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Nanosystems in nose-to-brain drug delivery: a review of non-clinical brain targeting studies

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Abstract

The treatment of neurodegenerative and psychiatric disorders remains a challenge in medical research. Several strategies have been developed over the years, either to overcome the blood-brain barrier or to achieve a safer or faster brain delivery, one of them being intranasal (IN) administration. The possibility of direct nose-to-brain transport offers enhanced targeting and reduced systemic side effects. Nevertheless, labile, low soluble, low permeant and/or less potent drugs might need a formulation other than the common solutions or suspensions. For that, the formulation of nanosystems is considered a promising approach, since it can protect drugs from chemical and/or metabolic degradation, enhance their solubility, or offer transport through biological membranes. However, the understanding of the factors promoting efficient brain targeting when using nanosystems through the nasal route is currently patchy and incomplete.

The main purpose of the present review was to evaluate the association between brain delivery efficacy (in terms of brain targeting, brain bioavailability and time to reach the brain) and nanosystem type. For that, we performed a systematic bibliographic search and analysis. Furthermore, study designs, nanosystem properties, and reporting quality were also analyzed and discussed.

It was found a high heterogeneity in of how pre-clinical brain targeting studies have been conducted, analyzed and reported in scientific literature, which surely originates a significant degree of bias and data dispersion. This review attempts to provide some systematization recommendations, which may be useful for researchers entering the field, and assist in increasing the uniformity of future reports.

The analysis of literature data confirmed that there is evidence of the advantage of the IN route (when compared to the intravenous route) and in using carrier nanosystems (when compared to IN solutions) for brain delivery of a large set of drugs. Among the most represented nanosystem classes, microemulsions had some of the lowest values in pharmacokinetic ratios, while polymeric micelles had some of the best. Nevertheless, brain targeting efficacy comparisons between nanosystem groups had little statistical significance, and the superiority of the polymeric micelles group disappeared when nanosystems were compared to the respective IN drug solutions. Some drugs reached the brain so efficiently, even as drug solutions, that further benefit from formulating them in nanosystems became less evident.

Keywords

Brain targeting, drug delivery, intranasal, *in vivo*, nanosystem, pharmacokinetics.

Contents

Abbreviations

ATCC - anatomical therapeutic chemical classification; AUC - area under the curve; BBB - blood-brain barrier; $B\%_{brain\ IN/IV}$ – comparative brain bioavailability (IN vs IV); DTE% - drug targeting efficiency; DTP% - direct transport percentage; IN - intranasal; IV - intravenous; NLC - nanostructured lipid carriers; PDI - polydispersity index; PK - pharmacokinetic; PM - polymeric micelles; PN - polymeric nanoparticles; $RB\%_{brain}$ - relative brain bioavailability; RDTE% - relative drug targeting efficiency; RDTP% - relative direct transport percentage; r_S - Spearman's correlation coefficient; r_{xy} - Pearson's correlation coefficient; SEM - standard error of the mean; SLN - solid lipid nanoparticles; Tmax - time required to reach maximum concentration

1. Introduction

Neurodegenerative and psychiatric disorders have always been an important focus of medical research due to their high incidence and severe consequences, including disability and death. For example, Alzheimer's disease and other dementias were among the top 10 causes of death in 2015, with 1.57 million deaths globally, and schizophrenia has been estimated to affect approximately 1% of the general population worldwide [1,2]. Given the chronicity and rapid deterioration induced by neurodegenerative diseases, and the ongoing aging of the western world population, the attention given to these disorders has further increased in the last few years. However, the treatment of said pathologies is quite complex, in part due to challenges in drug delivery.

The blood-brain barrier (BBB) is the most important physical barrier in brain drug delivery, being estimated that all macromolecular compounds, and over 98% of low molecular weight drugs, are unable to permeate it, leading to a very low drug bioavailability at the desired target site. Among the strategies to surpass the BBB's low permeability to non-lipophilic drugs, it is possible to consider the bypass offered by the nasal route, commonly called intranasal (IN) delivery.

The pathways of drug distribution from the nasal mucosa to the brain have been repeatedly and extensively reviewed over the years (for recent reviews see for example [3–5]). Put simply, the nasal cavity can be divided in the respiratory and the olfactory regions. In the respiratory region, drugs can either enter the systemic circulation or be directly transported to the brain tissues through the trigeminal nerve pathway. In the olfactory region, drugs can be transported or diffuse directly to the brain through the olfactory mucosa pathway, considered the most important direct pathway [3,5]. Of course, only part of the drug will likely reach the brain, since a fraction might be locally lost due to mucociliary clearance or enzymatic degradation. Moreover, part will be absorbed to the systemic circulation, distributed to non-target tissues and finally eliminated. Nonetheless, with lipophilic drugs, a fraction of what reaches the systemic circulation will still reach the brain through the BBB (indirect pathway) (Fig. 1).

