanocapsules coated with sodium caproyl hyaluronate (PCL_SCH), and hybrid lecithin/chitosan NP (LCN) [37]. As shown in Fig. 3, the simvastatin diffusion across the respiratory epithelium of rabbits was evaluated, finding the highest permeation ability when using Tween® 80 as a surfactant. Compared to the drug solution, the selective accumulation of these polymer nanocapsules within the epithelial tissue was attributed to their surface's composition and properties, ruling out the nanoparticle size as a factor, which was in all cases similar, around 200 nm. The authors proposed that these modified nanosystems could be intracellularly transported through the respiratory epithelium and, potentially, *via* the olfactory neural pathway to the brain. However, additional studies addressing the nanoparticle's uptake pattern in the olfactory epithelium should be considered.

Table 1
Summary of recent reports on polymer nanocarriers for brain delivery *via* the intranasal route.

Polymer Nanocarrier	Drug	Disease	Particle size (nm)	PDI	Z-Potential (mV)	Functionality	Main findings	Refs.
Lecithin/chitosan NP and PCL nanocapsules	Simvastatin	Glioblastoma	202-258	0.11-0.15	+40; -22; -39	Mucoadhesion/ mucopenetration	Enhanced bioavailability	[37]
PLGA NP	Diazepam Midazolam	Status epilepticus	148-337	0.04-0.45 0.099	-15; -29 -16	Mucopenetration	Improved brain accumulation	[38, 39]
Chitosan NP	Midazolam	Status epilepticus	241-381	0.389-0.483	n.r.	Mucoadhesion	Superior brain-targeting efficiency	[42]
Chitosan NP	Rotigotine	Parkinson's disease	75	0.368	+25	Mucoadhesion	Enhanced brain targeting efficiency and drug bioavailability	[43]
Chitosan-PLGA NP	Ropinirole hydrochloride	Parkinson's disease	468	0.290	+54	Mucoadhesion	3.22-fold increased permeation in sheep nasal mucosa	[44]
Chitosan-HSA	Sulforhodamine B sodium salt	-	261	0.10	+45	Mucoadhesion	High mucoadhesion in *ex vivo* rabbit nasal mucosa model	[45]
PSA NP	Thyrotropin releasing hormone	Suicidal depression	258	0.204	-41	Degradable	Beneficial for intranasal delivery *in vitro*	[47]
Lf-PEG-PCL NP	Octapeptide (NAP)	Alzheimer's disease	88	0.220	-24	Lf active targeting	Rapid accumulation in various brain areas	[55]
Lf-N-trimethylated chitosan-PLGA NP	Huperzine A	Alzheimer's disease	153	0.229	+36	Lf active targeting	High distribution of NP in the brain over a long time	[56]
Lf-PEG-PLGA NP	Rotigotine	Parkinson's disease	122	0.194	-21	Lf active targeting	Accumulation in the OB, striatum, and cerebellum	[57]
WGA-PEG-PLGA NP	NR2B9c peptide	Ischemic stroke	139	0.200	-23	WGA active targeting	Effective active-brain targeting	[49]
WGA-PEG-PLA NP	miR132	Alzheimer's	191	0.250	-26	WGA active targeting	Considerable increase of the gene bioavailability in the brain.	[50]
Solanum tuberosum lectin-PEG-PLGA	Haloperidol	Schizophrenia	132	0.174	-14	Solanum tuberosum lectin active targeting	Increase (3-fold) in the brain tissue drug concentration	[52]
Ulex europeus agglutinin I-PEG-PLA NP	6-coumarin	-	111-196	n.r.	n.r.	Ulex europeus agglutinin I active targeting	Improved brain-targeting efficiency of NP and accumulation in the olfactory region	[53]
OL-PEG-PLGA NP	Urocortin peptide	Parkinson's disease	83-115	0.186	-25	OL active targeting	Increased brain uptake and enhanced neuroprotective effects	[54]
TAT-PEG-PCL micelles	Anti TNF-α	Cerebral ischemia	62	n.r.	+19	TAT cell-penetration	Enhanced *BBB* permeation	[63]
Bombesin-PEG-PCL-TAT micelles	Camptothecin	Glioma	80	0.400	+8	Bombesin/TAT active targeting/ cell-penetration	Enhanced therapeutic outcomes in a brain tumour-bearing rat model	[30]

BBB, blood-brain barrier; BSA, bovine serum albumin; HSA, human serum albumin; Lf, Lactoferrin; NP: Nanoparticles; n.r., No reported; OB, olfactory bulb; OL, Odorranalectin; PCL, poly(caprolactone); PEG, poly(ethylene glycol); PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PSA, poly(sebacic anhydride); TNF-α: tumour necrosis factor-alpha; TAT, Transactivator of transcription; WGA, Wheat germ agglutinin.

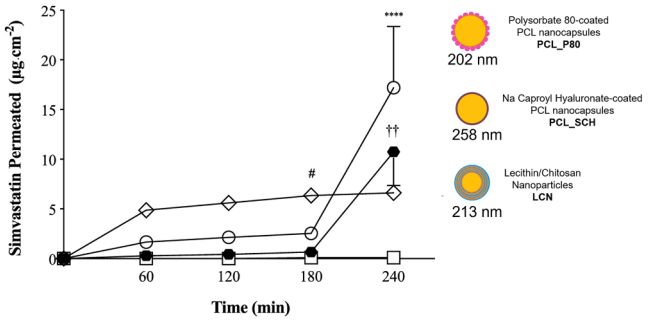

Fig. 3. Permeation profiles of simvastatin across the respiratory epithelium of rabbit for simvastatin suspension (empty square), polysorbate 80-coated poly (caprolactone) nanocapsules (SVT-PCL_P80, empty circles), sodium caproyl hyaluronate-stabilized poly(caprolactone) nanocapsules (SVT-PCL_SCH, black hexagons), and lecithin/chitosan hybrid nanoparticles (SVT-LCN, empty diamonds) across the nasal epithelium of rabbits. Reprinted from Ref. [37] Copyright® (2021), with permission from the American Chemical Society.

In two separate experiments, the benzodiazepines diazepam [38] and midazolam [39], commonly used to treat status epilepticus, were loaded in PLGA NP stabilized by Poloxamer 407. In both cases, the NP showed sustained drug release across sheep nasal mucosa, direct brain transport, and greater drug accumulation into the brain region than a drug solution following IN administration to rats.

NP coated with poly(ethylene glycol) (PEG) can reduce mucin interactions and have shown fast penetration into human respiratory mucus. Following this approach, Hanes et al. prepared mucus-penetrating PLGA-PEG NP to load small hydrophobic molecules and large hydrophilic proteins, like curcumin and bovine serum albumin (BSA), respectively [40]. The authors found that when NP are prepared using low Mw emulsifiers, dense brush PEG coatings are formed, allowing rapid NP permeation across human mucus. By contrast, high Mw emulsifiers, like poly(vinyl alcohol), interrupt the PEG coating on NP, triggering accumulation within the mucus. Besides, they demonstrated that the Mw of PEG (1-10 kDa) did not affect the formation of a dense brush-like coating on the surface of PLGA-PEG NP.

3.1.2. *Mucoadhesive polymer nanoparticles*

Mucociliary clearance is the primary physiological mechanism that significantly limits nose-to-brain transport. Therefore, NP-based formulations able to interact with mucin and prolong their residence time in the olfactory region have been investigated for decades [41]. In this sense, chitosan NP or polymer NP coated with this polysaccharide have been the workhorse in nose-to-brain delivery [22]. A recent example reported in the literature details the encapsulation of midazolam within chitosan NP [42]. A substantially higher brain concentration of midazolam was obtained using the nanoformulation than the drug solution (DTE, 271% vs. 191%), demonstrating the brain-targeting efficiency of these mucoadhesive and permeating chitosan NP.

Similarly, Bhattamisra et al. designed chitosan NP for IN delivery of rotigotine, a non-ergoline dopamine agonist that has displayed promising outcomes for treating Parkinson's disease [43]. This nanosystem showed superior brain accumulation ability and drug bioavailability in Sprague Dawley rats. An increase in alpha-synuclein levels is one of the critical clinical hallmarks of Parkinson's disease. Therefore, the authors demonstrated that rats treated with rotigotine-loaded chitosan NP produced a marked reduction in the levels of this neuronal protein at 51.10 ± 2.24 pg/mL, significantly greater than that for rotigotine solution, 62.78 pg/mL.

As an alternative to these biopolymer nanosystems, Borge et al. have proposed coating PLGA NP with chitosan and loading the anti-Parkinsonian therapeutic ropinirole hydrochloride [44]. The chitosan armour endowed these NP with mucoadhesive properties, notably increasing the permeation of the drug across sheep nasal mucosa by around thrice compared to unmodified PLGA NP. Additionally, the impact of chitosan coating on the properties of HSA NP was recently examined for nose-to-brain delivery [45]. The mucoadhesion and permeation ability of these nanosystems, encapsulating a fluorescent dye as a model molecule, was evaluated through *in vitro* release and uptake experiments. Two cell lines, Caco-2 and hCMEC/D3, were employed to represent the nasal epithelium and BBB models, respectively. The results with Caco-2 cells revealed that after incubation for 4 h, the permeability value of chitosan-coated HSA NP was higher, around 2-fold, than that for unmodified NP. The authors also suggested that both types of NP were mainly internalized by clathrin-mediated endocytosis. Permeation studies in hCMEC/D3 cells showed that the apparent permeability value obtained by the chitosan-modified NP was higher than with unmodified NP after 1 and 2 h of incubation. However, both permeabilities were quite similar after 3 h. On the other hand, *ex-vivo* studies in rabbit nasal mucosa proved strong chitosan/mucosa interactions unveiling the superior penetrating potential of chitosan-HSA NP compared to naked NP.

In another exciting approach to impart mucoadhesiveness, Sosnik et al. prepared two types of graft copolymers chitosan-*g*-poly(methyl methacrylate) and poly(vinyl alcohol)-*g*-poly(methyl methacrylate), able to self-assemble into NP [46]. Formulations combining these NP were assessed in terms of their permeation ability across a nasal septum epithelial cell line. The authors demonstrated that the mucoadhesive amphiphilic NP promotes tight cell junctions opening, permeating this *in vitro* model by the paracellular pathway.

At the other extreme of mucoadhesive NP, a recent innovative study has proposed using rapidly degrading poly(sebacic anhydride) (PSA) NP (250 nm) for immediate nasal delivery of thyrotropin-releasing hormone (TRH), a hydrophilic therapeutic peptide employed for treating suicidal patients [47]. Interestingly, the NP showed a burst-type release, where over 50% of the drug was delivered after 30 min. *In vivo* studies proved that IN delivery of PSA NP loaded with thyrotropin-releasing hormone did not provoke significant adverse side effects or abnormal behaviour in cynomolgus monkeys after 28 days of treatment.

Recently, several works have explored the integration of polymer NP within *in situ* gelling systems obtained from thermoresponsive (Poloxamer, chitosan, ethyl(hydroxyethyl) cellulose, and xyloglucan), pH-responsive (Carbopol®), and ion-responsive (gellan gum, and pectin) polymers. An interesting review article summarizing these systems has been recently published [48]; therefore, this approach will not be addressed in the present work.

3.1.3. *Bioconjugated polymer nanoparticles*

The use of biorecognition ligands to boost the nose-to-brain transport of polymer nanocarriers has been explored in the last years. Wheat germ agglutinin (WGA) and Solanum tuberosum lectin have proved to have a high affinity for the N-acetyl-D-glucosamine and sialic acid residues in the olfactory mucosa. Recent research has explored these ligands for the functionalization of NP for nose-to-brain delivery. For example, WGA-modified PEG-PLGA NP, carrying NR2B9c peptide, were designed to treat ischemic stroke [49]. After IN administration, NR2B9c-WGA NP could bypass the BBB and effectively transport the peptide into the brain and neurons. Following this approach, Han et al. have recently developed WGA-modified PEG-PLA NP loading miR132, a microRNA that can relieve the symptoms of Alzheimer's disease [50]. Nasal administration of this nanoformulation overpassed the BBB, considerably increasing the gene bioavailability in the brain.

