Intranasal Nanoparticulate Systems as Alternative Route of Drug

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Abstract: There is always a need for alternative and efficient methods of drug delivery. The nasal cavity can be considered as a non-invasive and efficient route of administration. It has been used for local, systemic, brain targeting, and vaccination delivery. Although many intranasal products are currently available on the market, the majority is used for local delivery with fewer products available for the other targets. As nanotechnology utilization in drug delivery has rapidly spread out, the nasal delivery has become attractive as a promising approach. Nanoparticulate systems facilitate drug transportation across the mucosal barrier, protect the drug from nasal enzyme degradation, enhance the delivery of vaccines to the lymphoid tissue of the nasal cavity with an adjuvant activity, and offer a way for peptide delivery into the brain and the systemic circulation, in addition to their potential for brain tumor treatment. This review article aims at discussing the potential benefit of the intranasal nanoparticulate systems, including nanosuspensions, lipid and surfactant, and polymer-based nanoparticles as regards productive intranasal delivery. The aim of this review is to focus on the topicalities of nanotechnology applications for intranasal delivery of local, systemic, brain, and vaccination purposes during the last decade, referring to the factors affecting delivery, regulatory aspects, and patient expectations. This review further identifies the benefits of applying the Quality by Design approaches (QbD) in product development. According to the reported studies on nanotechnology-based intranasal delivery, potential attention has been focused on brain targeting and vaccine delivery with promising outcomes. Despite the significant research effort in this field, nanoparticle-based products for intranasal delivery are not available. Thus, further efforts are required to promote the introduction of intranasal nanoparticulate products that can meet the requirements of regulatory affairs with high patient acceptance.

Keywords: Intranasal, nanoparticulate system, drug delivery, Quality by Design, regulatory, patients' expectations.

1. INTRODUCTION

Clear evidence of the nasal cavity as an effective route of administration has attracted research groups to concentrate on exploiting this region as an alternative means for systemic and brain delivery of drugs and vaccines to overcome the inconvenience of already available routes.

Over the past decades, nanotechnology has gained an advanced position in drug delivery approaches. A nanoparticulate system holds a great value over the manipulative characteristics of the applied therapeutics, such as solubility, permeability, and half-life. These features allow the extended use of nanoparticulates for cancer targeting and controlled release purposes. Many parenteral, oral, and topical nanoparticulate therapeutics are available on the market and clinical trial stages [1–8].

The term nanotechnology is also widely used and defined as the control and manipulation of matter at the nano-scale (10-100 nm). However, the particles within the size range of 1-1000 nm are considered as nanoparticles in practice. Nanoparticles are regarded as special due to the fact that particles on the nanometer scale have unique optical, electronic, and structural/functional properties distinctive from the normal size. Moreover, higher permeability, a large surface to volume ratio, and higher mucoadhesion can be achieved as a consequence of nanosizing [9–13].

Nanosystems form a special group regarding their regulatory acceptance. Related guidelines and relevant chapters of the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) must be applied during all manufacturing stages from material selection and formulation to the final production. Furthermore, the FDA has emphasized the application of the Quality by Design (QbD) methodology, which can be especially useful for novel, high risk dosage forms and administration routes. The adoption of the International Council on Harmonization (ICH) guidelines for pharmaceutical development-Q8, risk management-Q9 and quality system-Q10 provides great potential for careful planning during the formulation and development even in the early phase of the research [14–20].

A high number of successful applications of nanoparticulate systems in drug delivery motivated to apply this technology in the case of the intranasal route; in order to improve drug delivery and to overcome the limits of this administration route. A combination of novel nanotechnology developments together with increased knowledge on intranasal delivery can efficiently lead to substantial advances in drug delivery with enhanced bioavailability and patient acceptance.

2. NASAL CAVITY

2.1 Nasal Anatomy

As known from anatomy studies, the human nasal cavity (Fig **1**) is composed of two symmetrical chambers (nostrils) separated by the median septum, the area inside each chamber is divided into the nasal vestibule area and the main nasal cavity containing the respiratory and olfactory regions. The total surface area and volume of the nasal cavity are 150 cm^2 and 15 ml, respectively [21,22].

The nasal respiratory area is the largest part of the nasal cavity, it is confined between the septal and lateral walls and it contains the superior, middle, and inferior turbinates forming the slit-like area that is responsible for the humidification and temperature regulation of the inspired air [23].

The uppermost region of the nasal cavity is the olfactory region, which is responsible for the sense of smell. This area comprises 10% of the total intranasal cavity and the olfactory information is sent from the olfactory bulb via the olfactory neuron into the piriform cortex, amygdala, and entorhinal cortex; where this can promote direct brain transport [24,25].

The cell lining type varies along the nasal cavity; the vestibules are covered by non-ciliated squamous and transitional epithelium with poor blood perfusion, whereas the respiratory region is covered by epithelium consisting of ciliated, pseudostratified, and columnar epithelium cells with a rich blood supply from the underlying lamina propria. The presence of microvilli along with columnar cells intensifies the surface area available for drug absorption, as each cell covered with 300 microvilli, and their fine projections (cilia) are fundamental to mucus transport into the nasopharynx. The topographical and physiological features of the respiratory region are responsible for being the main region for permeation. Similar to the respiratory area, the olfactory region is covered by pseudostratified epithelium with a specialized refractory receptor for smell perception. Prior to transfer, the olfactory component must be dissolved in the serous fluid that is produced and secreted by Bowman's gland [26–29].

Besides the significance of the respiratory region for systemic absorption, it plays a crucial role in direct drug delivery into the brain through the trigeminal region. The olfactory and trigeminal structures act as the only available apertures of the central nervous system (CNS) entry [30].

The anatomical aspect plays a crucial role in nasal delivery. To get the benefit of the high surface area of the nasal cavity and its consequences on higher absorption, the formulation must be spread over a large mucosal area. The place of distribution inside the cavity is essential for the activity; for example, for local delivery, systemic delivery, and vaccines, broad distribution is required whilst in brain targeting the drug must be delivered into the upper parts of the nose containing the olfactory region in addition to covering the trigeminal nerve, which may have a contribution in targeting. Such factors must be considered in selecting the dosage form and designing the delivery devices to ensure the proposed deposition and coverage of the formulation to get the intended response [31,32].

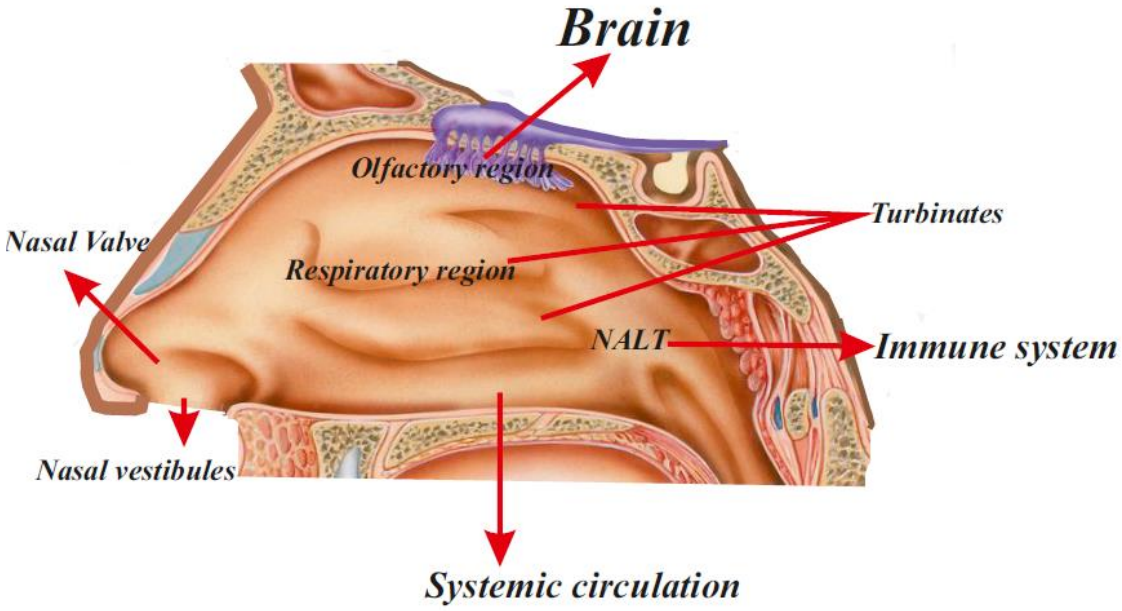

Fig. (1). Anatomical structure of the human nasal cavity.

2.2 Nasal Cavity as Drug Route of Administration

The distinction of the intranasal route is ascribed mainly to the anatomical and physiological characteristics of the nasal cavity. The nasal cavity offers a number of advantages, such as: avoiding first-pass metabolism, high surface area, high permeation, high vascularization, and having a nose to brain direct pathway as well as circumventing the blood brain barrier. Thus, the nasal route has the potential for the delivery of drugs that suffer from extensive first-pass metabolism, poor solubility, and degradation in the gastrointestinal tract. It is also an attractive site for vaccine and peptide delivery that have been parenterally administered so far. The intranasal route is a non-invasive, non-sterile, and easily administered method that can enhance patients' compliance [33–39].

On the other hand, many limitations could adversely affect nasal delivery, these include: mucociliary clearance, restricted volume of nasal administration (max. 200 ul), presence of enzymes and efflux transporters, pathological and environmental factors that affect intranasal blood supply. Moreover, the narrow nasal valve represents a potential obstacle to an efficient drug delivery [40–43].

2.3 Emerging Intranasal Application from Local to Systemic, Brain, and Vaccine Delivery

The intranasal delivery of drugs has been initially utilized for the local treatment of topical conditions. Various marketed drugs have been used to treat congestion, nasal allergies, infections, and nasal polyps. Decongestants, steroids, and antihistamines are the most common drugs that are nasally applied for their local action [44–48].

As a consequence of the previously mentioned advantages, the nasal cavity has evolved from local administration into a route for systemic, brain targeting, and vaccine delivery. This extension has opened up the possibilities for all drug delivery purposes, including cancer treatment [49–53].

Intranasal products with systemic effects are commercially available for certain drugs such as zolmitriptan, sumatriptan, ergotamine, butorphanol tartrate, and fentanyl as well as peptides such as calcitonin, desmopressin, buserelin, and nafarelin [54–61]. Other drugs have been nasally introduced for the treatment of urgent conditions such as migraine, seizures, opioid overdose, and pain breakthrough in cancer [62–67].