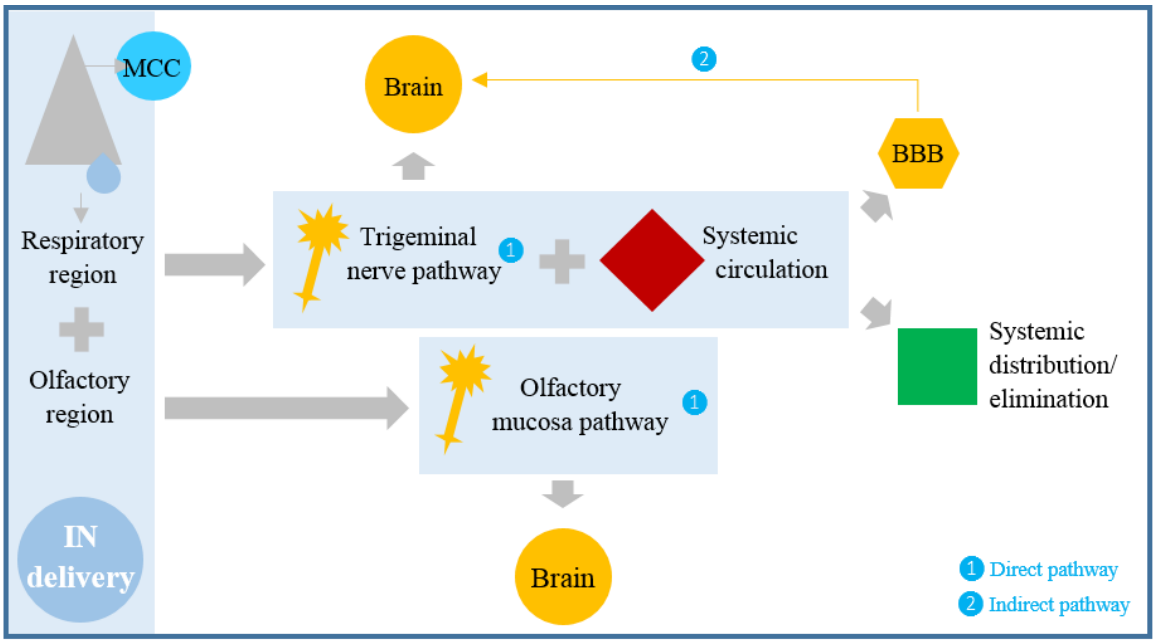

Fig. 1. Schematic representation of intranasal (IN) delivery's direct and indirect pathways. After instillation, the drug can either: a) suffer mucociliary clearance (MCC); b) reach the respiratory region, where it will either enter the systemic circulation or be directly transported to the brain through the trigeminal nerve pathway (direct pathway); or c) reach the olfactory region, where it will be transported to the brain through the olfactory mucosa pathway (direct pathway). The drugs that reach systemic circulation can be distributed to non-target tissues, followed by elimination, or still reach the brain through the blood-brain-barrier (BBB) (indirect pathway).

IN delivery can, however, offer advantages even in the delivery of BBB-penetrating drugs. Researchers working on IN delivery frequently cite the disadvantages of classic administration routes, such as those related with the invasiveness of parenteral routes (involving injections); the variable bioavailability, slower onset of action, and low patient compliance of the rectal route; or the unsuitability of the transdermal route in cases of emergency (passive delivery systems) [6–8]. In what concerns the oral and oromucosal routes, the limitations are related with altered physiological states of individuals (such as nausea and vomiting, or the hypersalivation and inability to swallow that happen during an epileptic seizure) [3,4,9–11], large inter-subject absorption rate variability, slow onset of action, drug-drug and drug-food interactions and cytochrome P450 induction [8,10,12–17]. Moreover, IN delivery offers the possibility of avoiding hepatic first pass metabolism and can reduce drug distribution to non-targeted sites, thereby minimizing systemic adverse effects [8,18–24].

Even though the nasal route usage in human medicine has been mostly limited to locally acting agents, in recent years a few systemically acting nasal drug products have been commercialized for menopausal symptoms, pain control, endometriosis and migraine headache. In addition, researchers exploring the IN delivery route have also focused on pharmaceuticals intended to treat neurodegenerative and psychiatric diseases [3].

Current marketed nasal preparations are mainly in the form of solutions or suspensions, but labile, low soluble, low permeant and/or less potent drugs might need a formulation that promotes drug bioavailability and, preferably, brain targeted delivery. For that purpose nanotechnology may provide some assistance. As the name suggests, all drug-carrier nanosystems are characterized by a very small size (although the upper limit is controversial, it is never over 1 µm), which can be suited to transport drugs to target tissues and cells, where they will ideally release their cargo. The authors give several reasons for their choice of this formulation strategy: protection of drugs from metabolism and/or chemical degradation; reduction of the issue of high plasma protein binding (in cases where the nanosystem itself might be absorbed); increased drug solubility and diffusion through biological membranes; increased half-life and reduction of P-glycoprotein efflux in the BBB [12,13,21,25–28]. These effects, by their turn, can result in enhanced brain targeting and/or bioavailability, and minimization of overall toxicity due to dose reduction, and increase the patient's compliance [4,25,26,29]. However, IN nanosystems could improve the drug's concentration in non-targeted areas of the brain, leading to possible neurotoxicity. Furthermore, the carrier systems themselves might originate safety concerns. Pulmonary complications have been reported for aerosol particles sized < 5 µm, due to aspiration to the lungs [4–6,30]. Consequently, each delivery system must be considered individually, and with caution.

IN delivery has other general requirements: the pH of the preparation should be similar to the human nasal mucosa's (5.0 to 6.5), in order to avoid irritation; tonicity should be high enough to promote absorption, but not so elevated that it causes toxicity to the nasal epithelium or enhances mucociliary clearance; careful consideration should be given to the preparation's viscosity, as a more viscous preparation enhances contact time with the nasal mucosa, which can increase permeation, but on the other hand might also reduce absorption because of decreased drug diffusion. Furthermore, it also has limitations: only a low volume can be administered (with the human nasal cavity usually retaining up to 200 µL), and therefore needing a relatively potent drug; it requires biocompatible excipients; and must not have aggressive associated odors [3,4,6]. All these aspects must be taken into consideration when formulating for IN administration.

Many variables can, therefore, influence the outcomes of *in vivo* studies comprising IN nanosystem administration. In the present systematic review, our aim was to analyze the reports of non-clinical brain targeting studies evaluating IN delivery of small molecule drugs within nanosystems. Our present analysis focused on study design, chosen nanosystem class and their characterization, and reported output pharmacokinetic (PK) parameters, with emphasis on searching for associations between brain delivery efficacy and nanosystem type.

2. Methods

2.1. Systematic bibliographic selection and data collection

Articles considered for data collection, and consequent statistical analysis, were obtained via search in the "Web of Science™" database, using the following terms: (nanoemulsion$ OR microemulsion$ OR submicron OR emulsion$ OR nanostructured OR miniemulsion$ OR nanoparticle$ OR nanosystem$ OR

liposome$ OR cubosome$ OR transfersome$ OR niosome$ OR polymersome$ OR exosome$ OR dendrimer$ OR nanocarrier$ OR micelles) AND brain AND ("in vivo" OR animal OR biodistribution OR pharmacokinetics OR pre-clinical OR non-clinical) AND (nasal OR intranasal). Date of last search was June 23th 2017 and no publication date restriction was applied. Screening for exclusion criteria was done by title, abstract and article content, if needed and in that respective order. Those criteria included: being a review article; not comprising an *in vivo* pharmacokinetic study; absent or insufficient presentation of the necessary pharmacokinetic data, more precisely brain and blood AUC values for IN nanosystem and IV formulation; inexistence of an IV comparison route; drug not having a low molecular weight (namely protein or gene derived entities); IN drug not being formulated in a nanosystem; being a vector study only, with no associated drug; being a toxicity study only; lack of information and interpretation quality (repeated errors); and lastly, impact factor inferior to 1. Data was extracted to a spreadsheet table format by one author and then thoroughly reviewed by a second author.