Solanum tuberosum lectin has also been conjugated to PLGA [51] and PEG-PLGA [52] NP, showing excellent brain targeting. Haloperidol-loaded functionalized PEG-PLGA NP (<135 nm) were used to treat schizophrenia, and their IN administration amplified the drug concentration in the brain tissue from 1.5 to 3-fold as opposed to unmodified NP and other routes of administration [52].

Although less explored so far, Ulex europeus agglutinin I is another lectin that has proved to specifically bind to L-fucose, highly expressed

in the olfactory epithelium. Thus, Jiang et al. employed this lectin to modify PEG-PLA NP carrying 6-coumarin, a fluorescent marker. The conjugation of Ulex europeus agglutinin I increased the brain-targeting ability of NP, which were accumulated mainly in the olfactory region [53].

Lectins seem to be an interesting option for brain targeting; however, their potential immunotoxicity has been discussed by several authors. As an alternative, shorter peptides possessing lectin-like capabilities, such as odorranalectin (OL, 1.7 kDa) secreted from frog skin, have been proposed as a biorecognition ligand of L-fucose. Lai et al. reported the conjugation of OL to PEG-PGLA NP encapsulating urocortin peptide to treat Parkinson's disease [54]. The NP were labeled with a fluorescent dye (DiR iodide), and their biodistribution after IN administration to a mouse model was evaluated. As shown in Fig. 4A and 4B, fluorescence images demonstrated that OL-NP accumulated preferentially in the brain region after 8 h. In addition, *ex vivo* results (Fig. 4C) of the fluorescence intensity of OL-NP in the brain were more substantial than unmodified NP. Moreover, intranasally administrated OL-NP effectively increased the brain uptake and improved the neuroprotective outcome of urocortin peptides in hemiparkinsonian rat models.

Lactoferrin (Lf) and transferrin are other important biorecognition ligands, as overexpression of their receptors (LfR and TfR) are found in neurons and brain endothelial cells forming the BBB. Chen et al. examined the nose-to-brain transport of Lf-modified PEG-PCL NP, carrying a neuroprotective octapeptide (NAP), for treating Alzheimer's disease in an animal model [55]. The authors performed *in vivo* biodistribution studies using fluorescent NP in rats, finding that Lf-modified NP showed a faster accumulation in various regions of the brain in contrast to the unmodified NP. In another study, PLGA NP were coated with (Lf)-conjugated mucoadhesive N-trimethylated chitosan (Lf-TMC) for IN delivery of Huperzine A, a reversible inhibitor of acetylcholinesterase used for Alzheimer's disease treatment [56]. *In vitro* mucin adsorption of the nanosystems demonstrated that Lf-TMC NP possesses high mucoadhesion and increased cellular uptake over native PLGA NP. Besides, the combined active targeting and mucoadhesion ability of the Lf-TMC formulation led to a high NP distribution in the brain over a long time. Lf-conjugated PEG-PLGA NP (120 nm) were also prepared for IN delivery of rotigotine for Parkinson's disease treatment [57]. The authors found that the conjugation of Lf promoted rotigotine accumulation in the OB, striatum, and cerebellum.

On the other hand, although transferrin-decorated NP have been extensively investigated for brain targeting *via* intravenous administration, the nose-to-brain delivery of these functional nanocarriers has been overlooked [58].

3.1.4. Cell-penetrating polymer nanoparticles

CPP are positively charged oligopeptides, rich in arginine and lysine residues, characterized by an extraordinary capacity to interact with biological membranes. Cellular uptake of biomacromolecules has been demonstrated to be favourable, either by direct cytoplasmic translocation or through endocytic pathways [23,24]. Recent advances in nose-to-brain drug delivery have evidenced the capability of such peptides to considerably enhance the infiltration of many nanocarriers across the olfactory epithelium [35]. The transactivator of transcription (TAT) peptide of the human immunodeficiency virus is perhaps the most popular CPP applied in nose-to-brain delivery. Guanidinium groups of arginine are responsible for their invasive capability, permitting hydrogen bonding and electrostatic interactions with the cell surface. The group of Prof. Takanori Kanazawa, has explored several polymer nanoformulations modified with TAT for treating brain diseases *via* IN administration [59–62]. For instance, TAT-conjugated PEG-PCL nanomicelles (60-100 nm) were designed to deliver small interfering RNA

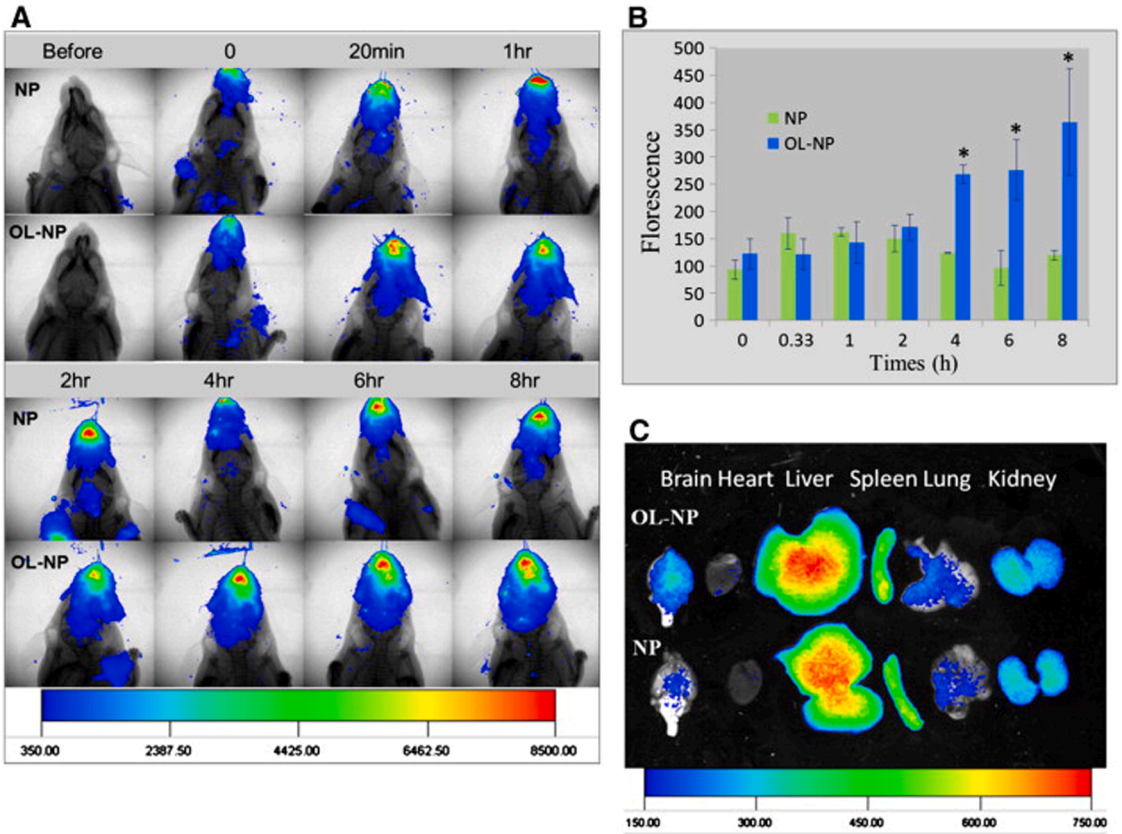

Fig. 4. (**A**) Biodistribution study in mice following intranasal (IN) administration of DiR-labeled nanoparticles (NP) and odorranalectin nanoparticles (OL-NP) over time. (**B**) Fluorescence intensity values in the brain region. (**C**) Bioacummulation analysis in different organs 8 h after fluorescent NP and OL-NP IN administration. Reprinted from Ref. [54] Copyright® (2011), with permission from Elsevier.

(siRNA) targeting tumour necrosis factor-alpha (TNF-α), an inflammatory cytokine, the hallmark of cerebral ischemia [63]. Recent advances in this approach have focused on combing membrane penetration and brain targeting in an all-in-one nanoplatform. Kanazawa et al. also used PEG-PCL-TAT together with stearoyl-modified bombesin to prepare tumour-selective polymer micelles (70-90 nm) loaded with camptothecin for glioma treatment [30]. Bombesin is a linear oligopeptide (14 amino acids) that binds specifically to the gastrin-releasing peptide receptor [64]. Upregulation of such a receptor on cell membranes is prevalent in several tumours encompassing prostate, brain (glioblastoma), breast, pancreatic, colon, and lung cancers compared with healthy tissue [65].

3.2. Lipid-based nanocarriers

Lipid-based nanocarriers are drug delivery systems formed from lipid and an aqueous phase, stabilised by surfactants. These systems are composed of biocompatible and biodegradable components and provide superiority concerning controlled release, drug protection, drug loading, stability, and surface versatility. In turn, lipid-based nanocarriers have a high potential to enhance brain drug delivery after their IN administration mainly due to their capacity to penetrate biological membranes as well as promote the partitioning of nanosized droplets in the nasal mucosa, resulting in an increased residence time.

In this way, an assortment of lipid-based nanocarriers has shown increased bioavailability of several drugs by nose-to-brain delivery, such as liposomes, nanoemulsions, lipid nanocapsules (LNC), solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC), which can be attributed to their small sizes and the presence of emulsifiers (Fig. 5). Liposomes and SLN were the oldest examined for nose-to-brain delivery, while many formulations of NLC have been reported for this route in recent years. Besides, the modification of lipid-based nanocarriers with mucoadhesive agents or their incorporation in mucoadhesive-based formulations has demonstrated promising results, suggesting a better contact with nasal mucosa and the potential for extended permanence in the nasal cavity and enhanced permeation rate. The latest relevant advances reported in the literature about lipid-based nanocarriers are summarized in Table 2 and described in the following sections.

3.2.1. Nanovesicular particles

Among the nanovesicular particles, classic liposomes were the most represented class, which are biocompatible and biodegradable vesicles of spherical shape consisting of one or more lipid bilayers mainly composed of amphipathic phospholipids, bearing a non-polar tail and polar head, and enclosing an interior aqueous[89]. Several variations of these particles have been developed, such as transfersomes, niosomes, and so on [19]. Over the last few years, several vesicular systems have been studied as vehicles for IN delivery of drugs achieving brain targeting, mainly evidenced by pharmacokinetics or efficacy studies. For instance, the IN administration of nanovesicular particles loaded with tramadol showed a significantly increased antinociceptive effect and a quick onset of action in a mice model compared to other nasal formulations and oral drug administration supported by high drug levels in plasma and brain. In addition, a pharmacokinetic study in sheep demonstrated a higher bioavailability after nasal administration of a nanovesicular system than IV route [66]. In another study, an IN formulation of niosomes loaded with pentamidine and coated with chitosan has been developed to explore a novel pharmacological approach to treat Parkinson's disease. The IN administration of this drug delivery system showed amelioration of motor performances in parkinsonism induced by subchronic intoxication with a pro-neurotoxin in C57BL-6J mice and a significant decrease in the glial-associated neuroinflammation by the reduction of specific gliotic markers in nigrostriatal areas, producing an improvement in parkinsonian motor dysfunctions [67].

Recently, IN administered rivastigmine liposomes developed to treat Alzheimer's disease showed significantly improved pharmacokinetics compared to the nasal rivastigmine free solution, nasal rivastigmine polymer NP, and oral drug solution [90]. Specifically, the nasal liposomal formulation exhibited higher C_{max}, improved systemic bioavailability, increased half-life, and reduced clearance rate in comparison to oral free drug solution. Moreover, nasal liposomal formulation reversed the memory deficit in *in vivo* models either induced by scopolamine or colchicine; and revealed a high correlation of acetylcholinesterase inhibition with pharmacokinetic values [68].