Maximum 2% of drugs are capable of reaching the brain after systemic administration due to the presence of the protective brain capillary endothelium. The exploitation of the intranasal route to target the CNS is an attractive approach to circumvent the blood brain barrier (BBB) and deliver the drug directly through the cribriform plate, olfactory and trigeminal regions. Alzheimer's disease, depression, migraine, schizophrenia, HIV consequences, and multiple sclerosis are all CNS diseases that systemic administration has failed to treat. The availability of an effective delivery rather than the drugs was the missing part for achieving considerable therapeutic outcomes [68–70].

The intranasal cavity offers easy administration for vaccines, inducing both mucosal and systemic immunity. The importance of this site has evolved from the nature of infections itself since the majority of viral and bacterial infections start from the mucosal tissues. Both innate and adaptive immune responses can be directly initiated after the delivery of the antigen via nasal-associated lymphoid tissues (NALT) through the distinctive M cells into the antigen sampling cells, dendritic cells, B-cells, and T-cells, being responsible for the humoral immune responses mediated by secretory IgA antibodies [71–74]. The pharmaceutical aspects of intranasal vaccination have been thoroughly discussed by Sharma *et al.* [75].

3. NANOTECHNOLOGY AS A FURTHER ASPECT OF THE INTRANASAL DELIVERY OF DRUGS

3.1 Rationale for using Nanotechnology for Intranasal Delivery

Within the last decades, tremendous efforts have focused on intranasal delivery and its potential for different applications beyond its local importance; to achieve systemic delivery and brain targeting in addition to mucosal and systemic vaccination.

Hypothetically, the ideal route is available. Nevertheless, many limitations can hamper its efficiency. The combination of nanotechnology as the drug preparation method and intranasal delivery as the route is supposed to provide an effective delivery system. Nanotechnology offers the criteria for achieving high solubility and dissolution rates, which are the key factors for drug absorption and activity. Furthermore, this technology can protect the drugs from nasal enzyme activity, counteract the mucociliary action to increase contact time and promote permeation. Table **1** lists the nanotechnology effects on the major nasal delivery limitations and Table **2** identifies the rationale for using the nasal passage as a route for administration and nanoparticles as a technology for various targets. Many risks can also be increased, such as toxicity or even the inhalation of the nano-scale particles. Thus the identification of the risks associated with the intranasal delivery of nanoparticles must be cautiously evaluated [76–78]

Table 1. Nanotechnology solutions for intranasal delivery limitations.

Limitation	Nanotechnology effects	Refs
Poor drug solubility	High ratio of surface area to volume Interactions between the groups of the polymer and drug molecule, such as electrostatic, and H-bonding Production of a microenvironment with special lower polarity inside the nanoparticles than in the aqueous bulk phase	[79–83]
Mucociliary clearance and short residence time	Localization of the formulation for a longer time Enhancement of contact time inside the nasal cavity	[84–86]
Poor penetration for large and hydrophilic molecules	Ability to open up tight junctions Possibility of high endocytosis Ability to change mucosal membrane properties	[87,88]
Enzymatic activity	Encapsulation of liable molecules	[89]
P-glycoprotein efflux transporter	Efficiency for bypassing and inhibition of P-glycoprotein	[90,91]

Table 2. Rationale for the use of the nasal route and nanoparticulate systems with various targets.

Target	Rationale for nasal delivery	Rationale for using nanoparticulate systems
Local	Rapid onset of action Minimum effective dose Minimum side effects	Enhancement of contact time with mucosa
Systemic	Ease of administration Rapid onset of action Avoidance of first-pass metabolism Avoidance of gastric deterioration and enzymatic degradation	Delivery of peptide, proteins, and high molecular weight therapeutics High systemic absorption
Brain	Circumvention of the blood brain barrier Noninvasive application of anti-tumor agents	Drug targeting Delivery of peptide, proteins, and high molecular weight therapeutics
Vaccination	Noninvasive Induction of mucosal and systemic immunity	Adjuvant activity

3.2 Pharmaceutical Factors of the Influence of Nanoparticles on Intranasal Delivery

The pharmacokinetics and bioavailability of the applied drug (vaccine) are ruled by different factors related to the properties of both the active pharmaceutical agent (API) and the formulations. These factors determine the mechanism of absorption through the nasal mucosa [92].

Nanomaterials possess distinctive physicochemical properties compared to their conventional counterparts, and these properties provide nanoparticles with beneficial characteristics. The physicochemical characteristics of nanoparticles which most influence their administration through the nasal route include size, shape, chemical composition, physiochemical stability, crystal structure/polymorphism, surface area, surface charge, and surface energy in addition to drug loading and drug entrapment efficiency.

Particle size is a critical evaluation parameter to assess the desired properties of nanoparticles due to its consequences on surface area and viscosity, and thus drug dissolution, release, absorption, and stability [93]. Due to their small size, nanoparticles are usually used as a drug carrier via passive transport, active transport, and endocytosis [94,95]. However, the mechanism by which nanoparticles enhance drug transport is not fully described. Some studies considered that nanocarriers interact with the mucus layer and release the drug in the mucus or at the mucus–epithelial cell interface, while other studies implied that the drug-loaded nanoparticles themselves cross the mucosal barrier. Both cases involve the uptake of nanoparticles into the respiratory or olfactory epithelium and then drug payload diffused into the systemic circulation or to the CNS. Surface charge also plays an important role in the interactions of nanoparticles with biological systems. For example, positively charged nanoparticles have been designed to improve nasal adhesion with the nasal mucosa via the electrostatic interaction with the sialic groups of mucin [96]. Furthermore, it has also been observed that the surface charge of nanoparticles alters blood-brain barrier integrity and transmembrane permeability [95].

There is no clear trend found which is concerned with the influence of nanoparticle size on drug uptake into the tissue [97]. Therefore, the effect of these important factors on drug permeability has been discussed in many studies. Some authors studied the *in vitro* transport across nasal epithelium, *ex vivo* across nasal mucosa or *in vivo* with animal models. Brooking *et al* [98] studied the transport of 12’1-radiolabelled latex nanoparticles by using a range of particle sizes and surface coatings across rat nasal mucosa. Among 20, 100, 500, and 1000 nm of non-modified nanoparticles, the 20 nm sized particles showed the highest

extent in the systemic circulation. The 20 nm sized nanoparticles showed 2-fold higher blood concentration than the 100 nm sized particles, while 500 and 1000 nm sized particles showed similar lower levels of uptake; half of these seen for the 100 nm sized particles. The surface modification of 100 nm sized particles changed the surface charge. This change had a significant effect on the uptake of the particles into the systemic circulation. Coating the particles with poloxamine 908 (-14 mV zeta potential) resulted in a significant reduction in uptake compared with the uncoated particles (-49 mV zeta potential). However, coating of the polystyrene particles with Poly-I-lysine (PLL) (25 kDa) and PLL (I28 kDa) with zeta potentials +33 and +19 mV, respectively, did not significantly change the levels of particles transported into the blood stream as compared to the uncoated particles despite these former particles had a positive surface charge. It is worth noting that these results contradicted what has been previously proved, namely that PLL is able to open the tight junction and increase the transport across the nasal membrane into the blood stream. It was expected that PLL-coated nanoparticles would give greater transport across the nasal membrane [99]. On the other hand, 100 nm chitosan modified particles resulted in a significant increase in the transport of particles into the blood stream due to its mucoadhesive effects and ability to open the tight junction.

Gartziandia *et al* [100] studied the transport of polymeric and lipid-based nanoparticles with the same surface charge (-23 mV) across olfactory monolayers in rats. 100 nm sized nanostructured lipid carriers (NLCs) penetrated to a higher extent compared to the 220 poly(lactic-co-glycolic acid) (PLGA) nanoparticles. Moreover, the positively charged chitosan-coated NLCs increased the transcellular transport by almost threefold compared to the uncoated NLCs. Mistry *et al* [101] studied the transport of different-sized fluorescent carboxylated polystyrene nanoparticles across excised porcine olfactory epithelium mounted in a vertical Franz Diffusion Cell. 20, 100, and 200 nm of the non-modified nanoparticles (surface charge -42 mV) were compared to Polysorbate 80-modified polystyrene nanoparticles (-21 mV) and chitosan-modified nanoparticles (+42 mV surface charge). Polysorbate 80-coated (PEGylated) particles penetrated deeper in the tissue compared to the uncoated and chitosan-coated nanoparticles.

A study by Ahmad *et al* [102] discussed the effects of nanoemulsion (NE) particle size on the permanence of the NE within the nasal cavity. NEs with droplet size of 80, 200, 500, and 900 nm were compared. The NEs were prepared from Labrafac®WL1349/Labrafac® CC and Soluto®. The results showed that the smaller the droplet size, the higher permanence within the nasal mucosa. The study also confirmed the translocation of 100 nm in the nasal mucosa and along the trigeminal nerve to the olfactory bulb. However, large nanodroplets (900 nm) were not transported to the olfactory bulb.

The shape of nanoparticles influences their stability, absorption, and cellular uptake. The spherical shape is the most stable thermodynamically. However, these effects are cross-linked with particle size and surface charge. Gratton *et al* [103] designed a series of particles with different sizes and shapes to study the interdependent effect of size and shape on their internalization by human cervical carcinoma epithelial (HeLa) cells. Nanoparticles and microparticles were prepared by using particle replication with non-wetting templates method. The particles were made of cationic, cross-linked poly(ethylene glycol) hydrogels. Three series were produced as follows: cubic-shaped particles (2, 3, and 5 μm cube side length), cylindrical particles with identical heights but varying diameters (0.5, and 1 μm diameters), and cylindrical shaped nanoparticles (100, 150, 200 nm diameters). The results showed a strong dependence of cellular internalization on the size and shape of the particles. 2 μm cubic particles showed a significant internalization by the most cells, whereas 3, and 5 μm showed insignificant internalization. The cylindrical nanoparticles showed the same level of internalization, which was higher than the 2 μm cubic particles. The cylindrical nanoparticles showed a very high degree of internalization. Moreover, it was found that 100 nm cylindrical particles were internalized to a lesser extent than the larger 150 nm cylindrical nanoparticles with the same ratio aspect. In another study, Chithrani *et al* [104] also used HeLa cells to investigate the intracellular uptake of spherical and rod-shaped gold nanoparticles. The results revealed that the uptake of rod-shaped nanoparticles was lower than that of their spherical counterpart. The difference in the surface chemistry between the two shapes could be one of the reasons for such uptake differences. However, the cellular uptake of rod-shaped structures with a lower aspect ratio (1:3) is greater than in the case of nanoparticles with a higher aspect ratio (1:5) although both of these rod-shaped gold nanoparticles were modified by cetyl trimethylammonium bromide.