2.2. DTE%, DTP%, $B\%_{brain\ IN/IV}$ and $RB\%_{brain}$ (re)calculation

Given the lack of uniformity or absence of the calculation of drug targeting efficiency (DTE%), direct transport percentage (DTP%), comparative brain bioavailability (IN vs. IV) ($B\%_{brain\ IN/IV}$) and relative brain bioavailability ($RB\%_{brain}$) ratios, whenever possible, these were recalculated from reported AUC data.

DTE% is a measure of brain targeting through IN administration, with the following mathematical formula:

$$DTE\% = \frac{(AUC_{brain}/AUC_{blood})_{IN}}{(AUC_{brain}/AUC_{blood})_{IV}} \times 100 \qquad (1)$$

where AUC is the "area under the curve", representing drug concentration variation over time (in brain or blood), for the duration of the study ($AUC_{0\text{-}t}$), and IN and IV indicate the administration route to which the AUC values correspond to [4,7]. As the given formula implies, the DTE% value can be interpreted as the relative propensity of the drug to accumulate in the brain when administered through the IN route, over the IV route. Values can range from 0 to $+\infty$. Values above 100% indicate a more efficient brain targeting through IN administration when compared to IV administration, and values below 100% represent the opposite [7]. The $\log_{10}(DTE\%)$ was also calculated (in attempt to normalize the distributions) and expressed as Log DTE%.

Even if useful, DTE% might not offer an easy interpretation of which drug fraction was transported through the olfactory and trigeminal nerve pathways and which was not [4]. As an alternative, the **DTP%** can be calculated from Eq. 2:

$$DTP\% = \frac{AUC_{brain_{IN}} - \mathrm{F}}{AUC_{brain_{IN}}} \times 100 \qquad (2)$$

where AUC values are $AUC_{0\text{-}t}$, and F is given by:

$$F = \frac{AUC_{brain_{IV}}}{AUC_{blood_{IV}}} \times AUC_{blood_{IN}} \qquad (3).$$

Since the expected contribution of the indirect pathway to $AUC_{brain\ IN}$ is subtracted from its total value, DTP% is, as the name suggests, the value that better represents the drug fraction suffering direct transport to the brain [4,7]. Values can theoretically range from -∞ to 100, although the expected value if there is no transport through the direct nose-to-brain pathways is 0. Values higher than 0 indicate the presence of brain targeting through the direct pathways, opposite to values from -∞ to 0, which indicate a more efficient brain targeting through the IV route [7]. A value of 100 is only possible if the drug does not cross the BBB at all ($AUC_{brain\ IV} = 0$), or if it is not absorbed to the systemic circulation when administered intranasally ($AUC_{blood\ IN} = 0$). An approximation of the first case is more likely to occur, and it means that drugs that are poorly permeable in the BBB are more likely to have the highest DTP% values. However, DTE% and DTP% can be high despite very low bioavailability in the brain.

Comparative brain bioavailability (IN vs IV) is a measure of brain drug accumulation through the IN route over the IV route, considering brain $AUC_{0\text{-}t}$ values only (and not blood's) [17]. The mathematical formula is:

$$B\%_{brain\ IN/IV} = \frac{AUC_{brain_{IN}}}{AUC_{brain_{IV}}} \times 100 \quad (4)$$

Values above 100 indicate a better brain drug accumulation through IN administration when compared to the IV administration. The $\log_{10}(B\%_{brain\ IN/IV})$ was used and expressed as Log $B\%_{brain\ IN/IV}$.

Relative brain bioavailability ($RB\%_{brain}$) is similar to $B\%_{brain\ IN/IV}$, only IN nanosystem delivery is here compared with an IN drug solution [31], the formula being:

$$RB\%_{brain} = \frac{(AUC_{brain_{IN}})\text{nanosystem}}{(AUC_{brain_{IN}})_{solution}} \times 100 \quad (5)$$

Values above 100 will indicate a better brain drug accumulation through IN administration of the nanosystem when compared to the IN solution. The $\log_{10}(RB\%_{brain})$ was used and expressed as Log $RB\%_{brain}$.

The comparison of the efficiency in brain targeting between IN nanosystems relatively to IN solutions was also verified by calculating, in analogy with Log RB%, the logarithm of **relative DTE%**, (Log RDTE%) and of **relative DTP%** (Log RDTP%). The following formulas where applied:

$$Log\ RDTE\% = \log_{10}\left(\frac{DTE\%_{IN_{nanosystem}}}{DTE\%_{IN_{solution}}} \times 100\right) \quad (6)$$

$$Log\ RDTP\% = \log_{10}\left(\frac{DTP\%_{IN_{nanosystem}}}{DTP\%_{IN_{solution}}} \times 100\right) \quad (7)$$

2.3. Statistical analysis

Statistical analysis was performed using Prism software, version 6.0, from GraphPad. Normality of variables distribution was assessed with the D'Agostino-Pearson omnibus normality test. For normal distributions, group mean values were compared with a reference value using the one sample t-test (also in the case of insufficient representation, with $n < 6$). In the case of non-normal distributions (and sufficient representation), median values and a Wilcoxon signed-rank test were used. Differences between mean

attribute values of nanosystem groups that were at least minimally represented ($n \geq 5$) were evaluated by one-way analysis of variance (ANOVA) with the Tukey multiple comparisons post-test, as were the general mean differences between IN solutions, IN nanosystems and IV formulations (when analyzed as a whole). The overall median differences between IN solutions and nanosystems were evaluated by the Mann-Whitney location test. Conservative outlier analysis was performed using the ROUT method (combined Robust regression and Outlier removal) setting Q at 0.1%.

3. Results and discussion

3.1. Bibliographic search results characterization and quality of reported variables

Of the obtained 243 search results, only 56 met the inclusion/exclusion criteria. Interestingly, the countries of origin were India (66%), Egypt (18%), China (12%), and Saudi Arabia (4%). All articles selected for analysis were published between 2004 and mid-2017, most of them (34%) being published in the year of 2016, and more than half in the last 3 years (2014-2016) (Fig. 2). Therefore, there seems to be an increasing interest in intranasal delivery of nanosystems and their *in vivo* brain targeting evaluation. Journal impact factors varied from 1.566 to 5.434 (median value of 3.773).