Nasr et al. studied an innovative composite composed of a hybrid microemulsion/vesicular system and other vesicular formulations loaded with piracetam and vinpocetine, which enabled the *ex vivo*

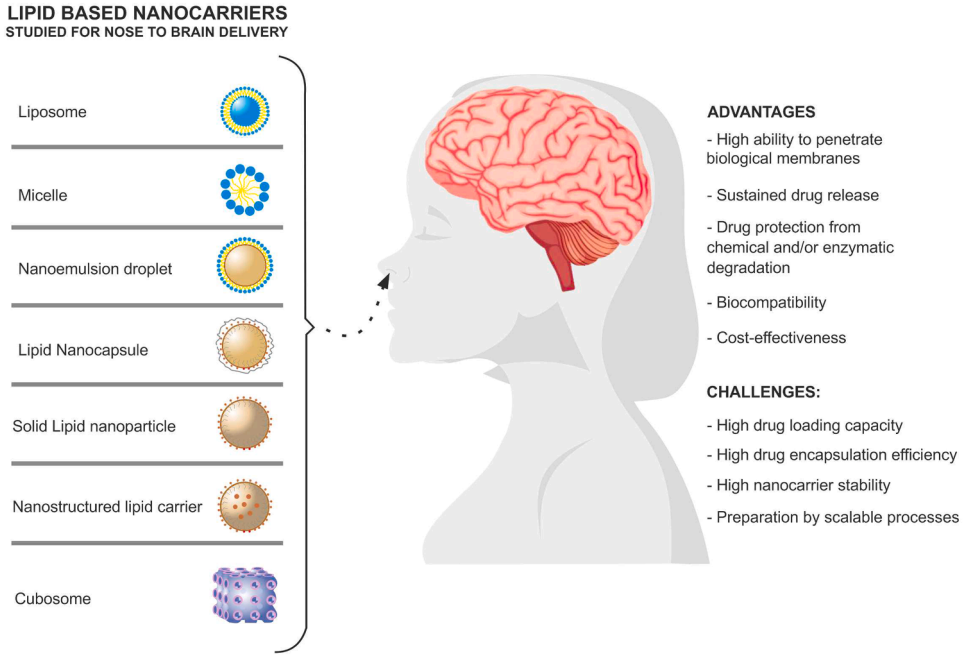

Fig. 5. Schematic representation of lipid-based nanocarriers studied for nose-to-brain drug delivery.

Table 2
Summary of recent reports describing the use of lipid-based nanocarriers developed for brain drug delivery *via* intranasal administration.

Lipid Nanocarrier	Drug	Disease	Particle size (nm)	PDI	Zeta potential (mV)	Lipid excipients/ Emulsifiers/Other excipients	Main findings	Refs.
Multilamellar vesicles	Tramadol	Acute and chronic pain	167	0.400	n.r.	Phospholipon® 90G/ Propylene glycol/Vitamin E	Enhanced bioavailability	[66]
Chitosan-coated niosomes	Pentamidine	Parkinson's disease	300	0.200	+39	DCP/Cholesterol/ Tween® 20/Chitosan	Effective in a mice model of Parkinson's disease	[67]
Liposomes	Rivastigmine	Alzheimer's disease	10000	n.r.	n.r.	Soya lecithin/Cholesterol	Enhanced pharmacokinetic: rapid absorption, enhanced systemic bioavailability, and half-life	[68]
Composite system based on a hybrid of the microemulsion/ vesicular system	Piracetam and vinpocetine	Alzheimer's disease	113	0.270	-15	Oleic acid/ Tween® 20/ phosphatidylcholine	High *ex vivo* permeation of both drug	[69]
Liposomes	Cholesterol	Huntington's disease	nr	n.r.	n.r.	Phosphatidylcholine	Brain targeting	[70]
SLN	Efavirenz	HIV	108	0.172	-21	Glyceryl tripalmitate/ Poloxamer 188	150 times increase brain targeting efficiency	[71]
SLN	Conjugate of geraniol with ursodeoxycholic acid	Parkinson's disease	121	0.164	-22	Compritol® ATO 888/ Span® 85/ Tween® 80	Induction of the prodrug permeation from nose to CSF of rats	[72]
SLN in mucoadhesive *in situ* gel	Levofloxacin and doxycycline	Meningitis	29	~0.200	n.r.	Stearic acid/ Compritol® 888 ATO/ Span® 60/ HPMC/ mucoadhesive polymer	Improved drug delivery to the brain after IN administration Higher drug concentration in the brain that drug free solution after IN administration	[73]
SLN in mucoadhesive *in situ* gel	Almotriptan malate	Migraine headache	208	0.400	-24	Phospholipon® 90H/ Precirol®/ Polyvinyl alcohol P / Tween® 80/ Poloxamer 407/Na-CMC	Enhanced drug distribution to the brain following IN administration	[74]
SLN in mucoadhesive *in situ* gel	Paeonol	Central neurodegenerative diseases	167	0.241	-9	Glycerine monostearate/ soybean Lecithin/ Poloxamer/ Tween® 80/ Deacetylated gellan gum/ HPMC	High brain targeting	[75]
NLC	Sumatriptan	Migraine headache	101	0.270	-32	Stearic acid/ Cholesterol/ Triolein/ Brij® 35	3 times increase of absolute brain bioavailability	[76]
NLC	Nicergoline	Dementia	111	0.251	-15	Precirol® ATO 5/Sesame oil/ Tween® 80	1.60-fold and 4.57-fold rise in the brain concentration that IV administration of the same formulation and IN drug solution, respectively	[77]
NLC	Ketoconazole	Meningoencephalitis	102	0.195	-2	Compritol® 888 ATO/ Miglyol 812 N/ Solutol® HS 15/ Tween® 80	High brain targeting	[78]
NLC	Pioglitazone	Alzheimer's disease	211	0.257	+15	Capmul MCM and tripalmitin/ Tween® 80/ Pluronic F68/ Stearyl amine (positive charge inducer)	Enhanced drug permeability across the nasal mucosa. Increased drug concentration in the brain	[79]
Chitosan-coated NLC	Almotriptan malate	Migraine	255	0.270	+34	Compritol®, Labrafil® / Tween® 80, Lauroglycol. l Chitosan	Increase of the drug C_{max} in the brain.	[80]
Mucoadhesive NLC *in-situ* gel	Olanzapine	Schizophrenia	89	0.310	-23	Compritol® 888/ Labrafil® M 1944 CS/ Gelucire® 44/ 14 / Tween® 80	Enhanced *ex vivo* drug permeation. Increase of drug C_{max} in the brain.	[81]
Mucoadhesive NLC *in-situ* gel	Teriflunomide	Multiple sclerosis	100	0.350	-22	Compritol® 888 ATO, maisine 35-1, Gelucire® 44/14/ Tween® 20. Poloxamer 407, HPMC.	Enhanced *ex vivo* drug permeation	[82]
Mucoadhesive NLC *in-situ* gel	Teriflunomide	Glioma	118	0.560	-22	Glyceryl dibehenate/ glyceryl mono-linoleate/ Gelucire® 44/14/ Gellan gum/ Carbopol 974P	Enhanced *ex vivo* drug permeation Increase of drug C_{max} in the brain High brain targeting	[83]
NLC *in-situ* gel	Flibanserin	Hypoactive sexual desire disorder	115	0.241	+8	Glyceryl behenate/ sweet almond oil/ L-phosphatidylcholine/ Gelucire® 44/14/ Gellan gum.	A 6-fold increase of drug concentration in the brain	[84]
NLC *in-situ* gel	Lorazepam	Epilepsy	72	0.210	-20		Improved efficacy	[85]

(*continued on next page*)

Table 2 (*continued*)

Lipid Nanocarrier	Drug	Disease	Particle size (nm)	PDI	Zeta potential (mV)	Lipid excipients/ Emulsifiers/Other excipients	Main findings	Refs.
						Glycerol monostearate, Oleic acid/Poloxamer 407/ βglycerol phosphate/ Chitosan.		
Chitosan-coated LNC	simvastatin	Glioma	185	0.160	+34	Poly (ε-caprolactone, sorbitan monostearate/ caprylic/capric triglyceride/ Lipoid® S75/ Chitosan.	Enhanced *ex-vivo* permeation	[22]
LNC	Nimodipine	Acute subarachnoid haemorrhage	36	0.146	-14	Labrafac/Lipoid®S75, Solutol® HS15.	Same drug brain targeting with a lower drug concentration in blood	[86]
Cubosomal thermogelling formulation	Saquinavir	HIV	120	n.r.	n.r.	Onoolein/ Poloxamer 407/ polyvinyl alcohol	Enhanced bioavailability	[87]
Cubosomal mucoadhesive *in situ* nasal gel	Donepezil	Alzheimer's disease	137-231	0.380-0.480	-40	Glycerol mono-oleate/ surfactant Poloxamer 407/ Gellan gum/ Konjac gum	Enhanced bioavailability	[88]

DCP, Dicetyl phosphate; HIV, human immunodeficiency virus; HPMC, hydroxypropyl methyl cellulose; IN, Intranasal; LNC, Lipid nanocapsules, Na-CMC, sodium carboxy methyl cellulose, NLC, Nanostructured lipid carriers; SLN, Solid lipid nanoparticles.

permeation of both active molecules across the nasal mucosa. In addition, formulations containing Polysorbate 20 (Tween® 20) showed an increased release of both drugs and may reduce the mucus viscosity due to a surfactant effect. Furthermore, the administration of the hybrid system *via* the IN route reversed the scopolamine-induced effect in a memory impairment model in rats, reduced the acetylcholinesterase activity and lipid peroxidation, and presented the highest anti-inflammatory and antiapoptotic effects [69].

Another study explored the IN administration of deuterium-labelled cholesterol liposomes as a non-invasive strategy for cholesterol delivery to the brain to manage Huntington's disease. The IN administration was effective in delivering the formulation into the brain, reaching measurable drug levels that persisted for at least 72 h. The acute IN administration showed an increase of deuterium-labeled cholesterol liposomes in plasma and the brain areas, from 61% in the cortex up to 105% in the striatum, while chronic administration (10 IN doses) revealed that it was distributed and accumulated without differences in the striatum, cortex, and cerebellum, suggesting that the formulation reached the CNS by both olfactory and trigeminal pathways [70].

3.2.2. Solid lipid nanoparticles

SLN are colloidal systems, useful for controlled drug release and drug protection against chemical degradation [89], consisting of a solid lipid core with a monolayer coating of surfactants [91]. Several IN formulations based on SLN have improved the bioavailability of different drugs in the brain. The latest developments are mainly related to Parkinson's disease, Alzheimer's disease, and HIV infections. SLN have been used to encapsulate efavirenz for HIV infections therapy as a strategy to overcome the low drug bioavailability by oral route, showing 150 times more brain targeting efficiency after their IN administration than a standard oral formulation. These results were related to direct nose-to-brain delivery and formulation features, including lipid content and nanoscale size [71]. In another study, SLN were also used to encapsulate a conjugate of geraniol and ursodeoxycholic acid to treat Parkinson's disease by IN administration. This approach overcame some drawbacks of geraniol, which is poorly soluble in water and muco-irritant and presents a short half-life in the bloodstream after oral administration. Interestingly, the IN administration of this SLN formulation exhibited higher geraniol concentration in the CSF of rats without inducing mucosal irritation, demonstrating its ability to promote the permeation of this molecule from the nasal cavity to the CSF in animal models [72].

The combination of SLN formulations containing emulsifiers with a high permeation effect and mucoadhesive polymers has been explored to enhance the bioavailability of drugs administrated by the nose-to-brain route. Abdel Hady et al. fabricated a mucoadhesive *in situ* gel formulation prepared from hydroxypropyl methyl cellulose (HPMC) containing SLN loaded with levofloxacin and doxycycline, which exhibited a significant rise in the brain peak concentration of both therapeutic agents after IN administration compared with IN free drug solutions. A higher DTE (over 150%) for both drugs than the IV route revealed a more efficient brain targeting for this formulation. The enhanced drug delivery mainly was accounted to the formulations mucoadhesive property provided by HPMC that favours residence in the IN cavity and the presence of lipid components and Span® 60, which may promote the passage through the BBB and act as uptake enhancers, respectively [73]. Another mucoadhesive *in-situ* gel formulation, composed of Poloxamer 407, sodium carboxymethylcellulose, and SLN was developed for brain delivery of almotriptan malate for migraine treatment by IN route, which showed rapid drug accumulation in the brain. Biodistribution studies revealed that IN *in-situ* gel formulation of SLN presented a higher brain/blood drug ratio than IN and IV administration of the drug solution. Moreover, values up to 6 h of the AUC, corresponding to the drug dosage profile in the brain, confirmed the superiority of both IN SLN and IN drug solution with a good targeting efficiency compared to IV drug administration [92].