Shi *et al* [105] developed a model to figure out the basic mechanisms for the uptake and release of nanoparticles in animal cells. The authors reported that there is an optimal particle size as well as an optimal shape for the maximum rate of particle absorption and release. Other studies showed the relationship between cellular uptake and nanoparticle size, shape, and surface chemistry, and the mechanism of cellular uptake were reported in literature [106–108]. The parameters affecting the loading and entrapment efficiency must be controlled to achieve a desirable and controlled release profile concerning the total amount

of the released drug and the release kinetics. Accordingly, the amount of the loaded agent, the composition of the nanoparticle forming materials, the molecular weight of the constituents, the ratio of the active agent to the additives, concentration, and the type of the used stabilizing agents, and the manufacturing process parameters that can affect these properties must be investigated prior to preparation [109].

In addition to the pharmacokinetic properties, interaction with the mucosal tissue and the bioadhesive properties of the nanoparticles inside the nasal cavity are significant factors that can influence the delivery of the API. Mucus represents a challenge for drug delivery. The barrier properties of nasal mucus are related to the dense fiber network of mucin containing highly glycosylated (negatively charged) parts. Thereby, the way to increase interaction with mucus is by applying nanocarriers with a positive surface charge.

Another strategy has been used to modify the interactions with mucus; a way to produce mucus penetrating nanoparticles is by PEGylated modification of nanoparticle surfaces [110,111]. Many studies have identified and discussed the effects of PEG on nanoparticle transport across the nasal mucosa. For example, PEG-modified polylactide-polyglycolide (PLGA) nanoparticles for the tetanus toxoid showed higher antibody levels following the intranasal delivery than those corresponding to PLA unmodified nanoparticles. Moreover, the fluorescence microscopy studies revealed that the PEG-PLA particles were able to cross the rat's nasal epithelium to the brain [112]. Another study reported that PEGylated liposomes had shown greater uptake of risperidone into the brain in comparison to liposomes and cationic liposomes [113].

In conclusion, it is essential to characterize the nanomaterial properties and their interaction with the biological agent to produce successful nanoparticle systems and novel delivery.

This review shows the most common nanoparticulate systems intended for intranasal application with recent literature studies.

4. NANOPARTICULATE SYSTEMS FOR NASAL DELIVERY

4.1 Intranasal Nanosuspensions/ Nanocrystals

Nanosuspensions can enhance the dissolution rate and the saturation solubility of poorly water-soluble drugs. Nanosuspensions also offer the advantage of high drug loading capacity, and thus a possibility to introduce the required dose within the limited volume of the nasal cavity. Furthermore, small particles can penetrate the mucosal tissue more easily and are able to pass to the brain directly through the olfactory region, resulting in enhanced bioavailability [79,114].

The development of a nanosuspension for intranasal delivery for systemic purposes is demonstrated by Kürti *et al* [115]. In this study, a nanosuspension of meloxicam with 140 nm particle size has been prepared by co-milling with PVP-C30. The *in vivo* results showed the significant enhancement of the maximum plasma concentration (C_{max}) and area under the curve (AUC) of the nasal meloxicam nanosuspension compared to the physical mixture (2.7-fold and 1.5-fold, respectively). The notable result was the tremendous differences in time required to reach C_{max} (T_{max}); 5 min for the intranasal nanosuspension compared to 90 min for the oral administration with 1.6-fold higher C_{max}. These results ensured the efficacy of intranasal delivery to achieve a rapid onset of action close to intravenous (IV) administration. Another example showed the importance of a lyophilized nanosuspension (nanocrystals) for brain targeting of resveratrol with deacetylated gellan gum as an *in situ* ionic sensitive gelling agent. Intranasal delivery showed both brain C_{max} and $AUC_{0-\infty}$ higher than IV administration (2.3- and 2.88-fold, respectively). This study confirmed the direct transport of resveratrol into the brain with 458.2% drug targeting efficiency (DTE%) and 78.18% brain drug direct transport (DTP%) [116]. Whether resveratrol has been confirmed for Alzheimer's disease treatment or not, this study ensured a way for delivery and maximizing brain concentration where other routes failed to produce tangible evidence for brain targeting. Examples of recent intranasal nanosuspensions are listed in Table **3**.

Table 3. Nanosuspension-based intranasal formulations.

API	Target	Composition	Particle size	Model/Compared compartment	Observations	Refs

Carvedilol	Systemic	Poloxamer 407, oleic acid, and gellan gum	190 nm	Rabbits/ Oral suspension and IV solution	2.4-fold higher C_{max} and 2.6-fold higher $AUC_{0-\infty}$ with 69.4 % absolute bioavailability were achieved	[117]
Meloxicam	Systemic	Polyvinyl alcohol, and sodium hyaluronate	135 nm	Rats/ Micronized (1.9 *μm* particle size) and raw meloxicam spray	3-fold higher plasma level was observed after 5 min	[118]
Ezogabine	Brain	Tween® 80, and Poloxamer 188	155-454 nm	*Ex vivo*/ Not recorded	Maximum 97.9% of ezogabine released within 6 h and no cilio-toxicity was observed	[119]

Studies on the nasal application of nanosuspensions reported an average size with the range 140-500 nm. Moreover, the use of a mucoadhesive agent such as chitosan and the preparation of *in situ* gel are common procedures in the practice. Besides the systemic target, brain delivery has been significantly considered in the recent studies.

4.2 Intranasal Lipid and Surfactant-Based Nanoparticulate Drug Delivery Systems

Lipid nanoparticles show a promising approach for intranasal delivery. The advantages of active agent protection from enzymatic degradation, capability for hydrophilic as well as lipophilic molecule delivery, low toxicity, good permeability, and the possibility of modifications and adaptations have justified their wide application for the intranasal route. These systems include liposomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), niosomes, nanoemulsions (NE) and nanocapsules (NC) [120,121].

4.2.1 Liposomes

Liposomes are spherical vesicles containing one or more lipid bilayers that encapsulate aqueous drug compartments with a diameter in the range of 400 nm-2.5 *μm*. The properties of the lipid structure have a significant effect on the liposome surface charge, membrane flexibility, and the surface hydration and particle size [122]. These factors affect the kinetics of liposomes, bio-distribution, and faith after administration [123]. Liposome properties on uptake enhancement, and toxicity minimization were earlier explored by Kimelberg *et al* [124].

The intranasal delivery of liposomes showed an efficient delivery of calcitonin based on what has been discussed by Law *et al.* [125]. The effects of the type and charge of liposomes on calcitonin loading efficiency were verified; anionic liposomes showed higher loading efficiency than neutral and cationic ones. Loading efficiency increased with calcitonin concentration. The evaluation of the effects of calcitonin liposomes on bioavailability was accomplished with *in vivo* studies. The intranasal absorption of calcitonin was enhanced compared to the calcitonin solution; particularly, with positively charged liposomes, these findings confirmed the effects of different factors on the intranasal application of liposomes. The positively charged liposomes showed higher calcitonin bioavailability than the negative liposomes due to their higher contact time with the negatively charged mucosal membrane, thus lowering mucociliary clearance [126]. Alternatively, Chen *et al* [127] evaluated the usage of ultra-flexible liposomes on salmon's calcitonin intranasal absorption. There were no differences in the absorption between negative and positive liposomes, which was attributed to the rapid absorption of calcitonin from the liposomes.

Insulin-loaded liposomes showed low permeability through the nasal mucosa in the rabbit model. On the other hand, the permeability of insulin entrapped in the liposomes was increased after using sodium glycocholate as a penetration enhancer [128,129]. The importance of liposomes for insulin delivery has been reported in the work of Jain *et al* [130]. The authors reported that chitosan-coated multiple vesicular liposomes were able to control the plasma glucose level in diabetic rats for two days. This sustained pattern can overcome the inconvenience of rapid increase followed by a rapid decline of insulin serum concentration after the intranasal administration. In spite of the feasibility of the intranasal use for sustained delivery, chronic application is controversial and to date, it is not practical [131].

The brain delivery of the intranasal liposomes has been investigated by using ovalbumin as a model peptide. Both transportation into brain and brain residence time have been enhanced compared to the solution preparation. In this study, cationic liposomes with an average size of 299 nm showed high loading efficiency and more than 90% drug delivery to the brain. Intranasal delivery depended on the concentration and volume of the administration in a pattern that smaller volumes of the liposomal preparation enhanced retention and reduced swallowing, thus promoting brain delivery [132].

Liposome contribution in intranasal mucosal vaccinations is related to the retention enhancement of the liposome inside the nasal cavity, therefore, the high chances of antigen delivery by M cells located on the NALT. Moreover, liposomes are able to induce immunoadjuvant activity [133,134]. Wong *et al* [135] showed that intranasal liposomes of hemagglutinin - the influenza antigen - induced serum the IgG levels higher than the naked antigen. Furthermore, modified liposomes showed enhanced delivery; for instance, chitosan-modified liposomes facilitated the interactions with the negatively charged mucosal surfaces and produced a great potential for DNA delivery. In another aspect, galactose-modified liposomes of ovalbumin showed a higher macrophage uptake and induced both mucosal IgA and serum IgG in a mouse model [96,136]. These findings highlighted the importance of modified liposomes in antigen delivery [137,138].

4.2.2 Solid lipid nanoparticles (SLNs)

Solid lipid nanoparticles are another example of lipid-based systems that have shown promising prospects for intranasal delivery. These systems are characterized by a 50-1000 nm range of particle size, they are composed of physiological lipids, and stabilized by nontoxic surfactants like Poloxamer and lecithin. The attractiveness of these systems is based on their safety compared to polymeric nanoparticles, and the low production cost compared to liposomes. They can be formulated by simple methods like high pressure homogenization and microemulsions [139]. Intranasal alprazolam-loaded SLN using Tween® 80 and Pluronic® F68 had an average diameter of 99.5 nm and entrapment efficiency of 40.3%. These SLNs showed higher brain bioavailability of alprazolam than with IV administration, with 55% DTP and 224% DTE. The intranasal SLN of budesonide showed higher permeation values than the free drug and the already marketed formulation of budesonide by 3.4- and 1.8-fold, respectively. [140,141]. In a study to prepare agomelatine SLNs with the emulsification solvent evaporation technique, the optimized SLN showed a particle size of 167 nm, polydispersity index of 0.12 and entrapment efficiency of 91.3%. This optimized formulation exhibited a substantial increase in each of the plasma peak concentration, the $AUC_{(0–360\ min)}$ and the absolute bioavailability compared to those of the oral marketed dosage form with the values of 759.00 ng/mL, 7,805.69 ng·min/mL and 44.44%, respectively. The SLN of agomelatine also revealed DTE of 190.02 and DTP of 47.37, thus higher brain targeting by the intranasal delivery than by the IV route [142].