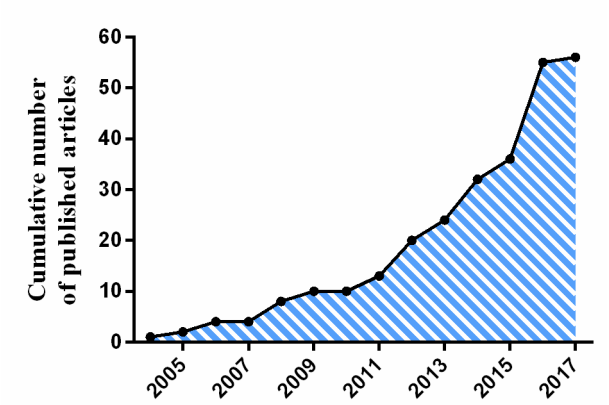

Fig. 2. Increase in the number of publications on the topic of the present review over time. Data correspond to cumulative publication frequency per year of publication.

Essential parameters such as animal model, study duration, IV formulation and IN nanosystem type, drug and analytical method were described in every single article. On the other hand, the analytical method validation was only mentioned in 30% of all publications, while one should expect to find the validation parameters described. Most articles indicated DTE%, $Tmax_{brain\ IN}$, $Tmax_{blood\ IN}$ and $Tmax_{brain\ IV}$. $Tmax_{blood\ IV}$ was only mentioned in 75% of publications because some consider it to be equal to zero, as the drug is readily available in the bloodstream (a possible reason for other values might be considering $Tmax_{blood\ IV}$ to be the minimum time at which the blood samples were collected). DTP% follows DTE% as the second most reported pharmacokinetic ratio. $B\%_{brain\ IN/IV}$ had a low report rate, and $RB\%_{brain}$ even lower (close to none). Instead, comparative brain AUC fold-changes were sometimes calculated, but they are basically the same (ratio instead of a percentage value).

DTE% and DTP%, being ratios that utilize the exact same data, were expected to have a perfect monotonic (non-linear) correlation between them. By examining the scatter-dot plot representations of the correlation between both ratios as mentioned in the articles (Fig. 3A), it can easily be seen that there was an inconsistency in some of the values calculated by the authors, with some very strong outliers standing out. Meanwhile, DTE% and DTP% values recalculated by us, using the above-mentioned formulas, had a perfect monotonic correlation (Spearman's correlation coefficient of 1, Fig. 3B).

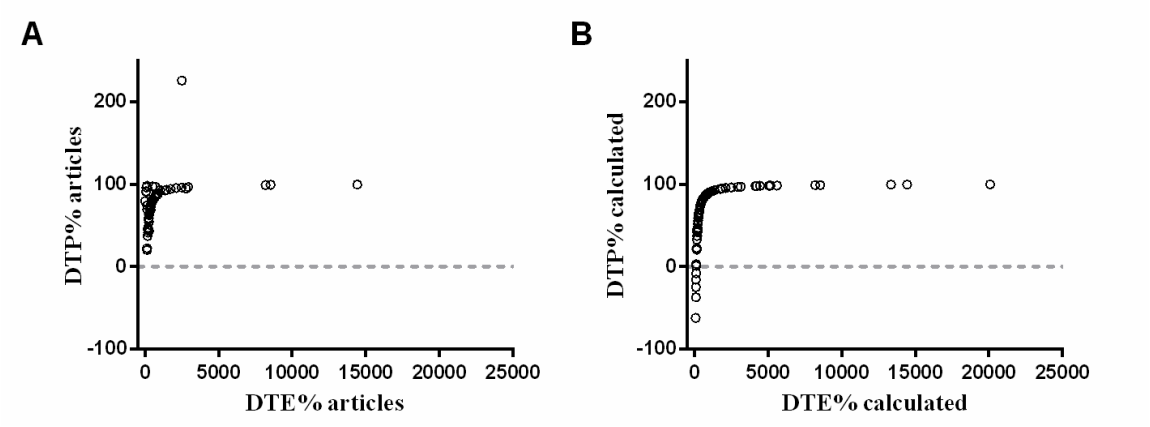

Fig. 3. Correlation of DTE% and DTP% ratios. Representation of DTE% vs DTP% values as given in the articles (r_S = 0.646) (A) and of DTE% and DTP% as recalculated (r_S = 1.000) (B). DTE - drug targeting efficiency; DTP – direct transport percentage; r_s - Spearman's correlation coefficient.

Of all pharmacokinetic ratios, DTE% was the most mentioned, however only 49% of all articles exhibited a value that could be considered equivalent to our recalculated ones. Some reasons for the discrepancies in DTE% were: the use of either AUC_{∞} or a different AUC_{0-t} value for the calculation, while we used AUC_{0-t} values corresponding to the duration of the experiment (considering time from zero to the last measurable concentration); the use of AUC_{0-t} for calculation, but then only presenting AUC_{∞} values, which we used since we didn't have access to additional data; calculation of DTE using the AUC_{0-t} of the nanosystems administered IV, while we recalculated them using AUC_{0-t} of the IV solutions when reported; and the use of a different formula (or non-disclosure of the formula) for the calculation [13,32–37]. For many other cases, we could find no further clear justification for the revealed discrepancies.

For DTP% and $B\%_{brain\ IN/IV}$, 63% and 35% of the articles reporting these values, respectively, displayed calculated values similar to ours. Furthermore, it is relevant to mention that $B\%_{brain\ IN/IV}$ values were either phrased as "absolute brain bioavailabity", "nasal bioavailability" or "comparative bioavailability", and also that it was sometimes hard to determine whether the authors were referring to brain or blood bioavailability, which made it confusing to interpret. Relative bioavailability values were only provided in 2 articles, both with natural discrepancies in comparison with the ones we calculated, since we used reported AUC_{0-t} and the authors used AUC_{∞} [11,31]. All these discrepancies, numeric or not, are evidence of a lack of systematization between studies.