In another study, the incorporation of paeonol-loaded SLN into an *in-situ* gelling formulation composed of HPMC and deacetylated gellan showed promising results concerning brain delivery of poorly soluble agents *via* the nose-to-brain route. Biodistribution studies revealed that SLN-*in situ* gel loaded with a dye agent was accumulated in the brain after administration across the olfactory area. Furthermore, a fluorescent signal was clearly visualised in the OB, cerebellum, and striatum, indicating that the neural pathway was the main route involved, allowing effective nose-to-brain transport [75].

3.2.3. Nanostructured lipid carriers

NLC are the second generation of lipid NP which present a less organised solid lipid matrix that results from a blend of solid and liquid lipids [93]. They were developed to increase drug loading and prevent drug leakage. In recent years, NLC were perhaps the most studied type of lipid-based carrier utilising the nose-to-brain route for drug delivery, displaying encouraging outcomes. Thus, pharmacokinetic studies revealed a rise in drug concentration within the brain after IN administration of sumatriptan-loaded NLC developed for migraine treatment, paralleled to IV administration of the free drug solution. Besides, the brain concentrations of the drug after IN administration of

sumatriptan-NLC in rats achieved more than 7-fold compared to sumatriptan solution, while plasma concentrations were less than 1.86-fold. The absolute plasma and brain bioavailability for sumatriptan-loaded NLC after their IN administration were 298.84% and 770.77%, resulting in 9 times and 3 times higher than the drug solution, respectively. Moreover, DTE and DTP were 258.02% and 61.23%, respectively [76].

In another study, the IN administration of sesame oil-based NLC loaded with nicergoline revealed a 1.60-fold and 4.57-fold rise in the brain concentration of the drug compared with the administration of an IV equivalent formulation and an IN drug solution, respectively. Moreover, IN sesame oil-based NLC loaded with nicergoline have shown a DTE and DTP of 187.3% and 56.6%, respectively, revealing an improvement in the bioavailability and high performing brain targeting [77]. The authors highlighted that the solubilizing ability of Tween® 80 present in the formulation led to better access to CNS through olfactory neurons, which in turn could inhibit the P-glycoprotein system.

Likewise, an NLC formulation containing Tween® 80 and Kolliphor® HS 15, designed as a strategical treatment of Cryptococcus neoformans-mediated meningoencephalitis, improved the penetration efficiency in capsulated C. neoformans cells, which are responsible for infection dissemination in the brain. The results revealed that fluorescent-dye-loaded NLC were successfully taken up into the cytoplasm of capsulated C. neoformans cells and entered the brain by the OB after 24 h of IN administration. Moreover, NLC facilitated brain dissemination *via* the OB route compared to the free fluorescent dye, showing an early NLC accumulation in the OB after 24 h, followed by continuous diffusion into the cerebrum, the pineal gland, and the cerebellum region after 120 h (Fig. 6). Furthermore, NLC loaded with ketoconazole showed increased antifungal *in vitro* activity against C. neoformans compared to free drug solutions and higher antifungal activity in mouse tissue after IN administration [78].

Nanocarriers with a positive surface charge have also been explored to improve the bioavailability of the dosage forms from the nasal mucosa and overcome the mucociliary clearance by interacting with the negatively charged mucus surface. A formulation of pioglitazone-loaded NLC containing stearyl amine as a positive charge inducer displayed a higher *ex-vivo* permeation rate across sheep nasal mucosa from NLC than the drug solution and a significant enhancement regarding the drug amount reaching the brain after IN administration. The authors suggested that positive surface potential might favour electrostatic interaction with the nasal mucosa and minimize drug loss *via* mucociliary drainage, thus improving the drug bioavailability [79].

In addition, the combination of NLC with mucoadhesive agents has demonstrated encouraging results, suggesting a better contact with nasal mucosa, prolonged retention time at the nasal cavity, and an enhanced permeation rate. Salem et al. developed mucoadhesive chitosan-coated NLC delivering almotriptan maleate to the brain through the nasal route, which displayed enhanced *ex-vivo* permeability across sheep nasal mucosa. Furthermore, IN administration of this formulation in albino rabbits demonstrated significantly higher brain C_{max} than a commercial oral drug product and IN drug solution [80].

The incorporation of NLC in gels has also been evaluated to optimize the nose-to-brain delivery of different agents. Gadhave et al. designed mucoadhesive formulations of olanzapine-loaded NLC prepared from Carbopol 974P and the combination of Poloxamer 407 and HPMC for IN administration, aiming to decrease the drug blood concentration and, consequently, the risk of agranulocytosis associated with the antipsychotic agent. Mucoadhesive formulations showed a higher *ex vivo* permeation through sheep nasal mucosa than olanzapine-loaded NLC without mucoadhesive properties, which was related to the ability of the

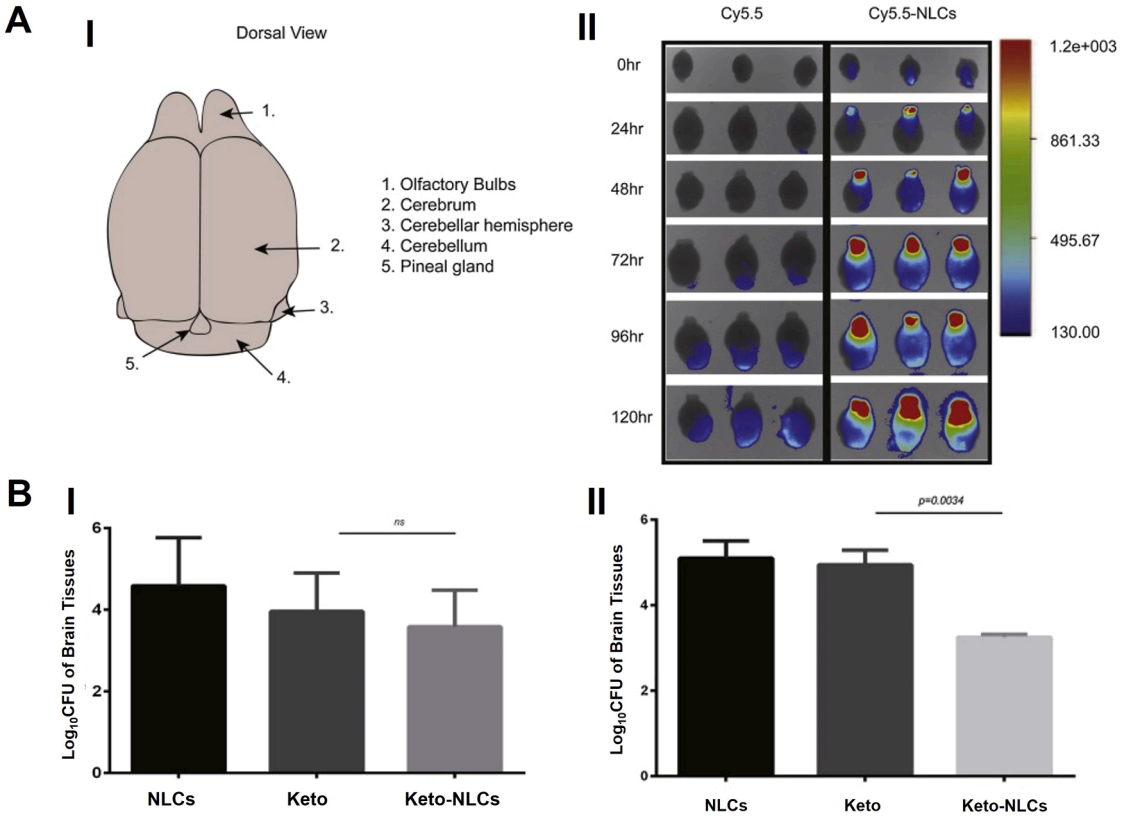

Fig. 6. (**A**) I-Schematic dorsal view of mice' brain. II- Imaging analysis of brain isolated from mice after IN administration of 50 μL of a 20-μg/mL solution of either Nanostructured lipid carriers (NLCs) or cyanine 5.5- Nanostructured lipid carriers (Cy5.5-NLCs). (**B**) Ketoconazole- NLC used intranasally significantly inhibit cerebral Cryptococcus neoformans proliferation in mouse brain tissue. Numbers of colony-forming units in brain tissues of mice isolated 5 days post-infection: I- intraperitoneally treated with nanostructured lipid nanocarriers (NLC), drug-free solution (keto), or ketoconazole- loaded NLC (keto-NLCs). (**D**) intranasally treated NLC, keto, keto-NLC. Reproduced with permission from Ref. [78] Copyright® (2020), Elsevier.

lipid NP to facilitate the permeability of lipophilic drug molecules and the mucoadhesive polymers to interact with nasal mucosa. In addition, mucoadhesive NP with Poloxamer 407 and HPMC showed better drug permeation and a higher brain C_{max} after IN administration compared to olanzapine-loaded NLC after IV administration and did not show any alteration in the haematological parameters [81]. In a follow-up study, the authors developed a similar mucoadhesive formulation of NLC loaded with the remyelinating agent teriflunomide intended for treating multiple sclerosis via IN administration. A combination of HPMC and Poloxamer 407 as a mucoadhesive agent and a gelling agent, respectively, was used. This formulation exhibited high mucoadhesive force, suggesting potentially prolonged retention in the nasal cavity. Furthermore, this *in-situ* gelling system exhibited rapid drug release and a higher permeation rate across the sheep nasal mucosa than NLC formulation without mucoadhesive polymers. *In vivo* assays revealed that the IN administration of the mucoadhesive formulation was safe and effective for remyelination in cuprizone-treated animals, rapidly reaching the target site and reducing the neuroinflammatory response compared with a negative control and the oral NLC formulation without mucoadhesive polymers [82].

Following this approach, a teriflunomide-loaded nanolipid-based *in-situ* gel was fabricated from gellan gum and Carbopol® 974P as gelling and mucoadhesive agents, respectively. This strategy achieved NLC (118 nm) with high mucoadhesive strength, higher *ex vivo* permeation across sheep nasal mucosa than raw teriflunomide-loaded NLC, and significantly suppressed the progress of malignant glioma cells. At the same time, it was nontoxic to normal healthy cells. Interestingly, technetium-labeled IN NLC-gel formulation showed 2-fold higher C_{max} in the brain than teriflunomide-loaded NLC after their IN and IV administration, which could be related to the combination of the surfactant with the natural gelling polymer, improving the drug permeability. Furthermore, biodistribution studies demonstrate quick delivery of the novel formulation to the brain, which has the potential as a safe and effective treatment for glioma, providing a reduced risk of liver and kidney toxicity [83].

Similarly, an optimized NLC formulation of flibanserin incorporated into gellan gum *in-situ* gel exhibited a 6-fold increase in the brain's drug concentration after IN administration in rats compared to the free drug-containing *in situ* gel. These results have been mainly associated with the nanosized and lipid nature of the carrier, which enabled an improvement in the drug solubility and permeability, thereby ameliorating its passage directly across the BBB *via* the nasal olfactory area. Moreover, gelling properties of gellan gum favour the reduction in the mucociliary clearance of the formulation [84].

Another formulation of thermosensitive *in-situ* gel containing a mix of chitosan and β-glycerol phosphate, and integrating lorazepam-loaded NLC, showed promising results after administration by the nose-to-brain route. The IN administration of this formulation achieved a significant reduction of pentylenetetrazole-induced seizures in rats or diminished the seizure intensity compared with a control group and those treated with an *in-situ gel* containing the free drug, both by IN route; suggesting that the incorporation of NLC into *in situ* gels could improve the therapeutic drug effect. Moreover, the authors related these outcomes with the small size of NLC, which might allow their transcellular passage by olfactory neurons towards the brain *via* endocytic pathways of neuronal cells [85].