4.2.3 Nanostructured Lipid Carriers (NLCs)

These systems, composed of both solid and liquid lipids as a core, offer the advantages of higher loading capacity than SLNs without undergoing polymorphic transition and drug explosion during storage [139]. Intranasal NLCs have been utilized for the brain targeting of temozolomide -an antitumor agent- in a recent study. In this study, NLC protected the drug from the p-gp system by the effects of Poloxamer 188, which also increased drug mucosal penetration. As a result, the brain concentration of temozolomide was higher than what has been achieved after IV administration with a sustained effect. Thus, it can provide a direct delivery for the treatment of brain tumors [143]. In another scope, the exceptional particle size, mucoadhesivity, and rapid release properties of tetrahydrocannabinol cationic NLC formulation raised the opportunity for the novel nasal spray to control cancer breakthrough pain [144].

4.2.4 Niosomes

Niosomes are structurally similar to liposomes in the concept of bilayer systems that entrap drugs with a chief difference in composition. Unlike liposomes, niosomes are composed of non-ionic surfactants that are responsible for a vesicle-like structure, thus providing more stability over liposomes by removing the inconvenience of oxidation and the purity variation of phospholipids [145]. The assembly of non-ionic surfactants into closed bilayers can be spontaneous or with the help of external stimuli, such as heat or shearing forces. Besides the activity of niosomes as hydrophilic and lipophilic drug carriers, they act as solubility enhancers, hence increasing the bioavailability of poorly water-soluble drugs. The main limitations of niosomes include aggregation, fusion, and leakages during storage. These adverse properties of niosomes can be minimized by additives such as cholesterol, fatty alcohols, charge inducers (dicetyl phosphate and stearylamine) or steric groups on the surface of niosomes [146].

Niosomes for intranasal application have been proposed to represent a promising approach for enhanced and controlled delivery. Intranasal folic acid niosomes intended for brain targeting have shown controlled *ex vivo* perfusion [147]. Regarding the systemic effects of intranasal niosomes, diltiazem-loaded niosomes have shown high half-life ($T_{1/2}$) and enhanced AUC $_{0-\infty}$ with a reduced elimination rate; such prolonged action for diltiazem is of great value compared to its low oral bioavailability due to extensive first-pass metabolism [148]. The study of using intranasal niosomes for vaccination with glycoprotein B of herpes simplex virus type 1 has shown *in vitro* controlled release and *in vivo* elicited plasma glycoprotein antibodies (IgG) and systemic T helper cells [149]. These outcomes demonstrated the efficacy of niosomes to produce immunity against genital herpes in the female murine animal model and generalized the activity of niosomes for vaccination.

4.2.5 Nanoemulsions (NEs)

Nanoemulsions are part of a nanoparticulate system with a typical particle size of less than 200 nm. These systems contain oil, water, and surfactants, and are characterized by simple preparation methods, biocompatible constituents, and robust stability against sedimentation, creaming, dilution, and temperature effects. NEs could enhance drug solubility and mucosal permeation [150]. Regarding their nature and the possibility for the addition of excipients, such as mucoadhesive and gel-forming polymers, NEs can provide a novel intranasal delivery system that meets the criteria of drug protection, mucosal adhesion, and permeation enhancement [151]. Intranasal NEs have been studied for their activity on systemic, brain targeting, and vaccine delivery, but not for local delivery due to the effect of absorption that would transfer the drug from the local site to the systemic circulation or even the brain. For example, intranasal nitrendipine NEs have shown a rapid onset of action with a relative bioavailability of 60% compared to the marketed oral tablets [152]. The preparation of NE *in situ* gel was proposed to be effective in enhancing systemic absorption based on the results of zaleplon; rapid absorption with T_{max} of 20 min and 8-fold higher bioavailability compared to the marketed tablets. These results could be attributed to the effects of gel-forming polymers on residence time enhancement and permeation improvement [153].

The utilization of NEs in vaccine delivery has been receiving focused attention in many studies. NEs may exhibit a strong and broad antimicrobial, antiviral, and antifungal activity and provide good adjuvant activity. Many studies have demonstrated the efficacy of NE-based mucosal vaccinations against many infections, particularly, influenza and respiratory syncytial viruses [154–158]. For example, a W805EC adjuvant NE with 400 nm globules successfully enhanced the immune humoral response in murine animal model [157]. A recombinant HIV gp120 antigen NE showed the mucosal adjuvant activity of the NE for multivalent HIV vaccines [156]. Moreover, Sun *et al* [159] reported the adjuvant activity of the NE against methicillin-resistant *Staphylococcus aureus* (MRSA). This study reported a novel NE containing MRSA recombinant protein with an average diameter particle size of approximately 31 nm. The mucosal vaccine showed improved immune responses without using additional adjuvant additives.

4.2.6 Nanocapsules (NCs)

Nanocapsules are composed of an oily core surrounded by a polymeric coat (shell) with a general range of particle sizes of 100–500 nm. NCs have been one of the systems in the focus of research due to their promising potential as an effective drug delivery platform for the transmucosal administration of peptides, vaccines, and hydrophilic and lipophilic drugs. The development of NCs has emerged from the ability to control particle size, surface properties, and composition. Therefore, control over stability and interaction with the mucosal membranes are attainable [160].

Importance has been given to the systemic delivery of intranasal peptides using NCs by the work of Prego *et al* [161]. In this study, salmon calcitonin was used as the model peptide and was incorporated into the chitosan-coated oil NCs. The results

showed that NCs with sizes in the range of 200-570 nm led to hypocalcemic effects that were considerably enhanced and prolonged compared to the corresponding salmon calcitonin nanoemulsion or to the aqueous solutions with chitosan.
Sallam *et al* [162] developed a locally acting nasal delivery system of triamcinolone acetonide using different nanosystems. The NCs composed of a Capryol® oily core and Eudragit RS100 provided the highest mucosal retention compared to the NEs and NLCs with the least permeation, thus the drug was retained on the nasal mucosa. Moreover, NCs also showed lower mucosal irritation and superior stability compared with NEs. The identification of the intranasal NCs as a brain delivery approach has been discussed in different studies. For example, Clementino *et al* [163] reported the NC systems for brain delivery of simvastatin. The drug was loaded in lecithin/chitosan and the system was characterized by a particle size of 200 nm, encapsulation efficiency of 98%, and zeta potential of +48. These NCs showed that around 20% of the dose was accumulated in the brain whilst the drug nanosuspension distributed the drug into other body organs and a very limited amount in the brain.
Vicente *et al* [164] reported a remarkable example for using NCs in the nasal system for the co-delivery of viral proteins and imiquimod for vaccination purposes. In this study, imiquimod, a lipophilic immunostimulant, was added in the oily core whilst the recombinant hepatitis B surface antigen (HB) was associated with the chitosan shell. The system showed a particle size of around 200 nm, zeta potential of +45 mV, and antigen association efficiency of 70%. As a result, the NCs containing imiquimod elicited a protective immune response and showed increased IgG levels and specific immunological memory. Moreover, a balanced cellular/ humoral response was achieved indicating the capacity of the NCs to modulate the systemic immune response upon nasal vaccination.

NCs are widely reported as NEs with mucoadhesive polymers. The advantages of NCs over conventional NEs were confirmed in many studies. For example, intranasal risperidone NE showed enhanced brain and plasma concentrations compared to the drug solution, and it was comparable to the IV injected formulations. However, the NE with chitosan (NCs) formulations showed a significantly higher C_{max} and AUC in addition to higher brain targeting (approximately 2-fold higher DPT%) [165] The same effects have been achieved with olanzapine. A mucoadhesive NE of olanzapine showed a higher brain $AUC_{0-\infty}$ compared to a NE without mucoadhesive polymer and also showed a 2-fold higher brain bioavailability than the IV injected drug [166]. Recently, Colombo *et al* [167] investigated the brain delivery of an intranasal NE containing kaempferol for glioma cell targeting. This study showed the enhanced delivery effects of the chitosan-based mucoadhesive NE compared to the NE without chitosan. A mucoadhesive NE of zolmitriptan is another example that showed the enhanced brain permeation of zolmitriptan from a chitosan-based mucoadhesive NE. A 2.8-fold higher brain $AUC_{(0-8)}$ compared to the IV and brain targeting parameters of 164.77 and 9.61 for DTE% and DTP%, respectively, were attained [168]. Other studies investigated hyaluronic acid for the development of mucoadhesive nasal NEs for brain delivery. For example, resveratrol and curcumin were formulated together into a lipidic NE using hyaluronic acid as the mucoadhesive agent. The NEs showed brain target ability in a manner of about 7- and 9-fold increase in brain $AUC_{0-7\,h}$ for resveratrol and curcumin, respectively [169].

Literature on lipid- and surfactant-based nanoparticles demonstrated the significance of these systems for nasal delivery. These systems were characterized as having a particle size in the range of 75-300 nm. However, the particle size was dramatically increased when proteins and peptides were loaded and when multilamellar vesicles were prepared as shown with the next tabulated examples. Several examples have shown the usage of gelling, mucoadhesive, functionalized polymers and other additives to ensure the nanoparticles' properties and efficiency. Therefore, the selection of the constituents and their concentrations is very valuable to get the attributed quality, safety, and efficacy [158]. Table **4** shows the recent researches of intranasal lipid nanoparticulate systems.

Table 4. Recent examples of intranasal lipid nanoparticulate systems.