As for formulation attributes, most articles reported nanosystem particle size (determined by dynamic light scattering) and zeta potential. However, zeta potential is a measure dependent on both particle charge and salt composition of the medium used for particle dilution, and these were not always described. It is often taken as a recommendation that its absolute value should be above 30 mV for maximum stability [15,20,21,38]. A direct electrostatic adsorptive interaction with the mucosa is usually expected with high positive zeta potential values, which justifies that chitosan, a natural cationic polymer, is one of the most mentioned mucoadhesive agents [12,25,39]. The polydispersity index (PDI) was also frequently but not always mentioned, and nanosystems' size is not completely characterized without it [32]. The PDI is also very important in drug pharmacokinetics, since a lower value indicates an enhanced probability of a more uniform absorption through the nasal mucosa and a higher value may lead to pharmacokinetic irregularity and variability in the therapeutic outcome [22,40]. It is usually recommended that the PDI is below 0.5 [12,20,33]. On the other hand, pH and viscosity were described fewer times. Little more than half of all articles reported formulation pH, and in those that did, it ranged from 4.62 to 7.00, which is fairly within the human nasal mucosa's physiologic range [3]. In addition to having a low reporting rate, viscosity had associated different measurement temperatures (mostly 25 ºC, but also 33 ºC), rotation speeds and spindle types, which makes it hard to interpret. All these formulation attributes can be critical in drug absorption and/or safety, and should be reported in every article.

Other important parameters such as *in vitro* release and *ex vivo* permeation of the drug, although mentioned in some of the articles, will not be considered for analysis. This is because only about one half and one third of all publications had, respectively, done these *in vitro* and *ex vivo* studies. Furthermore, with the reported release and permeation studies' duration, temperature and rotation speed varied substantially from one article to another, or were not mentioned at all, and membrane pore (*in vitro*) and nasal mucosa model (*ex vivo*) also varied greatly.

3.3. Delivered drugs

In a total of 56 articles, 39 different drugs were studied, with only one article studying 2 drugs simultaneously (herbal compounds borneol and geniposide, components of the same Chinese traditional medicine) [29]. They belonged most frequently to the antipsychotics, dopaminergic agents, antiepileptics and anxiolytics classes, but many other were represented (Table 1). This large heterogeneity, although justifiable by the need to innovate, and therefore to formulate different drugs, makes it difficult to compare, in a direct manner, values of different nanosystems without a great risk of introducing a substantial bias into data interpretation. Even in the case of the most studied drug, olanzapine, an atypical antipsychotic used for the treatment of schizophrenia (which was mentioned in 5 articles) there were 8 different nanosystems: mucoadhesive and non-mucoadhesive nano and microemulsions, polymeric nanoparticles, transfersomes, liposomes and nanocubic vesicular systems. These nanosystems, even if all studied in the same animal model (rat), had associated different analytical methods, IV comparison formulations, study durations, doses and excipients [31,38,41–43].

Table 1. Anatomical Therapeutic Chemical Classification (ATCC) of the formulated drugs*, and respective reference.

ATCC	Chemical entities (name)	Papers (n_i)	Ref.s
A04A Antiemetics and antinauseants	Ondansetron (hydrochloride)	1	[44]
C04A Peripheral vasodilators	Ergoloid mesylate	1	[45]
C05C Capillary stabilizing agents	Rutin	1	[35]
C08C Selective calcium channel blockers with mainly vascular effects	Nimodipine	2	[46,47]
G03F Progestogens and estrogens in combination	Estradiol	1	[48]
J05A Direct acting antivirals	Saquinavir (as mesylate)	1	[49]
L01A Alkylating agents	Temozolomide	1	[18]
M03B Muscle relaxants	Cyclobenzaprine (hydrochloride), Tizanidine	2	[39,50]
N02A Opioids	Tramadol	1	[36]
N02C Antimigraine preparations	Sumatriptan, Sumatriptan (as succinate), Zolmitriptan	4	[9,10,51,52]
N03A Antiepileptics	Carbamazepine, Clonazepam, Oxcarbazepine	5	[11,14,17,40,53]
N04B Dopaminergic agents	Bromocriptine, Cabergoline, Rasagiline, Ropinirole	6	[22,54–56]
N05A Antipsychotics	Asenapine (as maleate), Haloperidol, Olanzapine, Paliperidone, Risperidone, Quetiapine (as fumarate)	13	[19–21,25–27,31,34,38,41–43,57,58]
N05B Anxiolytics	Alprazolam, Buspirone (as hydrochloride), Clobazam, Diazepam	5	[8,12,28,59]
N06A Antidepressants	Duloxetine, Venlafaxine	3	[15,16,60]
N06D Anti-dementia drugs	Rivastigmine, Tacrine (as hydrochloride)	2	[33,61]
P01B Antimalarials	Artemether (as artemisinin methyl ether)	1	[62]
Experimental	Resveratrol, Tarenflurbil	2	[63,37]
Plant derivatives	Borneol, Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, Geniposide, Thymoquinone	5	[13,29,64–66]

$n_{i,}$ - absolute frequency; Ref.s – bibliographic references; * all ATCC obtained from the DrugBank Version 5.0.7 database [67]

3.4. Delivery nanosystems

From the 56 articles that were analyzed, 31 focused on the study of one formulation of only one nanosystem type, while 25 studied two or more. Reasons for studying more than one formulation included, more frequently, the addition of a mucoadhesive agent, but also other variations in constituents (different surfactants, PEGylation, variation of polymers' molecular weight, addition of cyclodextrins). Fewer times, there was also the comparison of two nanosystem types, or drugs (two different drugs, or chemical derivatives of the same drug).

Formulations were here classified using a two level classification system (Table 2). On level I we grouped them in a minimum number of nanosystem types: emulsions, polymeric nanosystems, lipid nanoparticles , and liposome related nanosystems. On level II, the detail in nanosystem distinction was increased, using a classical class definition. Rare nanosystems, containing phospholipids in their composition, occurring one

time only, were grouped in the same class, named "other" (niosomes, nanocubic vesicular systems, emulsomes and lipidic micelles).

Table 2. Frequency distribution of formulations by nanosystem class using a two level classification system.

Level I - groups	**n_i**	**Level II - classes**	**n_i**	**Formulations compared to IN solutions* (n_i)**
Emulsions	50	Microemulsions	36	26
		Nanoemulsions	14	12
Polymeric nanosystems	26	Polymeric nanoparticles	21	19
		Polymeric micelles	5	5
Lipid nanoparticles	8	Solid lipid nanoparticles	3	2
		Nanostructured lipid carriers	5	4
Liposome related nanosystems	10	Liposomes	4	0
		Transfersomes	2	0
		Other	4	0
N	94		94	68

n_i - absolute frequency; N - total number of events; IN – intranasal; * or drug dispersions (one case).