3.2.4. Lipid nanocapsules and cubosomes

LNC and cubosomes have recently been designed for IN applications to deliver drugs into the brain. Pharmacokinetics studies showed that IN nimedipine-loaded LNC (36 nm) could deliver an equivalent dose of the therapeutic agent to the desired site compared to IV administration of the drug solution in Wistar rats, minimizing the potential drug side effects. Moreover, high brain/plasma concentration ratio values following IN nimedipine-loaded LNC suggested that LNC could enhance the permeability through the BBB and deliver it into the brain tissues [86].

As for cubosomes, Hosny et al. developed an IN cubosomal thermogelling dispersion of saquinavir for brain delivery, which exhibited enhanced *ex vivo* permeation compared to an aqueous drug suspension. In addition, a pharmacokinetic study performed in albino rabbits showed that IN cubosomal thermogelling dispersion allowed an increase in the relative bioavailability 12-fold and 2.5-fold higher than the oral and IN aqueous drug suspension, respectively [87]. In another study, a cubosomal mucoadhesive *in-situ* nasal gel was designed for donepezil brain delivery, which displayed a higher C_{max} than unmodified cubosomal formulation and the drug solution. These results suggested that cubosomal mucoadhesive *in-situ* nasal gel presented enhanced bioavailability, which was attributed to the prolonged interaction of the formulation and the nasal mucosa after gelation of gellan gum [88].

Finally, it should be noted that recent studies have advanced in the functionalization of lipid nanoparticles with biorecognition ligands. Although IN administration studies have not yet been performed, *in vitro* results are very promising. A recent study explored the functionalization of SNL and NLC with the biorecognition ligand transferrin to mediate these particles' transport through the BBB, showing a 1.5-fold higher permeation of drug loaded in the nanocarriers in permeability studies using transwell devices with hCMEC/D3 cells monolayers [94]. In another example, cannabidiol was evaluated as a brain-targeting molecule of LNC. Results in one brain cell line (hCMEC/D3) consistently demonstrated a significantly greater BBB-targeting effect for CBD-decorated LNCs than unmodified LNCs of equal size. Moreover, the cellular uptake results were consistent with the permeability experiments: permeability coefficients across the endothelial monolayer were significantly higher for smaller LNC and CBD-decorated LNC [95].

3.3. Inorganic nanoparticles

The versatility of the nose-to-brain administration route was also explored using inorganic NP. These systems have a great variety of compositions (silver, gold, iron oxide, silica, graphene, etc.) and shapes (rods, prisms, spheres, stars, etc.). They are an ideal alternative for designing smart carriers for drug delivery, principally owing to their high chemical reactivity, allowing them to be combined with chemical or biological materials that add new functions for drug delivery, bioimaging, photothermal therapy, diagnosis of diseases, among other biomedical purposes (Fig. 7) [96]. Several authors evaluated different strategies to understand how to transport inorganic nanoparticles to the brain efficiently. For this purpose, studies have been carried out that compare the size and shape of nanoparticles, the use of external stimuli (ultrasound, magnetic fields), and the use of peptides, among others. Therefore, for a deeper understanding of the potential application of these systems, the following section will summarize recent works addressing the transport of the most relevant inorganic NP by the nose-to-brain route (Table 3).

3.3.1. Gold nanoparticles

Gold NP (GNP) have demonstrated a potential application for managing and diagnosing Alzheimer's disease and parkinsonism, among other CNS conditions [104]. Nevertheless, the BBB limits their accumulation in CNS after IV injection [105]. Currently, the IN route has been recommended for circumventing this barrier, but different physicochemical features of GNP will disturb the transport of nanomaterials to CNS from the nose. Then, the analysis of NP of different sizes and shapes may help understand this process. Gallardo-Toledo et al. reported the use of gold nanospheres (GNS) (47 nm) and nanoprisms (GNPr) (78 nm) with similar surface areas to assess their transport to the brain after IN administration. The authors used PEG and D1 peptide (GNS-D1 and GNPr-D1) as functionalizing agents [97]. The results exposed that even though both NP had comparable surface area, size, and zeta potential, their different shapes considerably influenced their translocation to the brain from the nasal cavity, being more substantial for nanospheres (GNS-D1) (Fig. 8). The concentration of gold located in the brain after IN

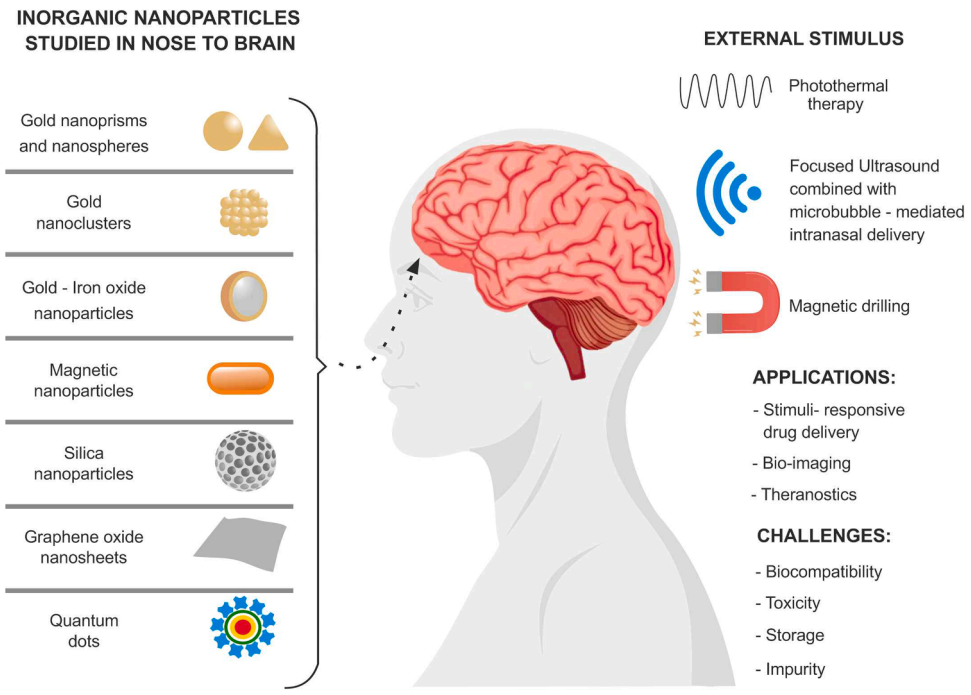

Fig. 7. Inorganic nanoparticles studied for drug delivery applying the nose-to-brain route.

Table 3
Summary of recent reports describing the use of inorganic NP to achieve drug accumulation in the brain.

Carrier composition	Functionalization agent	Drug	Disease	Particle size (nm)	PDI	Zeta Potential (mV)	Main findings	Refs.
GNPr and GNS	PEG and D1 peptide	n.r.	Alzheimer	GNPr 78 GNS 47	n.r.	GNPr -25 GNS -31	Higher gold concentrations by IN route (55 times) in comparison to IV.	[97]
^{64}Cu-alloyed AuNC	n.r.	n.r.	-	5	n.r.	n.r.	Lower ^{64}Cu-AuNC accumulation in organs (liver, spleen, lungs, kidney, blood, and heart) induced by IN administration in comparison to IV administration. FUSIN improved targeting toward FUS-targeted brain region.	[98]
Gold/iron oxide NP	β-cyclodextrin-chitosan (CD-CS) hybrid polymer and peptide PEG-T7	miRNAs and TMZ	Glioblastoma	53	n.r.	+4	Efficient translocation of miRNAs in treated mice and significantly increased their survival plus systemic cotreatment with TMZ.	[99]
Magnetic nanorods	n.r.	n.r.	-	250	n.r.	n.r.	Helical dynamic gradients improved transportation of NP to the brain in comparison to linear magnetic gradients.	[100]
MSN	n.r.	Chrysin and Curcumin	Oxidative CNS disorders	220	0.130	+24	pH-dependent release of phytochemicals from MSNP. NP with sizes under 500 nm would be able to be taken up through olfactory cells.	[101]
GO nanosheets	n.r.	n.r.	-	10 × 550	n.r.	n.r.	Size-dependent nose-to-brain translocation was observed. The Smallest GO sheet size category was associated with microglia and was preserved over one month (but underwent biodegradation-related changes).	[102]
CdSe/ZnS Quantum dots	PEG, phosphatidyl ethanolamine	n.r.	-	15-20	n.r.	n.r.	Rapid uptake and axonal carriage to the brain. Microglial cells activation (pro-inflammatory response)	[103]

AuNC, gold nanoclusters; GNPr, Gold nanoprisms; GNS, gold nanospheres; IV, Intravenous; IN, Intranasal; MSP, Mesoporous silica nanoparticles, nanoparticles; OB, Olfactory bulb; PEG, Polyethylene glycol, TMZ, Temozolomide.

administration of GNS-D1 was 55 times higher than IV injection. Additionally, authors performed a qualitative analysis of nanoparticle biodistribution (Fig. 9). The results show that there was generally not much difference in biodistribution of gold between the IN and IV routes, except the olfactory bulbs. Authors calculated the percentage of gold loci for each region. It was revealed that a greater percentage of GNS-D1 was observed in the basal forebrain, thalamus, and cerebellum after IV injection rather than after IN administration, whereas a major percentage of the nanoparticles after IN administration was observed in the periaqueductal gray, perirhinal and entorhinal cortex, olfactory bulb, and hippocampus region. Neocortex region showed the highest percentage of gold after either IN or IV administration, with values of 28% and 34%, respectively. Future experiments should be designed to study the distribution pattern in time course, using more advanced technologies, such as computed tomography. Finally, because isolation of the trigeminal nerve was not performed, the extent of NEO-D1 and NPrO-D1 transport through this nerve cannot be estimated and should be investigated in the future.

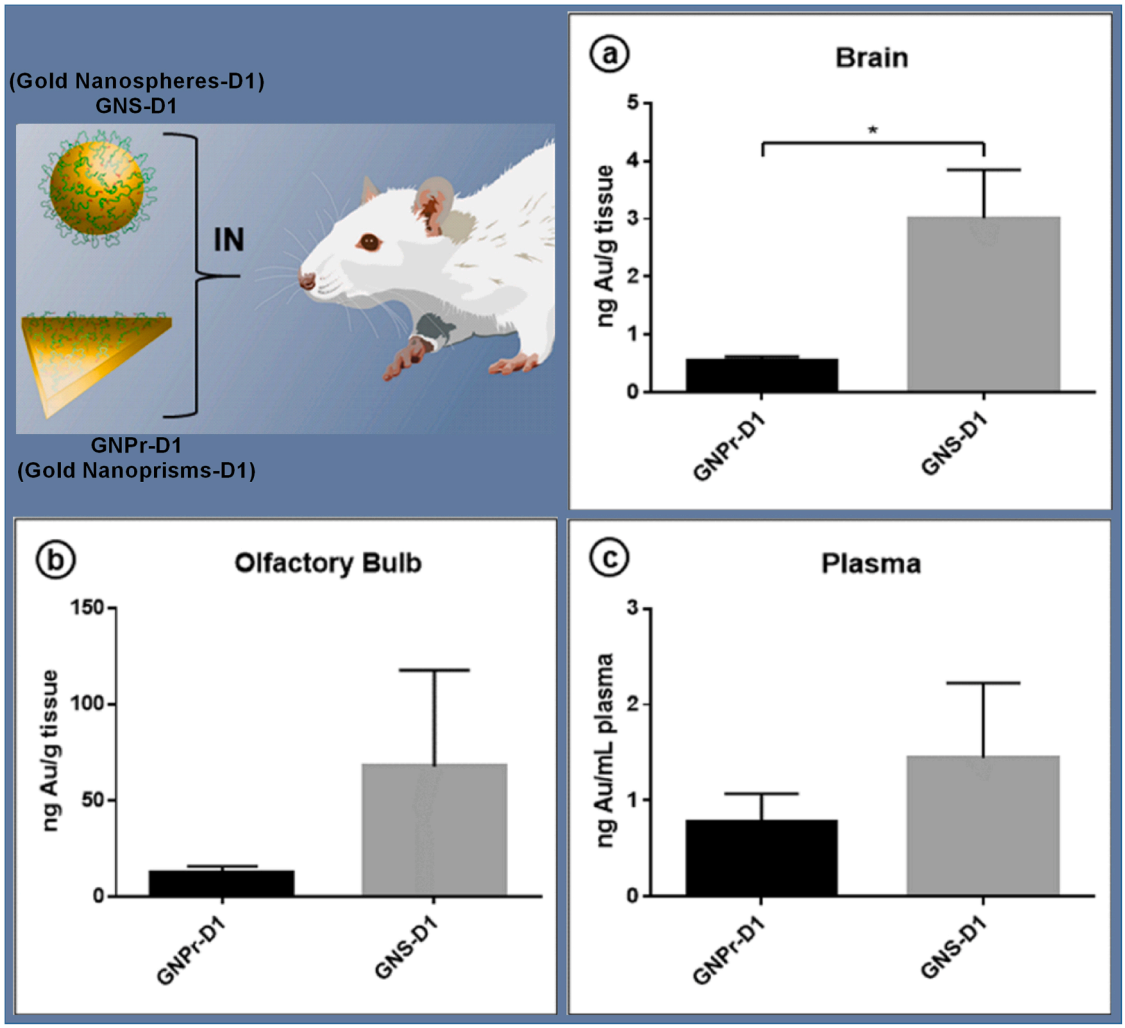

Fig. 8. Gold quantification at 30 min after IN administration of gold nanoprisms functionalized with D1 peptide (GNPr-D1) or gold nanospheres functionalized with D1 peptide (GNS- D1). Brain (a), olfactory bulb (b), and plasma (c) gold concentrations. Reproduced with permission from ref. [97] Copyright® (2020), Elsevier.