API	Target	Systems	Composition	Characterization	Model/compared parameter	Results	Ref.
Acyclovir	Systemic	Liposomes	DPPC, CHOL, PVP, and PEG 600	627.4 nm and 43.2% entrapment efficiency	Rabbits/ IV	The bioavailability of acyclovir has been increased to 60%	[170]

Fexofenadine	Systemic	Liposomes	DPPC, DPPG, CHOL, and chitosan	359 nm and 66.1% entrapment efficiency	Rat/ Oral	Chitosan-coated liposomes showed 5-fold higher bioavailability with slower release, lower C_{max}, and 1.3-fold higher $T_{1/2}$	[171]
Risperidone	Brain	Liposomes	Stearylamine and MPEG-DSPE	90-100 nm with 50-60% entrapment efficiency	Rats/ Pure drug IV bolus	PEGylated liposomes had 2.3-fold higher brain C_{max}, 1.7-fold higher $AUC_{0-\infty}$, 4 times shorter T_{max}, and 2.6 higher $T_{1/2}$	[113]
Rivastigmine	Brain	Liposomes	Lecithin, DDAB, and PEG-DSPE	478 nm and 48 % entrapment efficiency	Rabbits/ IN drug solution	The stealth liposomes showed 1.6-fold higher brain C_{max}, 5-fold longer T_{max}, 5.5-fold higher $AUC_{0-\infty}$, and 4.2-fold higher plasma $AUC_{0-\infty}$ compared to the IN drug solution	[172]
Donepezil	Brain	Liposomes	CHOL, PEG, and DSPC	102 nm and 84.9% entrapment efficiency	Rats/ Oral, IN free drug	The liposomes showed higher C_{max} for IN delivery with reduced T_{max}. Moreover, enhanced brain and plasma bioavailability were achieved as the liposomes had shown 2-fold higher plasma $AUC_{0-\infty,}$ 2-fold higher C_{max}, and 1.5-fold higher brain $AUC_{0-\infty}$ compared to the IN free drug	[173]
Astaxanthin	Brain	SLN	Stearic acid, Poloxamer 188, and lecithin	213.2 nm and 77.4% entrapment efficiency	Rats/ IV SLN	SLN showed 2-fold higher brain level after 1 h with lower blood level compared to the IV delivery	[174]
Quetiapine	Brain	SLN	glycerol monostearate and Span-80	117.8 nm with 97.5% encapsulation efficiency	Rats/ Tail IV, oral drug	The *in situ* gel of quetiapine showed similar blood and brain concentration as the IV delivery of the drug, but higher than the oral delivery	[175]
H102 Peptide	Brain	Liposomes	EPC, PEG-DSPE, and CHOL	112.2 nm and 71.35% encapsulation efficiency	Rats/ IN drug solution	Liposomes effectively delivered the peptide into the brain. The liposomes showed higher H102 concentrations at different brain regions with the maximum concentration being identified in the hippocampus	[176]
Galanthamine hydrobromide	Brain	Liposomes	PG, SPC, and CHOL	112 nm and 83.6% encapsulation efficiency	Rats/ Oral drug solution	The flexible liposomes showed 3.52-fold higher C_{max}, 3.36-fold higher $AUC_{0-\infty}$, and T_{max} shortened to half compared to the orally	[177]

						administered drug	
GDNF	Brain	Liposomes	DOPC, CHOL, and stearylamine	194 nm and 95% loading efficiency	Rat/GDNF solution in PBS	The liposomes showed 10-fold more GDNF delivery than the PBS with the same neuroprotective efficacy	[178, 179]
Haloperidol	Brain	SLN	GMS and Tween® 80	140 nm, 71% entrapment efficiency and 23% drug loading	Rats/ IN drug solution	SLN showed 3.6-fold higher brain C_{max} and 3.5-fold higher $AUC_{0-\infty}$	[180]
Protein antigen HBsAg	Vaccination	Liposomes	EPC, CHOL, and PAA	773 nm with 53.3% encapsulation efficiency	Mice/ IM	Gel core liposomes induced serum and mucosal immunity with comparative serum IgG to IM. Moreover, IN induced significant sIgA that IM failed to produce with significant 14th day boosting	[181]
Lipopeptide-based against Group A streptococcus	Vaccination	Liposomes	DDAB, DPPC, and CHOL	160 nm and 98% encapsulation efficiency	Mice/ IN unmodified peptide	The prepared cationic liposome containing the lipopeptide induced both mucosal and systemic immunity and a high level of titer after 5 months' post-immunization. Furthermore, high IgG and IgA titers were measured	[182]
OVA	Vaccination	Liposomes	DOTAP and DC-chol, CHOL	57-846 nm	Mice/ Nasal naked OVA	Liposomes were prepared by using DOTAP and DC-chol or by DOTAP and CHOL. The cationic liposomes induced a Th2 immune response with high levels of IL–4 expressions with adjuvant activity. DOTAP/DC-chol liposomes induced potent antigen-specific IgG serum responses that were superior to DOTAP/chol liposomes. Moreover, the liposomal activity was independent of particle size	[183]
DNA-hsp65	Vaccination	Liposome	EPC, DOTAP, and DOPE	244.5, 985.9 nm[a] 616.7, 2749.6 nm[b]	Mice/IM naked DNA	Liposomes contained DNA or were complexed with the DNA on the surface and produced a significant reduction in the number of bacilli in	[184]

						the lungs with 16-fold reduction in the required DNA amount. These liposomes were cationic with no toxic effects	
BSA as model antigen	Vaccination	Liposomes	SPC, DMPG CHOL, SA, and alginate, chitosan, and TMC	303-996.4 nm with 60-69% encapsulation efficiency	*Ex vivo*	The particle size was increased dramatically after coating with polymer. TMC amongst others showed the best mucoadhesive capabilities. However, the TMC-coated liposomes showed a low mucosal penetration due to due to their high particle size	[185]
Streptomycin sulfate	Brain and systemic	SLN	Compritol® 888 ATO, Tween® 80, and soy lecithin	140 nm and 54.8% entrapment efficiency	Mice/ IN Free drug	Streptomycin-SLN showed 3.15- and 11-fold higher brain and plasma concentrations and less accumulation in the kidneys, liver, and spleen with 3.3, 12, and 4 times lower concentrations, respectively, being observed	[186]
Rizatriptan benzoate	Brain	SLN	Lecithin, Pluronic® 127, and GMS	145-298 nm and 59-80% encapsulation efficiency	Rats/ IV free drug, oral marketed drug	The optimized rizatriptan SLN showed an enhanced $T_{1/2}$ and higher CSF concentrations by 1.3- and 5.46-fold compared to the IV and oral, respectively. The SLNs also showed a shortened T_{max}	[187]
Venlafaxine	Brain	NLC	Compritol® 888 ATO, and Capmul® MCM	75 nm and 81.4 % entrapment efficiency	*Ex vivo*	NLC of the venlafaxine showed a 1.5-fold higher flux and 1.5-fold higher diffusion coefficient compared to the free drug solution across the goat's nasal mucosa	[188]
Asenapine	Brain	NLC	GM and oleic acid	167.3 nm and 83.5% encapsulation efficiency	Rats/ IN free drug	NLC showed a higher brain concentration of Asenapine compared to the IV delivery for the drug with 1.8- and 2.7-fold C_{max} and AUC_{0-24}, respectively, being achieved. Moreover, Asenapine showed 276.7% brain bioavailability. There were marked increases of the antipsychotic effects	[189]

Olanzapine	Brain	NC	poly(ε-caprolactone) and Poly(MMA-b-DMAEMA)	254.9 nm and 99% encapsulation efficiency	Rats/ Olanzapine solution	The olanzapine-loaded amphiphilic methacrylic copolymer-functionalized PCL NC enhanced the amount of the drug in the brain (1.5-fold higher compared to the drug solution)	[190]
Loratadine	Local	Niosomes-*in situ* gels	CHOL, various Span surfactants, Carbopol® 934, and Poloxamer 407	266 nm with 89-97% drug content	*Ex vivo*	Niosomes were formulated into an *in situ* gel and they showed a high residence time and sustained drug release.	[191]
Melatonin	Systemic	Niosomes	CHOL, Span 60, and SDC	100 nm and 84-94% encapsulation efficiency	Rats/ IV melatonin solution	SDC increased encapsulation efficiency. The niosomes of melatonin with SDC showed 98.7% bioavailability compared to the IV delivery	[192]
Quetiapine	Brain	NE	Transcutol® P, Capmul® MCM, PG, and Tween® 80	144 nm and 91% drug content	Rats/ IV pure drug solution	QTP-loaded NE showed a 267.89 DTE% and 63.63 DTP%, thus the superiority of brain targeting.	[193]
Tramadol	Brain	NE	IPM, Soya lecithin, and Poloxamer 188	136.3 nm and 99.16% entrapment efficiency	Mice/ IV and nasal drug solution	Tramadol-loaded NE enhanced antinociception at most measurement time points compared to the nasal and IV solution. Moreover, NE showed 116.89 DTE% and 98.06 DTP% parameters.	[194]
Quercetin	Brain	NE	Oleic acid, PEG 400, Tween® 80, Labrasol®, and Transcutol® HP	91.6 nm and 99.8% drug content	Rats/ IV NE	Quercetin-NE improved neurobehavioural activity and reduced infarction volume effects in middle cerebral artery occlusion. Moreover, 4.8-fold higher brain C_{max} and 5.3-fold higher brain AUC_{0-t}, 9333.3% DTE, and 2181.8% DPT were achieved	[195]
Thymoquinone	Brain	NE	Oleic acid, Carbitol™, Tween® 20, Labrasol®, and Cremophore EL	94.8 nm and 99.9 drug content	Rats/ IV solution	The mucoadhesive NE improved the neurobehavioural activity in middle cerebral artery occlusion and showed 628.6 DTE and 90% DTP brain parameters for thymoquinone	[196]

Saquinavir mesylate	Brain	NE	Capmul® MCM, Tween® 80, and PEG 400	176 nm with 96% drug content	Rats/ IV NE	NE showed 2919.3 DTE% and 96.6 DTP%, suggesting brain targeting	[197]
Sertraline	Brain	NE	Capmul® MCM, Tween® 80, and PEG	78 nm whilst the drug content was not recorded	*Ex vivo*	*Ex vivo* showed a 62% nasal absorption for sertraline through a goat's nasal mucosa within 4 h	[198]
TNFα siRNA	Brain	NE	Flaxseed oil, DOTAP, Lipoid E80, and Tween® 80	69-166 nm and 70% encapsulation efficiency	Rats/ Naked siRNA	5-fold higher brain uptake and the NE of TNFα siRNA markedly reduced the unregulated levels of TNFα in an LPS-induced model of neuroinflammation	[199]
OMP antigen-Burkholderia cenocepacia bacteria	Vaccination	NE	The compositions were not recorded. However, NE was supplied by BlueWillow Biologics (Michigan, USA)	Not recorded	Mice/ OMP-PBS	OMP-NE-loaded antigen elicited high OMP-specific IgG antibodies with response to booster immunisation (13-30-fold higher than OM-PBS). Also a high rate of pulmonary clearance of bacteria was observed	[200]
OVA antigen	Vaccination	NE	Oleic acid, mannide monooleate, and Tween® 80	153 nm whilst the content of the OVA antigen was not recorded	Mice/ OVA antigen	Oleic acid NE showed high IgA and serum IgG for the 45th day and induced mucosal immunity with single booster immunization	[201]
W805EC	Vaccination	NE	NE was supplied by BlueWillow Biologics (Michigan, USA)	424-774 nm whilst the content of the W805EC was not recorded	Mice/Vaccine in phosphate buffer	The NEs showed that high hemagglutination titers of serum and high influenza-specific IgG and IgA titers, also high IgA levels in the bronchoalveolar lavage were achieved in comparison to the vaccine in the phosphate buffer. However, NEs with 1:6 ratio of cationic-to-non-ionic surfactants and 450 nm globule size elicited significantly higher influenza-specific IgG serum antibody titers than any other formulation	[202]
Recombinant hepatitis B surface antigen (HBsAg)	Vaccination	NE	NE was supplied by BlueWillow Biologics (Michigan, USA)	349 nm whilst the content of the HBsAg was not recorded	Mice/Antigen in PBS	Robust and sustained systemic IgG, mucosal IgA, and strong antigen-specific cellular immune responses were observed. Moreover, this vaccine induced a Th1 associated cellular immunity	[203]