Regarding the frequency of formulation types in the analyzed data, emulsions were the most studied delivery nanosystem group, comprising more than half of all formulations (Table 2). Within that group, microemulsions were the most studied class.

Nanometric emulsions (colloidal liquid in liquid dispersions) include **nanoemulsions** and **microemulsions**, not always clearly distinguished in literature. Theoretically, while the first have thermodynamic stability, the later do not. Nanoemulsions have, nonetheless, a relatively high kinetic stability [14,27,36], a higher surface area and higher free energy than macroemulsions, being more stable against sedimentation, flocculation, coalescence and creaming [37]. Given that water is added to a mixture of oil, surfactant and co-surfactant, both types of emulsions are said to form spontaneously [3,29]. Droplet size range is generally lower in microemulsions (10-100 nm) than in nanoemulsions (20-200 nm), but it is questionable whether it is accurate to simply identify a system as microemulsion when it is translucent. It only means that most droplets are very small in diameter, and partially translucent systems could either be macroemulsions or nanoemulsions. Their lipophilic nature, good permeability and solubilizing effect also make them promising systems for IN delivery, in particular of lipossoluble drugs [20,22,38,49,36]. However, a few reports stated irritation of gastrointestinal or nasal mucosa with the use of these preparations, justified by the existence of surfactants in large amounts [45,51]. They also suffer from rapid nasal clearance, but a mucoadhesive agent can be added to the formulations to overcome mucociliary clearance, leading to a higher residence time at the site of absorption, and therefore improving bioavailability [12,39].

Polymeric nanosystems followed emulsions in frequency, **polymeric nanoparticles** (PN) being the most frequent class in that group (Table 2). PN are compact colloidal systems with a highly variable size range within the nanometric scale, composed of natural or artificial polymers [3,12]. Drugs will be dissolved, entrapped, encapsulated or attached to the polymeric matrix [8,12]. Surface hydrophobicity, high drug loading and controlled release capacity, and the ability to prolong the duration of therapeutic effect, are a few of their advantages. As in the case of mucoadhesive nano and microemulsions, the integration of

mucoadhesive polymers into the PN formulation is expected to lead to a higher residence time on the nasal mucosa [12,39]. Although there is scientific evidence suggesting the biodegradability and biocompatibility of the polymers that are generally used, some reports mention toxicity, and also formation of aggregates with a large size and lack of stability in aqueous dispersion, leading to phase separation [10,28].

Even if made of polymers as well, **polymeric micelles** (PM) differ in composition from PN: they are comprised of amphiphilic block copolymers that will self-assembly, forming a hydrophobic core, and a hydrophilic corona. The core can solubilize and incorporate a lipophilic drug,whyle the hydrophilic corona will serve as a stabilizing interface between the hydrophobic core and the external aqueous environment. This kinetic stability and self-assembly in water will only occur above the critical micelle concentration, which is, however, usually lower than that of small molecule surfactants. Described drawbacks include the formation of aggregates with a large particle size and lack of stability in aqueous dispersion, resulting in phase separation [11,31,47].

Within the lipid nanoparticles group, the best known are perhaps the **solid lipid nanoparticles** (SLN**)**, which are solid matricial nanoparticles made of solid lipids dispersed in water or an aqueous surfactant solution [28]. They are said to have increased drug stability, good biocompatibility and tolerability, and high drug loading, also increasing nasal retention time due to an occlusive effect and mucous membrane adhesion [28,57]. Despite many claims of controlled delivery of hydrophobic drugs, some reports have reached opposite results, which is theoretically explained by authors as drug and lipid solidification in phase-separated crystals that precipitate either in the core or on the surface of the nanoparticles, with a consequent slow or pronounced burst release [17]. Reports of leakage during storage by lipid polymorphism have also been mentioned [21,44]. In their turn, instead of only having a solid lipid in their composition, **nanostructured lipid carriers** (NLC**)** have a blend of solid and liquid lipids that form an imperfect crystal matrix in which drugs can be accommodated in [3,62]. General advantages include rapid uptake, absence of burst effect and good tolerability. Stated advantages over polymeric nanoparticles comprise avoidance of the use of organic solvents in production, and over SLN higher drug loading, smaller particle size and no drug leakage or expulsion during storage, with improved long-term stability [18,21,44,60,62,68].

Within the liposome related nanosystems group, classic **liposomes** were the most represented class. These are biocompatible and biodegradable vesicles composed of phospholipid (and cholesterol) bilayers enclosing one or more aqueous compartments [3,5]. Several variations of these particles have been developed. In **transfersomes** the lipidic bilayers specific composition and membrane incorporated edge activators give the vesicles high flexibility, making it easier for them to interact with the membranes and pass through small fenestrations [43]. **Niosomes** are prepared with non-ionic surfactants such as monoester of polyoxyethylene fatty acids, free fatty acids and cholesterol, and have a high capacity central core that can deliver a large volume of active ingredient [10]. N**anocubic vesicular systems** have polymeric non-ionic surfactants integrated in their phospholipidic bilayer, whose very specific ratio in relation to the phospholipid gives the vesicles cubic shape [42]. **Emulsomes** have the combined characteristics of emulsions, SLN or NLC and liposomes: a lipidic core in a solid or liquid crystalline state (instead of an oil fluid phase), surrounded by at least one phospholipid bilayer envelope, with an aqueous interface in between (hydrophilic heads facing

outwards and hydrophobic tails facing inwards). Emulsomes allow high loads of lipophilic drugs and a prolonged release time. When compared to other lipid carriers, such as SLN and liposomes, these particles are described as not showing the common preparation methods shortcomings, such as high pressure induced drug degradation, lipid crystallization, gelation and co-existence of several colloidal species [17].

Lastly, **lipidic polymeric micelles** are, just like regular micelles, made of amphiphilic block copolymers, but those copolymers are now also attached to a phospholipid, which gives origin to a more lipophilic, although larger, nanosystem [47].

Furthermore, in the majority of studies (about 72% of all articles) the nanosystems were compared with the respective drug solutions or, in one case, drug dispersion (Table 2).