A different experiment conducted by Ye et al., investigated the biodistribution of gold nanoclusters (AuNC) (5 nm) after intranasal administration. They assessed the short-term safety and viability of focused ultrasound coupled with microbubble-mediated intranasal delivery (FUSIN) for AuNC release into the brainstem [106]. The experimental results showed a significantly reduced bioaccumulation of ^{64}Cu-AuNC in the liver, spleen, lungs, kidney, blood, and heart due to IN administration compared to IV administration. FUSIN improved the ^{64}Cu-AuNC transportation at the FUS-targeted brain region. No tissue injury was noticed within the brain, nose, or trigeminal nerve. Moreover, FUSIN has shown to be an auspicious non-invasive method to provide safe yet spatially targeted release of gold NP in the brain.

3.3.2. *Gold-Iron oxide nanoparticles*

Sukumar et al. examined the nose-to-brain administration of miRNAs (miR-100 and antimiR-21) surface-loaded in polyfunctional gold-iron oxide NP (polyGION) to treat glioblastoma in mice [99]. The nanocarriers allowed pre-sensitization of glioblastoma cells for the systemically delivered chemotherapeutic drug temozolomide and *in vivo* anatomic and molecular multimodality imaging of NP treatment effects and biodistribution. The GION were formulated and functionalized using a β-cyclodextrin-chitosan (CD-CS) hybrid polymer and co-loaded with miR-100 and antimiR-21. Subsequently, using β-cyclodextrin-adamantane host-guest chemistry, a PEG-T7 peptide was added to the surface of the NP. The resulting polyGION (53 nm) showed improved serum stability and efficient miRNA loading. The U87-MG glioblastoma cell-derived orthotopic xenograft model was used for the *in vivo* IN delivery studies in mice. *In vivo* optical fluorescence imaging along with magnetic resonance imaging (MRI) exhibited effective bioaccumulation of Cy5-miRNAs in animals IN treated with T7-targeted polyGION. Overall, a substantial increase in survival was observed in animals co-treated with T7-polyGION loaded with miR-100/antimiR-21 and systemic temozolomide, compared to temozolomide alone, non-targeted polyGION-miR-100/antimiR-21, or the untreated control group. In conclusion, this innovative IN theranostic nanocarrier could potentially boost temozolomide treatment effects in patients with glioblastoma.

3.3.3. *Magnetic nanoparticles*

Magnetic nanoparticles are used in several biomedical applications such as hyperthermia treatments, magnetic targeting, and as contrast agents in MRI. This NP can be composed of one or more metals with magnetic behaviour, and can have different forms such as core-shell, hollow or solid. Additionally, these particles can be coated with several stabilizing molecules such as mesoporous silica, silicon dioxide, natural or synthetic polymers, and noble metals, including gold or silver [107]. Jafari et al. sought to increase the efficiency of transportation of magnetic particles across the cribriform plate after IN administration to target the brain [100]. Previously, they have revealed that nanoparticles could be focused using remote pulsed magnetic fields [108], without

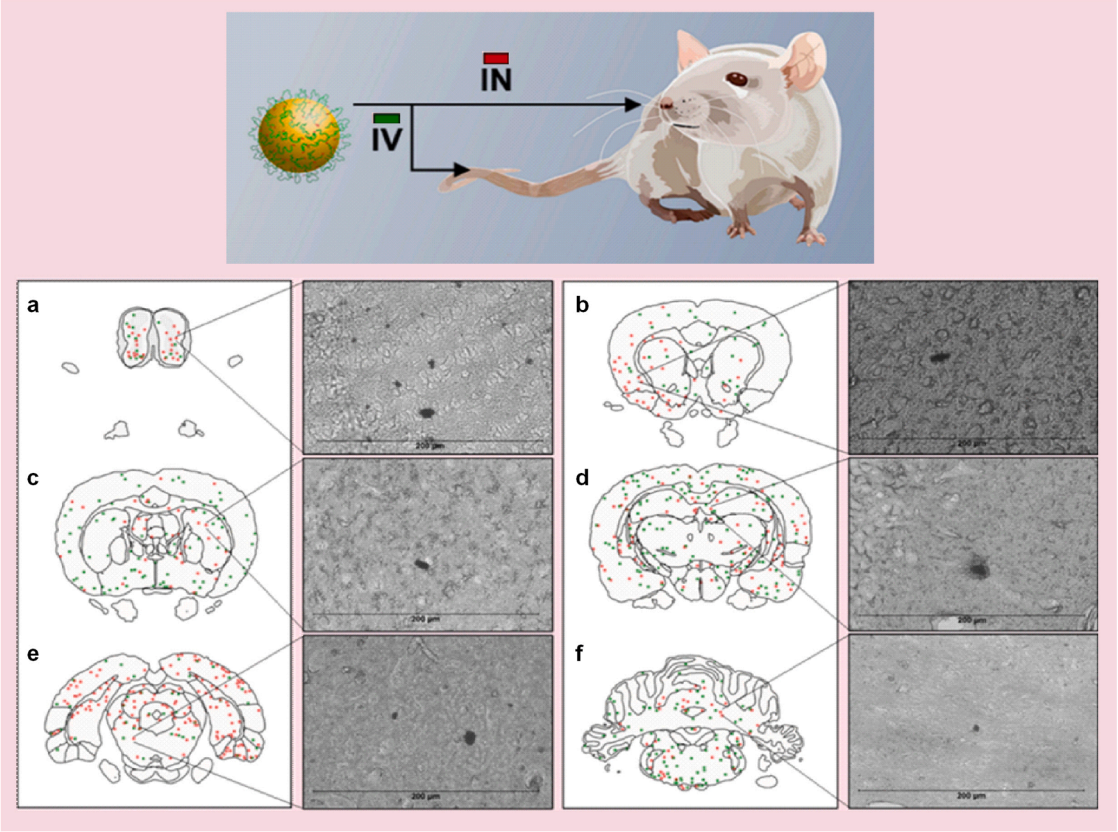

Fig. 9. Biodistribution of GNS-D1 in the brain 45 min following administrations by IN or IV routes: a) +9.18 mm, (b) + 1.99 mm, (c) − 0.2 mm, (d) − 3.32 mm, (e) − 6.76 mm, and (f) − 10.82 mm ant Bregma GoldEnhanceTM was employed to determine and compare GNS-D1 localization using light microscopy. Reproduced with permission from Ref. [97] Copyright® (2020), Elsevier.

affecting neuronal function [109]. In this case, the authors performed experiments combining IN administration of magnetic rods (250 nm) and different combinations of magnetic fields in mouse cadavers. Improved delivery of NP to the brain was shown using linear and rotational helical dynamic gradients instead of just linear magnetic gradients. As stated by histological examinations, no significant damage to the brain structures was evidenced during the transport process [109]. As observed, dynamic helical magnetic fields could improve the transport of magnetic NP by the nose to brain pathway in a cadaveric rodent model. However, more studies should be conducted in live animals to corroborate that no morphological and functional damage occurs in neurons and attached tissues.

3.3.4. *Based on silica nanoparticles*

Nanoparticles based on silica present different conformations, including hollow, solid, and mesoporous. Such NP represent a promising tool for drug delivery due to their biocompatible and non-toxic nature. Particularly, mesoporous silica NP (MSN) have tailored sizes and present high drug loading due to the large surface area of their internal mesopores [110]. Additionally, these NP are valuable carriers for phytochemicals with poor solubility, providing an innovative strategy that can bypass the BBB targeting such molecules in the CNS, as demonstrated by Lungare et al.[111]. These authors developed MSN loaded with chrysin and curcumin to evaluate their use for nose-to-brain delivery. For this purpose, spherical MSN particles (220 nm), with high curcumin and chrysin loading efficiency (11-14% for curcumin and chrysin, respectively), and high surface porosity were developed. This study demonstrated a pH-dependent release of phytochemicals from nanoplatforms and proved that NP below 500 nm could be taken up by the olfactory cells. No toxic effect against olfactory neuroblastoma cells OBGF400 was observed using loaded MSN. Additionally, confocal laser scanning microscopy revealed that NP with sizes less than 500 nm could bioaccumulate within cells with FITC-loaded MSN following 2 h incubation.

3.3.5. *Graphene derivatives*

Graphene and its derivatives have been shown as multipurpose materials in biomedicine, applied for imaging, photodynamic therapy, drug delivery, or theranostics. These materials could be used to develop drug carriers with high loading efficiency and with stimuli response release behaviour (magnetic, temperature, changes in pH) [112]. Due to the potential applications of materials based on graphene oxide (GO), it is critical to understand their interactions with biological systems. Newman et al. explored nanosheets with different controlled lateral dimensions of single- to few-layer GO to study the ranslocation degree to the brain of these NP after IN administration [102]. Using confocal Raman analysis and mass spectrometry, the authors demonstrated that the nose-to-brain translocation of trace amounts of GO is contingent on size, favouring smaller particles. The highest coverage and quantity of transportation to the brain was achieved by GO-sheet (ultrasmall GO-Sheets, 10 × 550 nm). Moreover, an association between ultrasmall-GO with microglia was observed after *in vivo* studies, and trace amounts of ultrasmall-GO were preserved after one month, presenting changes related to biodegradation.

3.3.6. *Quantum dots*

Quantum dots (QD) are semiconductor NP composed of elements from groups III and V, II and VI, or IV and VI. These NP have shown outstanding optoelectronic properties to be applied in biomedicine. Particularly, these particles could be loaded with drugs and targeting agents and used to develop theranostic tools for treating and diagnosis diseases due to their fluorescence properties that allow determinating

their biodistribution. Since QD are usually made of cadmium, an element with known neurological effects, Hopkins et al. assessed the effect of inhalation of CdSe/ZnS QD in adult C57BL/6 mice, evaluating the nose to brain transport of these particles [103]. For this purpose, the animals were exposed for one hour to aerosolized QD (15 nm), and NP were sensed in the olfactory tract and OB three hours post-exposure using several techniques, such as visualization *via* transmission electron microscopy and fluorescence. After short-term administration of this NP, the authors observed a fast olfactory absorption and axonal transport to the brain/OB. Additionally, a pro-inflammatory response was observed as microglial cells were activated. As demonstrated, nose-to-brain transport through the olfactory sensory neurons when inhaled is achievable, but a more extended period is needed to detect their potential transport to other brain areas. Moreover, additional investigation of *in vivo* inhalation is required to evaluate the potential neurological effects as well as the toxicity of these particles on the brain.