Abbreviations: DPPC, L-α-dipalmitoylphosphocholine; CHOL, cholesterol; PVP, polyvinylpyrrolidone; PEG, polyethylene glycol; DPPG, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol; DSPE-PEG, distearylphosphatidylethanolamine-mPEG; DDAB, Didecyldimethyl ammonium bromide; DSPC, 1,2-distearyl-sn-glycero-3-phosphocholine; EPC, egg phosphotidylcholine; PG, propylene glycol; SPC, Soya phosphatidyl-choline; DOPC, dioleoylphosphatidylcholine; PBS, phosphate buffer saline; GMS, Glyceryl monostearate; PAA, poly acrylic acid; DOTAP, 1,2-dio- leoyl-3-trimethylammonium-propane; DC-chol, 3β-[N-(N',N'-dimethylaminoethane)-car- bamoyl]; MMA, methyl methacrylate; DMAEMA, 2-(dimethylamino)ethyl methacrylate; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; SPC, soy phosphatidylcholine; DMPG, phospholipid dimyristoyl phosphatidylglycerol; TMC, trimethyl chitosan; SDC, sodium deoxycholate; IPM, isopropyl myristate; LPS, lipopolysaccharide; GDNF**,** Glial cell-line derived neurotrophic factor; OVA, Ovalbumin; PBS, phosphate-buffered saline; IL-4, Interleukin 4; Th2, T helper cells 2; min, minutes; h, hour; IN, intranasal. **Notes:** [a] entrapping DNA-hsp65, [b] complexing DNA-hsp65

4.3 Intranasal Polymeric-Based Systems

Polymeric-based nanoparticles have become one of the most applied methods for drug delivery due to their characteristics of high drug loading, stability, and a variability of loading substances, including peptides, vaccines, and genes in addition to surface modification possibilities and the ability of controlled release. Polymeric-based nanoparticles include degradable and nondegradable polymers as well [204].

4.3.1 Biodegradable nanoparticles (BNPs)

Many polymers have been introduced for the intranasal delivery of nanoparticles like polysaccharides (chitosan); polyester derivatives, such as polylactic acid (PLA) and poly (lactide-co-glycolide) PLGA; proteins (Lectins); poly(ethylenimines); and poly(alkylcyanoacrylates). The selection of the proper type and modifications depend on many factors, such as encapsulation efficiency, particle size, and stability. Amongst these polymers, both chitosan and PGLA have been found to be the most promising. Both polymers are safe according to the FDA and are characterized by biocompatibility, biodegradability, and the ability for encapsulation of a wide range of hydrophilic, lipophilic, small, and large molecules with protection capabilities, in addition to the possibility of the modification to improve the BNPs properties and their interactions with the biological materials [205,206].

The positive charge of the chitosan derivative is of high value for the intranasal delivery through increasing the contact time with the mucosal tissue. Its contribution in intranasal delivery has been proved via many types of research that have covered many aspects. Levodopa-chitosan-loaded nanoparticles were formulated as a thermoreversible gel for brain targeting. 74% of the drug was retained in the brain. However, gel formulation may hinder delivery due to its high viscosity and its effects on the ciliary beating [207,208].

Intranasal delivery of PLGA-tarenflurbil nanoparticles can prevent the elimination of this drug as a possible drug candidate for Alzheimer's treatment when its poor BBB penetration was responsible for its failure in phase III clinical trials [209]. Muntimadugu *et al* [210] showed that intranasal delivery of PLGA nanoparticles successfully targeted the brain with a 4-fold higher tarenflurbil brain concentration than with oral delivery and 1.5-fold higher than in the case of IV delivery. This study also showed the superiority of polymeric nanoparticles over SLNs in terms of loading efficiency and brain delivery. However, SLNs are still an option for direct drug to brain delivery.

The variability of nanoparticles to include mRNA possesses a great value for vaccination against tumors. The nasal delivery of cancer vaccination was tested by Phua *et al* [211] on mice. The results showed delayed tumor progression in both prophylactic and therapeutic models compared to the naked delivery. This effect can be attributed to the protection against enzymes and an efficient delivery as expressed by 24-hour-long luciferase expression. In the same field, Matsuo *et al* [212] reported the effect of ovalbumin- poly(γ-glutamic acid) nanoparticles. The results showed the activity of nanoparticles against E.G7-OVA tumor cells; tumor growth was suppressed and survival time was enhanced in mice models. Moreover, the inhibitory effect was extended to lung metastasis in a similar way as with subcutaneous (SC) injections. In another research, siRNA-loaded chitosan nanoparticles with 141 nm size and 81% encapsulation capacity targeted Galectin 1 (Gal1), the potent immunosuppressive

protein regulator in glioblastoma that induces the apoptosis of T cells and is the main contributor to glioblastoma resistance against temozolomide. The results showed a significant reduction of Gal 1 in both murine and human tumor cells with the subsequent reduction in Gal 1 mobility. The *in vivo* studies showed the applicability of the siRNA delivery to the brain via intranasal nanoparticles with a significant reduction of Gal 1. Chitosan offered the rapid attachment of the cells and protected the siRNA from RNAse degradation [213].

4.3.2 Dendrimers

Dendrimers are highly branched three-dimensional nanomaterials which have been introduced as drug carriers either via covalent conjugation with the drug molecule or drug (guest) hosting. These two options are attributed to their structure; they contain a large number of surface functional groups and a hydrophobic cavity that can enhance drug penetration through the mucosal membranes [214]. The advantages of dendrimers include internal cavity, particle size, and morphology control, in addition to their solubility enhancement, which allows the use of dendrimers for many drug formulations. Moreover, modified dendrimers have been targeted as nanomedicine against tumors, viral, and bacterial infection particles. Some of these products have been marketed successfully [215–218].

The first and most reported dendrimer type used in intranasal delivery was polyamidoamine (PAMAM). Kim *et al* [219] investigated using PAMAM as a siRNA gene carrier for the high mobility group box 1 (HMGB). The results of the reduced brain infraction volume were a proof for the activity of siRNA dendrimers as an efficient knock-down of HMGB [212]. In another study, the PAMAM formulation of haloperidol intended for brain targeting showed 100-fold higher solubility with significant brain and plasma concentrations, resulting in a 6.7-fold smaller dose being required to produce a response similar to the intraperitoneal (IP) injection [220]. Additionally, PAMAM showed substantial effects on the nasal absorption of poorly absorbed molecules, namely calcitonin, insulin, and fluorescein isothiocyanate-labelled dextran, as it was pointed out via *in vivo* studies using a rat model [221]. Both plasma concentration and AUC were increased. This study revealed a positive relationship between PAMAM molecular weight and its delivery enhancing effect. Beyond the benefits, toxicity is the major challenge for dendrimers. Toxicological effects are related to the generation number, concentration, contacting time, and is connected to their absorption enhancement capacity [222].

The application of dendrimers in intranasal delivery should be evaluated in regard to the host-guest relationship with paying attention to the guest properties, such as hydrophobicity and size for efficient binding or encapsulating. Furthermore, possible toxicity must be addressed cautiously [223,224].

According to the scientific literature, polymeric-based systems can meet the need for efficient intranasal delivery. Table **5** displays the most recent studies of these systems.

Table 5. Recent examples of intranasal polymeric-based nanoparticles.

API	Target	System	Composition	Characterization	Model/compared parameter	Observations	Refs .
Olanzapine	Systemic	BNP	Chitosan and TPP	208-322 nm and 87% encapsulation efficiency	Rabbits/ IV free drug	Olanzapine-loaded chitosan showed 51% absolute bioavailability and T_{max} similar to the IV	[225]
Donepezil	Brain	BNP	Chitosan and TPP	150-200 nm	Rats/ Free drug suspension	Brain C_{max} and $AUC_{0-\infty}$ were enhanced by 3.7- and 1.57-fold, respectively.	[226]
Acetazolamide	Brain	BNP	Chitosan and TPP	153-277 nm	*Ex vivo*/ Free drug	Maximum 64% of acetazolamide was released within 4 h and no toxicity was observed	[227]