3.6. PK study designs and drug assays

Only two animal models were used, rat (75%) and mice (25%). Study duration varied significantly, ranging between 2 and 72 h, with a median value of 8 h, which was also the duration of almost half of all studies. Every study used the IV route as a parenteral comparison route, although not all animal subjects were given an IV drug dispersion, with 39% of all articles administering the nanosystem itself. This is a major inconsistency, since the PK profile of the intravenously administered drug, in solution or associated to a nanosystem, can be quite different, and the calculation of DTE%, DTP% and $B\%_{\text{brain IN/IV}}$ of the IN formulation should be made using IV drug solution data. For drug assay, the most utilized analytical method was liquid chromatography (57%), which included high and ultra-performance systems, and was often coupled with a tandem mass spectrometry detector, followed by scintigraphy (43%).

3.7. Nanosystem attributes

In what concerns mean particle size (Fig. 4A), the most homogeneous formulations between studies, with the smallest range of values, belonged to the microemulsions class. It also had the lowest mean value, which was significantly lower than that of the PN. PN had the highest range, having the most heterogeneous particle size distribution out of the 4 groups, which might be explained by the different subtypes of nanoparticles and their respective composition and preparation methods. PN also had a significantly higher mean than the PM and the nanoemulsions classes (aside from the microemulsions). All these results are in agreement with the theoretical definition of nanosystems size.

Homogeneity of particle size distribution is characterized by PDI values (Fig. 4B), of which microemulsions had the lowest mean and PN the highest. The difference between means of these two classes was significant, and the mean of the PN class was also significantly higher than that of the PM and nanoemulsions classes, which is in accordance with what has been mentioned above. All the PDI values were below the recommended 0.5 threshold.

As previously mentioned, zeta potential values can be either positive or negative, depending on the combined charges and quantities of the drugs and excipients that compose the formulation. Positively charged formulations have, in theory, the advantage of interacting with the negatively charged nasal mucosal membrane due to the formation of electrostatic bonds with the sialic acid residues that are part of its

composition. This facilitates both adhesion and transport by increasing contact time with said region and amplifying the opening of the tight junctions that exist there [32,35,68]. About half of all nanosystem groups included negatively charged nanosystems only, and for those that comprised both positively and negatively charged formulations, most values were also negative (Fig. 4C). Out of the 5 considered classes, microemulsions had the most negative mean value, and PN the most positive, with the differences between the two classes being significant. PN also had significant differences when compared to the PM, nanoemulsions and NLC classes. The more positive values attributed to the PN class might be justified by the frequent presence of the positively charged polymer chitosan in their composition.

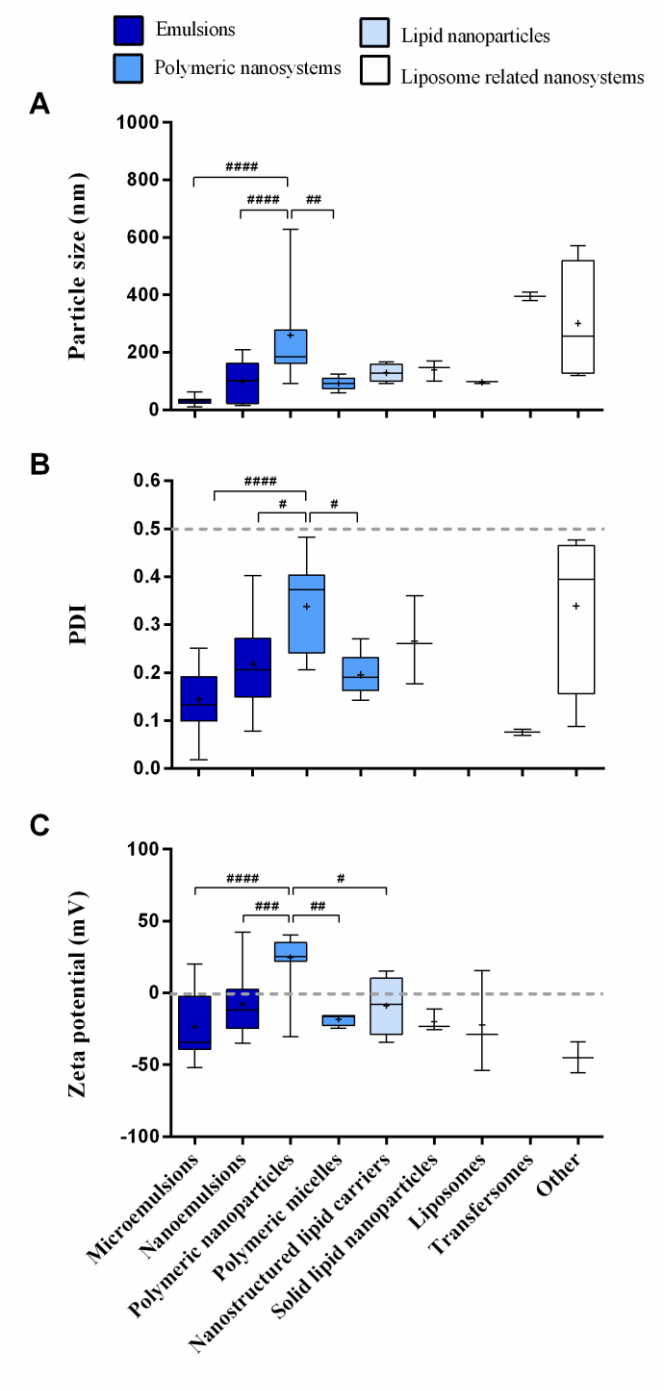

Fig. 4. Summary of attributes of the drug delivery nanosystems per class. Particle size (A), PDI (B) and zeta potential (C) are shown. Data correspond to median ± inter-quartile interval and range (box-plot) plus mean indicated by a small "plus" signal (+). Statistical analysis was done by applying a one-way ANOVA with the Tukey multiple comparisons post-test; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$; $^{\#\#\#\#}p < 0.0001$. PDI – polydispersity index.

3.9. Drug pharmacokinetics

The median values of Log DTE%, DTP% and Log B%$_{brain\ IN/IV}$ of all nanosystem formulations grouped together was superior and significantly different from the median values of the 42 solutions that were also evaluated (Fig. 5A, B and C). Furthermore, their range of values was extremely high, not only in the nanosystems group, where one could already expect it, but also in the solution group. This big variability in drug delivery performance of IN solutions can only be due to either the drug itself or confounding variables related with study design, such as differences in study duration and administered dose, both of which can greatly influence the AUC values and, consequently, their ratios. Even a conservative outlier analysis (ROUT, Q = 0.1%) identified several possible outliers. The very high Log DTE% values in the solution group (Fig. 5A) corresponded to plant derived drugs [13,35], and are probably due to their high hydrophilicity and consequent difficulty in permeating the BBB (low brain bioavailability through the IV route). Some very low DTP% values (negative or close to 0, Fig. 5B) correspond to the nanosystems group, more specifically to risperidone liposomes, olanzapine liposomes, olanzapine transfersomes and olanzapine nanocubic vesicular systems (3 independent studies [19,42,43]). Curiously, all these formulations belong to the LRN group.