3.4. Drug nanocrystals

The production of drug nanocrystals (NC) is one of the most favoured strategies for the delivery of poorly soluble drugs. NC are, in essence, crystalline NP (<1 μm) formed only by the drug and stabilized by a surfactant layer [113]. Drug NC are frequently produced in a liquid suspension, with the final product commonly known as nanosuspensions (NS), whereas solvent removal by freeze- or spray-drying leads to obtaining solid re-dispersible materials. When drug particles are comminuted to the nanometre range, the specific surface area exposed to solvents is enormously enlarged, leading to an increased dissolution rate, saturation solubility, and bio-adhesion [114]. NC were firstly developed in the early 1990s with the aim of increasing the bioavailability of low aqueous soluble drugs when administered orally, leading to the FDA approval of five NC-based products between 2000 and 2005. Unlike other nanoparticle-based drug delivery systems, i.e., lipid-based NP or polymer NP, NC possess multiple distinctive benefits namely, higher drug loading, increased long-term stability, feasible organic solvent-free and neutral pH manufacture, and scalable manufacturing techniques (Fig. 10). All these aspects make of drug NC an attractive platform for tissue targeting, which is supported by a large plethora of reports where NC have been used for drug accumulation in specific tissues, including the CNS *via* the nose-to-brain route [115,116].

When administered intranasally, NC can promote drug permeation across the mucosal barrier prolonging the residency of particles in the mucus and the contact area available. Crucially, it has been reported that drug NCs can remain as undissolved drug particles in the body for days [117], yet this will be strongly dependent on the administration route, size and shape of the NC, and physicochemical nature of the drug [118]. Given the low volume of dissolution media available in the nasal cavity, the NC dissolve slowly, allowing for time for the particulate drug to penetrate to the CNS before dissolution. Table 4 shows a series of reports describing the use of NC-based formulations administered intranasally for brain targeting.

In 2016, Hao et al., described the formulation of resveratrol NC loaded into ionic-sensitive *in situ* gel for IN delivery [119]. The authors obtained a NS with a particle size of 241 nm using a precipitation method followed by solidification by freeze-drying. The NC were subsequently loaded into deacetylated gellan gum enabling production of an *in situ* gel-forming formulation. Pharmacokinetic experiments in mice demonstrated that the IN formulation exhibited significantly larger concentrations of the drug in the brain when paralleled to an IV administration. Moreover, the plasma concentrations versus time curves for the IV and IN formulations presented similar profiles, with no significant differences found between these groups. In a different study, the antipsychotic drug zotepine was delivered intranasally in the form of NS to target the brain in Wistar rats [120]. Here, the NS were produced by precipitation followed by HPH and stabilised with Pluronic® F-127 (0.3%w/v), soya lecithin (0.4%w/v), and HPMC-E15 (0.3%w/v). Interestingly, the administration of the novel NS by bolus injection and intranasally presented markedly different pharmacokinetic profiles. On the one hand, the plasmatic concentrations observed for the NS intranasally were lower in plasma than those observed for the IV solution, whereas in the brain, the opposite occurred, and the IN NS had a greater absorption than the control solution.

Chen et al., reported the formulation of a breviscapine NS stabilised with Tween® 80 [121]. Like other works discussed above, the drug with multiple therapeutical applications was used as a model compound. After the HPH process, the resultant NS (mean particle size 527 nm and zeta potential −46.4 mV) were loaded into an *in situ* gel-forming formulation containing deacetylated gellan gum, glycerol, and ethyl 4-hydroxybenzoate for IN delivery. After physicochemical characterization of the NS and the gel, including rheology, the *in vitro* release of

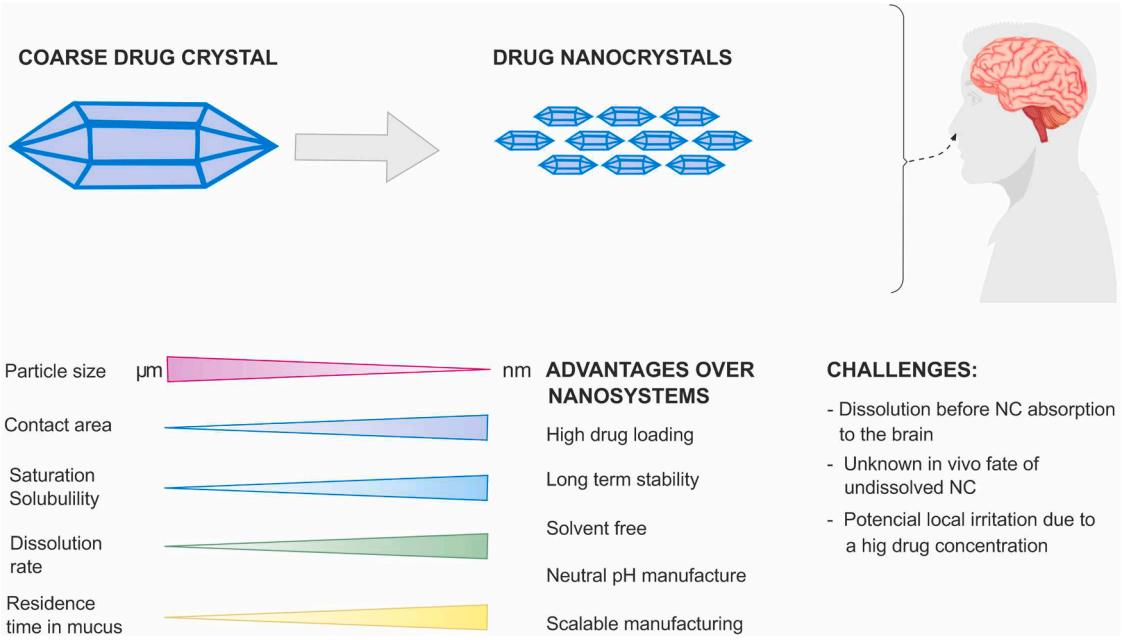

Fig. 10. Main advantages for nose-to-brain drug delivery using nanocrystals.

Table 4
Summary of reports describing the use of NC-based formulations to produce drug accumulation in the brain.

Drug	Disease	Particle size (nm)	PDI	Zeta potential (mV)	Stabilizers	Dosage form administered	Production method	Main findings	Refs.
Resveratrol	Model drug	241	0.234	-8.8	DGG	*In situ* gel formulation based on ionic-triggered DGG	Precipitation	Increased absorption in the brain in comparison to IV injection	[119]
Zotepine	Psychosis	330	330.2 and 519.26	-21.7, -18.26	P127, HPMC and soya lecithin	NS directly instilled in the nostrils	HPH and precipitation	Enhanced drug accumulation in the brain while plasma levels were lower than the control	[120]
Breviscapine	Model drug	527	n.r.	-46.4	Tween 80	*In situ* gel formulation based on gellan gum	HPH	Preferential distribution of the drug in brain, cerebellum, and OB	[121]
Paeoniflorin	Parkinson's disease	156	0.102	-23.2	TPGS	NS directly instilled in the nostrils	Precipitation	Enhanced transmucosal permeation and referential distribution of the drug in the brain	[122]
Clozapine	Psychosis	281	n.r.	-0.83	TPGS and PVP	NS directly instilled in the nostrils	HSH	Increased transmucosal permeation and accumulation in the brain	[123]

n.r., not reported; P127, Poloxamer 127; DGG, deacetylated gellan gum; HPH, high-pressure homogenisation; TPGS: D-α-tocopheryl polyethylene glycol 1,000 succinate; HPMC, hydroxypropyl methyl cellulose; HSH, high-speed homogenisation; IV, intravenous, OB; olfactory bulb; PVP, polyvinylpyrrolidone.

the formulation was assayed in Franz cells using a semipermeable dialysis membrane between compartments and simulated nasal fluid as release media. Following administration of the *in situ* gel-forming formulation, drug distribution was preferential in the CNS, with significantly greater drug concentrations observed in the plasma, brain, cerebellum, and OB tissues of male Sprague-Dawley. Another work reported the formulation of paeoniflorin NC for IN delivery [122]. The drug, an anti-Parkinson Chinese medicine with poor bioavailability, was nanosized by an antisolvent precipitation technique and stabilised using TPGS. The transmucosal release of the drug from the NC-based formulation (particle size of 150 nm) and the bulk drug was evaluated in Franz cells using porcine nasal mucosa. Here, the NC showed a substantially increased permeation compared to the pure active. Pharmacokinetic experiments in male Sprague-Dawley rats demonstrated, again, a preferential distribution of the drug in the brain versus plasma. As observed in Fig. 11, the plasmatic profiles of the IN and IV treatments did not show substantial differences after 30 min of the administration. On the other hand, the brain pharmacokinetic profiles revealed significantly higher drug absorption of the NC formulation compared to the control cohort.

In a recent work, the nose-to-brain delivery of clozapine, a antipsychotic drug, formulated as a NS was reported [123]. The authors used quality target product profile (QTPP) to obtain an optimised NS stabilised with TPGS using a high-speed homogenization technique. The *ex vivo* transmucosal drug release experiments performed using Franz cells and sheep mucosa, proved that the formulation, containing NCs with a particle size of 281nm, led to a faster permeation of the drug through the mucosal tissues (Fig. 12A). These results agreed with those observed in the pharmacokinetic experiment carried out in rats, demonstrating that the IN administration of the NS led to an improved drug absorption compared to the animal group treated with a conventional suspension orally administered, as observed in Fig. 12B.

The reports described above highlight the potential of the NC in the administration of hydrophobic drugs. A special effort has been devoted to diseases that affect the CNS, primarily psychosis, Alzheimer's, and Parkinson's disease, whose currently available treatments are associated with side effects related to their systemic distribution after oral administration. The nose-to-brain route offers many advantages that can be exploited to develop optimized treatments that can lead to increased drug accumulation in the brain. However, the limited data regarding *in vivo* experiments in more representative animal models, different than rodents, must be addressed. This information could help drug delivery

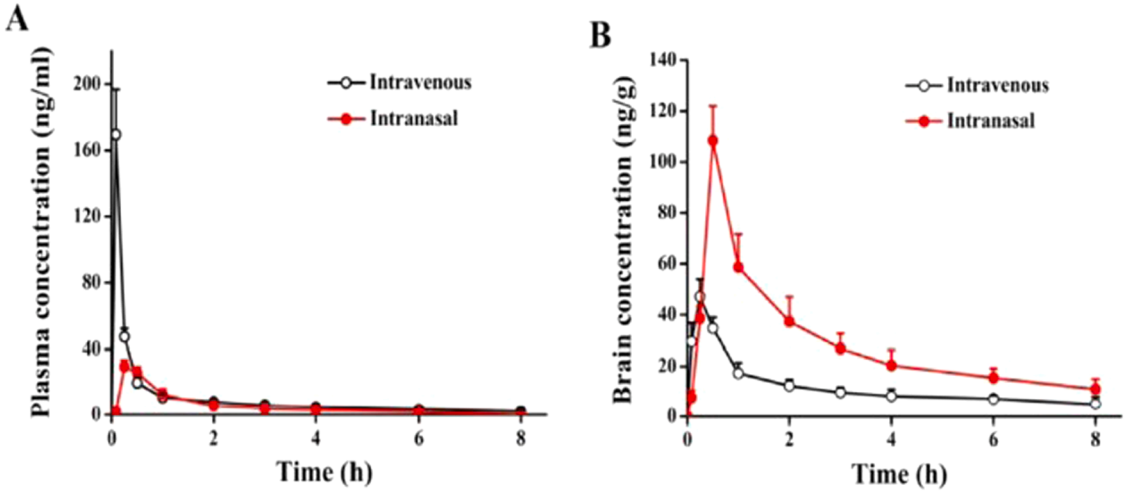

Fig. 11. Pharmacokinetic profiles obtained from plasma (A) and brain (B) samples after intranasal administration of paeoniflorin NCs and a drug solution. Reproduced with permission from Ref. [122], Copyright® (2020), Elsevier.