Olanzapine	Brain	BNP	PLGA and Poloxamer 407	91 nm and 68.9% entrapment efficiency	Rats/ IV and IN drug solution	The nanoparticles showed brain concentration 6.35-fold higher than the IV and 10.86-fold higher than the IN delivery of the drug solution after 3 h	[228]
Lorazepam	Brain	BNP	PLGA and Poloxamer 407	153.7 nm and 83.8% drug entrapment	Rats/ IV and IN free drug solution	Lorazepam-PLGA-loaded NPs showed higher brain/blood ratios at all sampling time points compared to the delivery of the drug solution	[229]
Rotigotine	Brain	BNP	PEG, PLGA, and Lactoferrin	122 nm and 19% conjugation efficiency	Rats/ IN NPs-PLGA	The modified nontoxic NPs showed heterogeneous brain distributions and higher targeting than the unconjugated NPs	[230]
NT-I	Brain	BNP	PLA and sodium cholate	65 nm and 35.5% entrapment efficiency	Rats/ IV NPs-PLGA and IV of NT-I	PLA-NPs of NT-I displayed lower brain T_{max} and higher C_{max}. The AUC_{0-4h} values of IV-NP and IN-NP were 196% and 160%, respectively	[231]
Didanosine	Brain	BNP	Chitosan and TPP	269-382 nm, 9.1, and 47.3% loading efficacy and 90.7, 94.6% encapsulation efficiency for 10 and 50% didanosine theoretical loading, respectively	Rats/ IV and IN solution	The NPs had CNS/ systemic distributions of 1.9, 2.5-3.3, and 8.1-8.9 for the brain, olfactory bulb and CSF, respectively. The NPs also showed 70.9% and 38.9% bioavailability compared to the IV and IN delivery of the drug solution, respectively	[232]
Carboplatin	Brain	BNP	PCL and PVA	311.6 nm and 27.95% entrapment efficiency	Rats/ *In situ* nasal solution	*In situ* nasal studies demonstrated better nasal absorption for carboplatin from the NPs than the drug solution	[233]
Piperine	Brain	BNP	Chitosan, TPP, and Poloxamer 188	248.5 nm and 81.7% encapsulation efficiency	Rats/ IP donepezil pure drug and blank NPs	Significant cognitive function improvement as with donepezil with the antioxidant and acetyl choline esterase inhibitor	[234]
bFGF	Brain	BNP	STL, PEG, and PLGA	118.7 nm, 69.21% encapsulation and 0.0462% loading efficiency	Rats/ IV and IN- bFGF	The conjugated NPs showed 1.79–5.17 and 0.61–2.21-fold higher brain concentrations than the IV and IN delivery of the unmodified NPs, respectively. The modified solution also demonstrated an	[235]

						improved cognitive function	
Rasagiline	Brain	BNP	Chitosan glutamate and TPP	151.1 nm and 96.43% encapsulation efficiency	Mice/ IV and IN rasagiline	The rasagiline NP had 2.8 and 1.7 higher brain concentrations than the IV and IN, respectively. DTP was only 69.27%	[236]

Abbreviations: NP, nanoparticle; bFGF, Basic fibroblast growth fact; IP, intraperitoneal; CSF, cerebrospinal fluid; STL, Solanum tuberosum lectin; NT-I, Neurotoxin-I; PCL, polycaprolactone, PLA; polylactic acid.

Biodegradable polymeric NPs are mainly based on chitosan and PLGA polymers. Their particle sizes are mostly in the range of 50-400 nm with high encapsulation efficiency. Moreover, modifications of the nanoparticles are common procedures and mainly applied by PEG and other materials, such as PLA and Poloxamer. These modifications are useful in achieving the delivery goals that have been determined.

4.4 Topicalities of Intranasal Nanoparticles on the Pharmaceutical Market

In the last decades, intranasal products for systemic delivery have been marketed successfully. Examples of such products are zolmatriptan (Zomig®, Impax Laboratories Inc, USA) and sumatriptan (Imigran®, GlaxoSmithKline, UK) for treatment of migraine, fentanyl (PecFent®, Kyowa Kirin Services Ltd, Japan) for treatment of breakthrough pain in cancer and nicotine (Nicorette®, McNeil AB, Sweden) for smoking cessation. Moreover, various nasal peptide products are currently on the market, such as calcitonin (Miacalcin, Novartis®, USA) desmopressin (Desmospray®, Ferring, UK), buserelin (Suprecur®, Sanofi-Aventis, UK) and nafarelin (Synarel®, Pfizer Service, Belgium) oxytocin. On the other hand, an influenza virus vaccine has been introduced (Flumist®, Medimmune, USA).

Despite the significant research efforts in the nanoparticles field, there is still no FDA approved nanoparticle-based product for intranasal delivery. Nanoparticulate systems for nasal delivery represent a relatively new approach. Despite the unavailable data from company pipelines about the clinical trial and development of these products, BlueWillow Biologics, Inc. showed some clinical trials for NEs as vaccines (Table **6**). Research in this area should be continued at an even higher pace to investigate the possibilities of introducing nanotechnology as a delivery system via the nasal route for different targets.

Table 6. Examples of intranasal NEs for clinical consideration.

Target	Status
Seasonal influenza	Phase1
Pandemic influenza	Preclinical
RSV	Preclinical
Anthrax	Preclinical
Pertussis	Preclinical
HSV-2	Preclinical
Chlamydia	Preclinical
Abbreviation: RSV, respiratory syncytial virus	

5. REGULATORY ASPECTS AND PATIENT EXPECTATIONS OF NASAL DELIVERY

5.1 Regulatory Aspects and QbD Implementation in Nasal Dosage Form Developments

Prior to the development of intranasal formulations (similarly to other administration routes as well), the objectives, materials, methods, delivery systems, and expected outcomes should be identified clearly to end up with a product that can compromise between patients' demands and industrial expectation alongside with the regulatory guidelines of the FDA or EMA [237–239].

In 2005, the FDA enforced the submission of QbD with new drug application requests. The QbD approach provides a holistic view that can help in understanding and controlling the variables from the material selection to the scale up and commercialization of a medicinal product. Such designs offer the rewards of transferring the chemistry manufacturing control

(CMC) of the new abbreviated drug into the pharmaceutical quality assessment, thus saving the time of development and submission, saving the time of regulatory authorities' approval, and defining the probability of out of specification and out of tolerance [240].

To implement QbD methodology, the quality target product profile (QTPP) must be defined initially. QTPP describes the profile of the drug delivery that is aimed to be reached; this will give the framework for the further adjustments during the development process. QbD-based development, later on, identifies the relations of the critical quality of attributes (CQAs), critical material attributes (CMA), and critical process parameters (CPP) with the product properties and control strategy according to ICH guidelines Q8, Q9, and Q10 to assess the product quality by referring to its efficacy and safety. For QbD, it is essential to apply a risk assessment and evaluation of the effects of the variables on production or the effects of the materials on safety and stability, thus it can help in the determination of the CQA and CMA, which highly affect the quality of the final product.

The application of QbD for intranasal nanoparticles has been introduced by Pallagi *et al* [241]. This study pointed out the importance of the risk assessment-based QbD on the early stage production of intranasal meloxicam nanosuspension by co-grinding with PVP-C30 stabilizer. The application of QbD provided an indication about the effects of parameters on the product quality, thus the researchers prioritized the study for the parameters of the greatest influence. Accordingly, the investigations revealed the importance of CPP (grinding time, rotation speed and meloxicam: PVP-C30 ratio) on CQA, particularly, particle size and dissolution rate. The optimization of 2 h grinding time, 400 rpm rotation speed and 1:1 meloxicam: PVP-C30 was able to produce a nanosized product with 140 nm particle size and showed approximately 100% dissolution rate in the first 15 min. The nanosized product was formulated into nasal hydrogel with sodium hyaluronate and it showed C_{max} at 5 min. Shah *et al* also applied the QbD approach for optimization rivastigmine SLN using homogenization and ultrasonication method. In this study, the authors set low particle size, low particle size distribution and high entrapment efficiency as CQAs. The effects of independent variables (drug: lipid ratio, surfactant concentration and homogenization time) on the previously determined CQAs were evaluated and a space design was built to determine the optimized formulation. The optimized formulation showed 82.5 particle size, 0.132 polydispersity index and 66.8% entrapment efficiency, also it showed 65.9% *ex vivo* diffusion compared to 32.8% for the drug's solution. In another study, Shah *et al* reported the use of QbD and risk assessment and optimization of venlafaxine-loaded nanostructured lipid carrier. QbD was built and the CQAs were identified. From the design space, the optimized formulation was characterized by a particle size of 77 nm, a particle size distribution of 0.234 and 81.3 % entrapment efficiency. Moreover, it displayed a higher diffusion rate of 14.2 mg/cm^2/h through nasal mucosa in comparison to 9.62 mg/cm^2/h for the solution [188,242].

In accordance with intranasal literature, QbDs have been applied mainly to the formulations rather than the final product. Chudiwal *et al* reported the development of budesonide suspension nasal spray with QbD as a case study. In this study, the delivery device variables were recognized alongside the material and process variables [243].

Nanoparticles can be formulated into different dosage forms, such as liquids, gels, sprays, aerosols, and powders. The selection of the dosage forms depends on the drug type and formulation properties, the required effects and the targeted patient population. All traditional and new olfactory region targeted technologies, such as bidirectional (Optinose)®, Controlled Particle Dispersion (CPD)® (Kurve), and Pressurized Olfactory Device (POD) can widen and specify the intranasal delivery for efficient outcomes [244,245].

Paying attention to the delivery system to meet the challenges of proper deposition and nasal cavity geometrical barriers is highly required. All technological parameters such as plume geometry and applied force directly affect the deposition of drugs; whereas, particle size has massive effects on the deposition as large particles tend to deposit in the anterior part without deep penetration.

The used delivery system must meet the FDA and EMA guidance requirements for nasal formulations, especially related to reproducibility and dose uniformity, besides the efficiency for delivering the formulation into the suitable regions. Such guidance offers great management for the quality aspects of nasal products and clarifies the requirements for regulations and the industry [237,246].

Fig. (**2**) represents the Ishikawa diagram. It shows the most important CPP and CMP, and the factors affecting drug products; whereas Fig. (**3**) shows the general flow chart for designing intranasal formulations. One can conclude from the complexity of the job (nanotechnology-based intranasal formulation), as illustrated in the figures, that a careful selection of the composition and the preparation method should be performed together with an initial risk assessment in order to directly get a product with the required quality parameters.