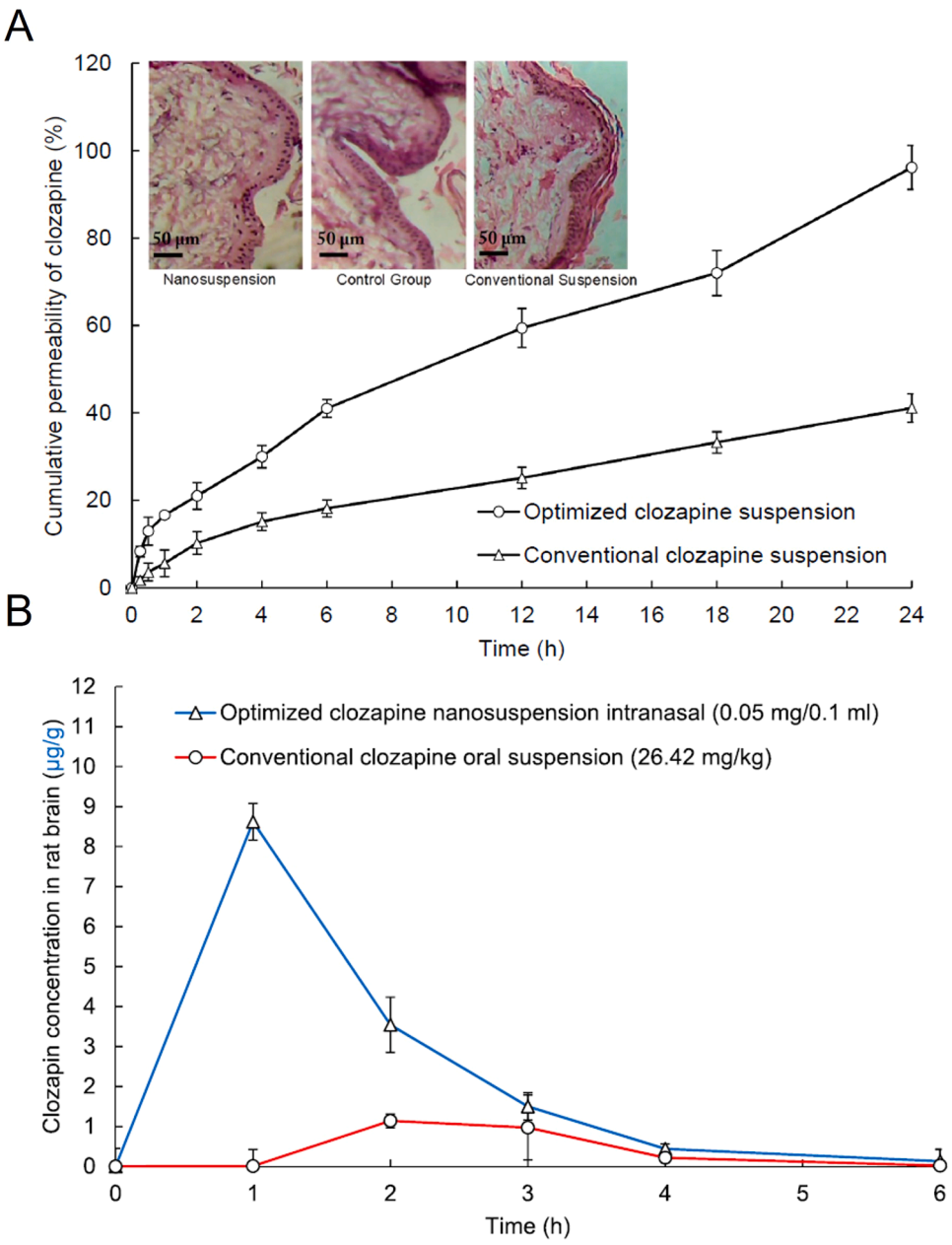

Fig. 12. **A**- *Ex vivo* transmucosal permeation studies performed in Franz cells for the optimised clozapine nanosuspension (NS) and conventional drug suspension. Inset showing histopathology images of undamaged nasal mucosa. **B**- Pharmacokinetic profiles of clozapine NS (intranasal) and clozapine conventional suspension (oral) in the brain. Reproduced with permission from Ref. [123], Copyright® (2020), Elsevier.

scientists to assess the retention of the formulations in the IN cavity and characterize in-depth the phenomena's of mucus secretion, ciliary movement, and passage of the formulation to the digestive system which can imperil the absorption of therapeutic agents through the nasal mucosa.

4. General discussion and concluding remarks

Besides reaching the brain quickly by evading the BBB, nose-to-brain delivery is a non-invasive, convenient, and patient-friendly administration route, showing a fast onset of action, precise drug targeting, and fewer systemic side effects [124]. However, the clinical translation of IN nanoformulations still has a long way to go. Indeed, mucociliary clearance, enzymatic degradation of the drug, scaling-up and stability of the nanoformulation, low efficiency of NP translocation, mucosal toxicity, and brain neurotoxicity are typical limitations associated with this drug delivery approach. In particular, the long-term biosafety of NP is a relevant concern; and the development of nanocarriers with low or no toxicity to the organism and the environment is one of the most significant challenges associated with their design. Although the most explored building blocks in polymeric NP for IN delivery are natural polymers or bioresorbable materials like PLA, PLGA, or PCL, the role of secondary components in the formulation is often passed over. For instance, the potential risks of the most common lectins used as bioligands in these NP should be evaluated deeply.

Regarding the biodegradability of lipid NP, they are generally composed of physiologically compatible lipids such as fatty acids, glycerides or other fatty acid esters, sterols, sterol esters, waxes, and so on. For this reason, they are considered members of the I or III classes in the "nanotoxicological classification system" (NCS) described by Keck and Müller [125]. Among lipid-based nanocarriers, the development of NLC and LNC has a great research interest due to their low toxicity and

stability obtained from physiological lipids such as mono, di- and triglycerides, fatty acids, and waxes [126].

On the other hand, the biocompatibility and toxicity of inorganic nanoparticles is a field of extensive study, so we recommend reading review articles that detail this topic in depth [127,128]. In general terms, these nanoparticles can cause damage to mammalian cells through oxidative stress, immune system dysfunction, or autophagy [105]. In this sense, materials such as metallic nanoparticles and Quantum dots have been shown to generate an overexpression of intracellular reactive oxygen species [129]. Furthermore, they can also interact with microglia cells, modifying their function and shape, leading to neurotoxicity if it occurs for prolonged periods. However, the biocompatibility and toxicity of these NP are a subject of scientific controversy because some articles mention biocompatibility and neuroprotective effects [130–132]. This controversy is based on the lack of standard tests for measuring these parameters, which allows the results to be compared between different articles [130]. Undoubtedly, this is the main challenge for these NP to expand their application in clinical trials.

Another significant challenge for NP in nose-to-brain drug delivery relies on the possibility of determining the spatial distribution of their cargo in the brain tissue (neural and glial cells, and potentially the endothelial cells of the BBB). Many studies have suggested that the major routes are the direct transport of drugs to the brain through neuronal pathways such as olfactory or trigeminal nerves and the indirect transport of drugs through the vasculature and lymphatic system. Nevertheless, the exact mechanisms of drug absorption have not been fully understood yet, as well as if one single mechanism or several ones may participate in the transport process [133]. The most common approach related to this challenge involves the inclusion of a marker in the NP, a fluorescent compound imaged by fluorescence microscopy. In this sense, there is an urgent need for high-resolution imaging methods to follow nanomedicines distribution from the nasal cavity to the brain and differentiate their localization among intravascular, extracellular (perineural and perivascular), and intracellular (neuronal and glial) sites. The coating of nanoparticles and the addition of specific ligands may play a significant role in the pathways that NPs take to penetrate the CNS in general, and to reach particular regions of the brain [134]. Again, further development of *in vitro* and *in vivo* models are required to gain understanding on these mechanisms [135].

One central aspect of drug administration *via* the nose using NP is the increased residency of the formulation within the nose cavity. Therefore, different types of bioadhesive hydrogels have been extensively reported in the studies reviewed here [13,48]. *In-situ* gel-forming formulations are exceptionally attractive since they are liquid at room temperature, allowing their administration using sprays, aerosols, or droppers. Once in the nose, at physiological temperature, they form a gel that remains in the IN cavity for prolonged periods. Multiple reports covered in this review have used this approach, showing promising results [74,75,84,88,119,121]. When using nanoparticles, the addition of surface ligands that can interact with the mucus, enhance its penetration capacity, and remain in the nose for prolonged periods was reported [21,36,38–40].

Another critical point is the drug loading capacity of nanoparticles, which can be detrimental for the required volume to be administered or produce sub-therapeutic drug concentrations in the brain. While nanocarriers like SLN, NLC, and polymer NP have a limited drug loading capacity (5-30%w/w) owing to the need for carrier materials, NC can load up to 100% of the drug. Therefore, this strategy holds promise, especially for drugs that require large doses to exert their pharmacological effect. The *in vivo* fate of drug NC when administered intranasally still remains unclear. NC dissolution before the unmodified drug particles are absorbed in the olfactory region is a possible scenario. However, due to the reduced aqueous volume available in the IN cavity, this is unlikely to occur. Surface NC modification can further avoid this phenomenon by decorating the particles with suitable polymers or lipids [136–138]. Similarly, the other types of NP covered in this review could release their drug cargo before traveling across physiological barriers. In this sense, complementary imaging and cell culture experiments would be necessary to provide clarification on this matter.

The *in vivo* models used in the vast majority of the reports found in the literature are rodents. Although there is a certain degree of similarity between the anatomical structures of the nose of rodents and humans, there are some aspects that need to be considered. For instance, the respiratory region in humans accounts for up to 80–90% of the nose, while in rodents, it only covers 50% of the nasal cavity. These aspects, together with other translational factors related to IN formulations have been well discussed in a recent review by Keller et al. [10].

Direct delivery of drugs to the brain is greatly desirable since it could avoid the severe systemic side effects of currently available treatments. For instance, antiparkinsonian drugs are associated with severe behavioural and gastrointestinal side effects [139], while antipsychotic drugs can produce mild to life-threatening side effects such as weight gain, myocarditis, and agranulocytosis [140,141]. In this sense, utilization of the nasal cavity to deliver drugs directly to the brain offers unique advantages compared to other administration routes.

The delivery of antiretroviral drugs (ARV) to the brain using the IN route is a promising approach [142,143]. The human immunodeficiency virus (HIV) has already claimed 36.3 million lives, and nearly 38 million people are currently living with HIV in 2020 [144]. One of the most challenging issues in managing HIV infection is the eradication of the viral reservoirs that are formed in the CNS, among other tissues [145]. Since available ARV regimens cannot produce therapeutically relevant drug concentrations in the CNS, increasing the brain bioavailability of these drugs *via* the IN route is an attractive target for further investigations.

An interesting strategy to improve the delivery of drug nanoparticles to the brain is the use of microneedle-based devices [146]. Microneedle patches are formed by a base plate from which microneedles (100 to 1500 μm in height) protrude from [147]. These patches are widely used for delivering hydrophilic and hydrophobic drug cargoes transdermally, both for topical and systemic effects [148–150]. The microneedles could physically help to circumvent the barriers of the intranasal route by piercing the outermost layers of the mucosa and producing drug accumulation in the brain [151]. However, microneedle patches to be applied intranasally must be carefully designed to avoid piercing the profuse nasal vasculature and producing bleeding.

Markedly, long-acting delivery of drugs intranasally seems challenging using currently available technologies, and treatments aimed at this route would need repeated applications, potentially affecting patient compliance and treatment adherence. Once NP have been absorbed into the brain, they could accumulate and circulate in the extracellular fluids and be absorbed by cells, in both cases, gradually releasing the drug. However, the physicochemical properties of such a drug coupled with the particle components would strongly influence this. Again, NC could hold promise in this sense due to their slow dissolution, which could offer a long-acting release profile. Unlike NC, lipid and polymer NP provide more flexibility in terms of controlled drug delivery. Polymer NP can be prepared using suitable combinations of stimuli-responsive building blocks and low and high molecular weight polymers to produce a long-acting release. Similarly, adjustment of drug release rate is possible *via* modification of the nature of the constituent lipids.

Finally, multiple aspects related to the manufacture of NP must be considered for the development of a product that can reach patients. Ease for scaling-up, and long-term physical, chemical, and microbiological stability are key parameters to be considered as well as the safety of the formulation [10]. In this sense, synergistic interaction between industrial, academic, and regulatory parties will be required.

Declaration of Competing Interest

The authors declare to have no conflicts of interest regarding the

present work.

Data availability

No data was used for the research described in the article.

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