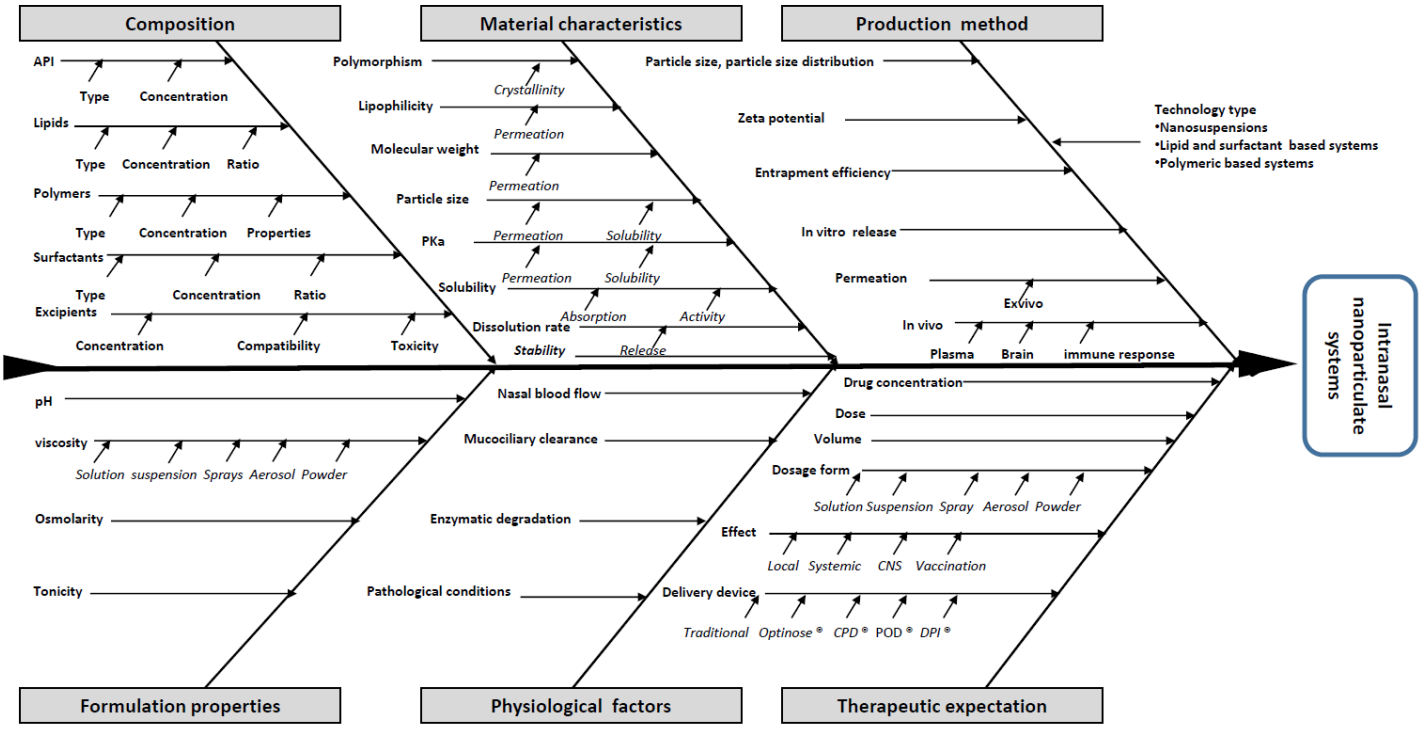

Fig. (2). Ishikawa diagram illustrating the parameters influencing the quality of an intranasal nanoparticulate system.

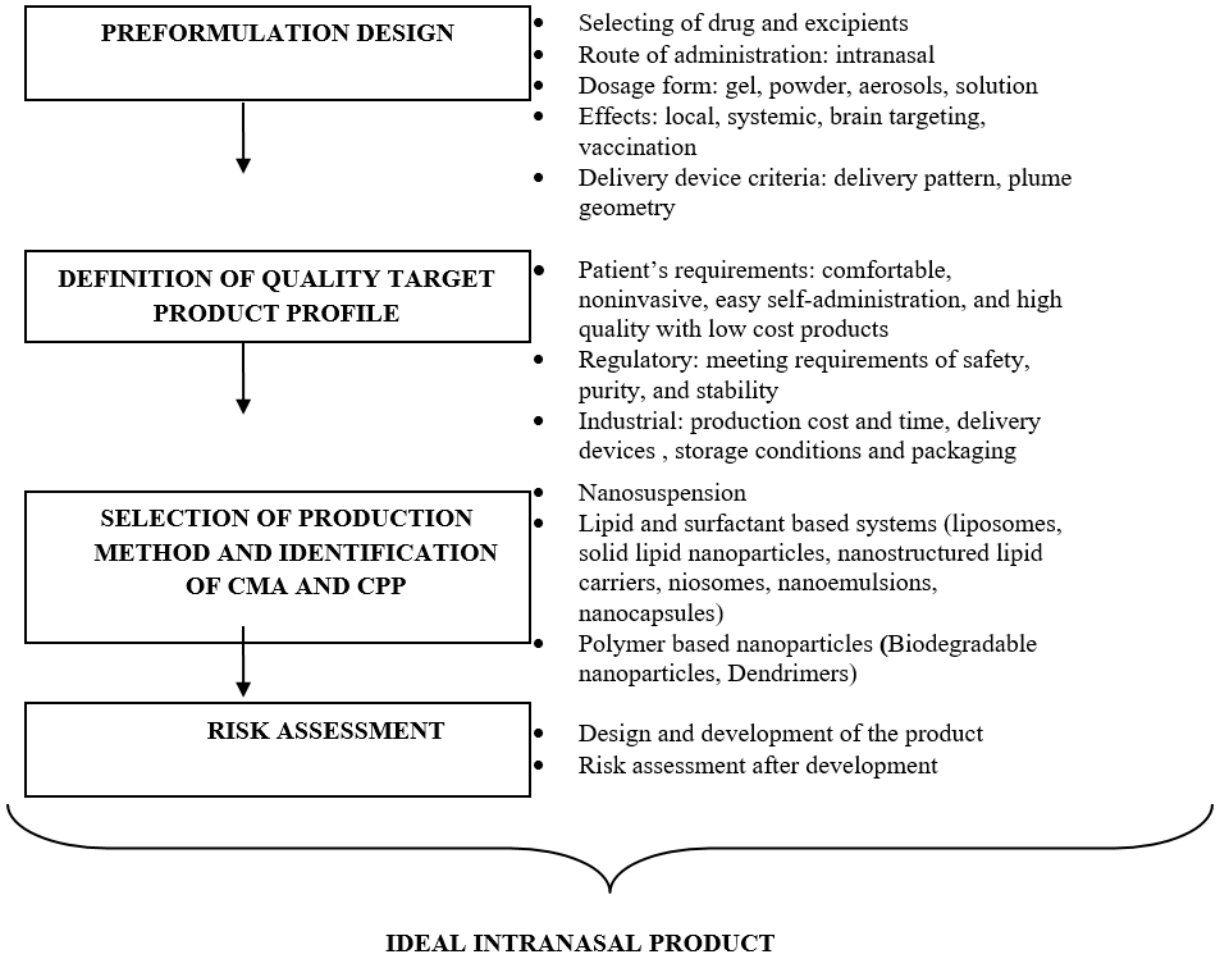

Fig. (3). General method development strategy for intranasal nanoparticulates.

5.2 Evaluation of Patients' Expectations of Intranasal Delivery

Patients -as users of the medicinal products- have an increasing role in therapeutic success. Identifying what is expected or is critical for patients and reflecting these aspects during research and development is the first step to achieve patient acceptance and the required therapeutic outcome. This field has been ignored for many years, but as the effects of customers' preferences increase, it should be considered more as part of R&D thinking [247–249].

Patient requirements have extended the need for safe and efficient drug delivery to other concerns such as the comfort of both formulation and applicator device in the nasal cavity, the ease of application, confidence in the delivered amount, and a warning about the remaining dose (dose counting). All these factors can enhance patients' satisfaction and, therefore, their adherence [250,251]. In order to improve efficiency and productivity, manufacturers must understand the best tools, methods, and analysis. They have to define their goals on the basis of the patients' voices before proceeding into the production stage. Integrating the voice of the customer can help to assess the patients' convenience for their product and induce manufacturers to develop patient-friendly products. These considerations have not been kept in the theoretical framework or companies, but have transferred to the regulatory agencies that seriously consider patients' preferences and their assessments of using formulations and delivery devices [252].

6. EXPERT OPINION

The successful delivery of intranasal nanoformulations depends on many mutually dependent parameters**:** the selection of the nanotechnology method that can provide accepted encapsulation capacity and protection, the active pharmaceutical agent, the nanoparticulate system and the formulation properties, and the proper delivery system for the intended purposes**.** It is not an easy task to compromise between all aspects. Whilst various successful nanotechnology-based formulations have reached the market, intranasal products are still not available. Based on the previous literature and market research works, most marketed nasal products are available as nasal sprays and aerosols for local delivery, whilst the number of systemic and brain targeting products is lower and only one nasal vaccine is available.

The utilization of nanotechnology in intranasal delivery has shown promising results. The majority of the studies has focused on the efficiency of the delivery and how to modify the system to achieve acceptable outcomes, whilst the potential toxicity of these systems has not been adequately revealed. Lipid-based systems represent a high percentage of the studies. On the other hand, polymeric nanoparticles have focused mainly on chitosan, PLGA, and their modifications. Although the marketed products and patents for nanotechnology-based products are low, nanoemulsion adjuvant vaccines are under different clinical trials.

Nanoparticles/ nanocrystals have shown effective systemic, brain targeting, and vaccine delivery. However, it is not yet fully figured out if nanoparticles/ nanocrystals enhance delivery due to their design properties or due to their effects on residence time enhancement or/ and protection of the encapsulated agents against enzymatic degradation and the pg-efflux system.

The proper selection of the best system should not be separated from the final goal of providing a product combined for efficiency, safety, stability, and patient acceptance for acute and chronic use. Due to complexity, the implementation of QbD can offer a tool for early-stage assessment for constituents, methods, and delivery system selection in addition to the possibility of risk assessment before drug development. It is highly recommended to be adopted.

7. CONCLUSION AND PROSPECTIVE FUTURE

Intranasal delivery has shown the possibility of being a simple and direct method that can replace many traditional routes. Local delivery offers higher efficiency and lower side effects, systemic targeting can improve the bioavailability of many agents and nasal vaccination produces rapid mucosal and systemic immunity that has not been achieved by the parenteral route. Nanoparticles showed the advantages of drug protection, enhancement of contact time, enhancement of drug solubility, ability of being easily functionalized and using of GRAS excipient. Therefore, they have the potential to overcome the traditional limitation of the nasal delivery. The suitability of intranasal nanoparticles for brain targeting and bypassing the blood brain barrier has demonstrated a way for treatment of unresolved CNS conditions and opened a new scope for treatment of aggressive brain tumors either by drugs or vaccine delivery. The intranasal administration of nanoparticles for a systemic effect showed effective results with regard to bioavailability, plasma maximum concentration, and time to reach the maximum concentration. Moreover, different studies on nanoparticle-based vaccines displayed the ability of these systems to elicit mucosal and systemic immune responses with adjuvant activity.

The successful formulations can map the future of intranasal delivery. However, there are still many challenges to face. Increasing knowledge of nanotechnology is the first step towards successful delivery. The two-branch approach of utilizing nanoparticles coupled with intranasal delivery can provide the opportunities for efficient and convenient drug (vaccine) delivery. Accordingly, the future decades will most likely witness the production of intranasal formulations that overcome the current limitations.

Declaration of interest

The authors state no conflict of interest and have received no payment for the preparation of this manuscript.

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