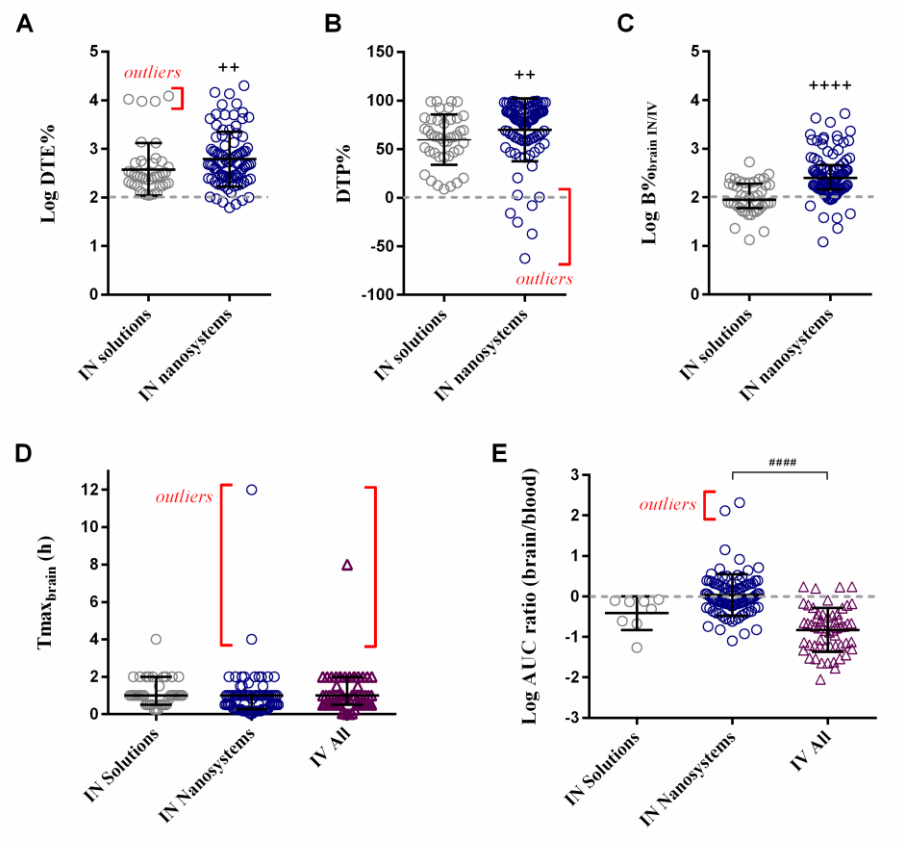

Fig. 5. Comparison of overall drug delivery by nanosystems and solutions. Log DTE% (A), DTP% (B), Log B%$_{brain\ IN/IV}$ (C) of IN solutions and IN nanosystems, Tmax$_{brain\ IN}$ (D) and Log AUC ratio (brain/blood)$_{IN}$ of IN solutions, IN nanosystems and all IV formulations are shown. Potential outliers are signaled by brackets. Data correspond to individual values plus median ± quartiles. Statistical analysis was done by applying Mann-Whitney U test when comparing IN nanosystems to IN solutions (control), $^{++}$ $p < 0.01$, $^{++++}$ $p < 0.0001$; and by applying one-way ANOVA with the Tukey multiple comparisons post-test when comparing IN nanosystems, IN solutions and IV formulations, $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$; $^{\#\#\#\#}$ $p < 0.0001$. B%$_{brain\ IN/IV}$ – comparative brain bioavailability (IN vs. IV); AUC – area under the curve; DTE% – drug targeting efficiency; DTP% – direct transport percentage; IN – intranasal; IV – intravenous; Tmax – time required to reach maximum concentration.

In the matter of the amount of time it takes the drug to reach maximum concentration in the brain ($Tmax_{brain\ IN}$), three possible outliers were identified (Fig. 5D): one IN zolmitriptan nanoemulsion, one IN thymoquinone PN, and one IV thymoquinone PN, all having Tmax values of 4 h or greater [51,64]. In the case of the IN zolmitriptan nanoemulsion, the obtained Tmax might be justified by the fact that the authors quantified the drug in the cerebrospinal fluid, instead of the brain (most articles), a compartment that the drug may take more time to reach. In the case of the thymoquinone preparations, the high Tmax value could be due to a slow drug release from the nanosystem, as suggested by the release studies performed for those same formulations in the considered article.

Log AUC ratio (brain/blood) also had a couple of extremely high values identified as possible outliers (Fig. 5E), both corresponding to the IN geniposide microemulsions [29]. These high values might be justified by the drugs rapid elimination from the blood, leading to low blood AUC [69,70].

The parameters DTE%, DTP% and $B\%_{brain\ IN/IV}$ showed an ample value range within each nanosystem class, with DTE% reaching 4 digits (Fig. 6A, 6C and 6E). This high variability could be due to several factors, such as nanosystems' composition (both drug and excipients used) and their properties, in addition to confounding variables (study design), as discussed. Considering only the most represented nanosystem classes, all mean/median values of Log DTE%, DTP% and Log $B\%_{brain\ IN/IV}$ were significantly higher than the respective reference values (0 for DTP%, 2 for Log-transformed ratios). The highest Log DTE% mean/median value belonged to the PM class, being significantly different from the Log DTE% of microemulsions (one-way ANOVA). This class also had the second highest Log $B\%_{brain\ IN/IV}$ mean value, which was significantly better than the microemulsions class, and, even though no statistical significance was found, the highest DTP%. Microemulsions had some of the lowest mean/median values, having a significantly lower $B\%_{brain\ IN/IV}$ than PN and NLC, besides PM (one-way ANOVA).

It may strike the eye that liposomes and transfersomes behave poorly, and they were already discussed as possible outliers. However, all liposomes and transfersomes data came from only two articles, therefore possibly being isolated cases of poor performance.

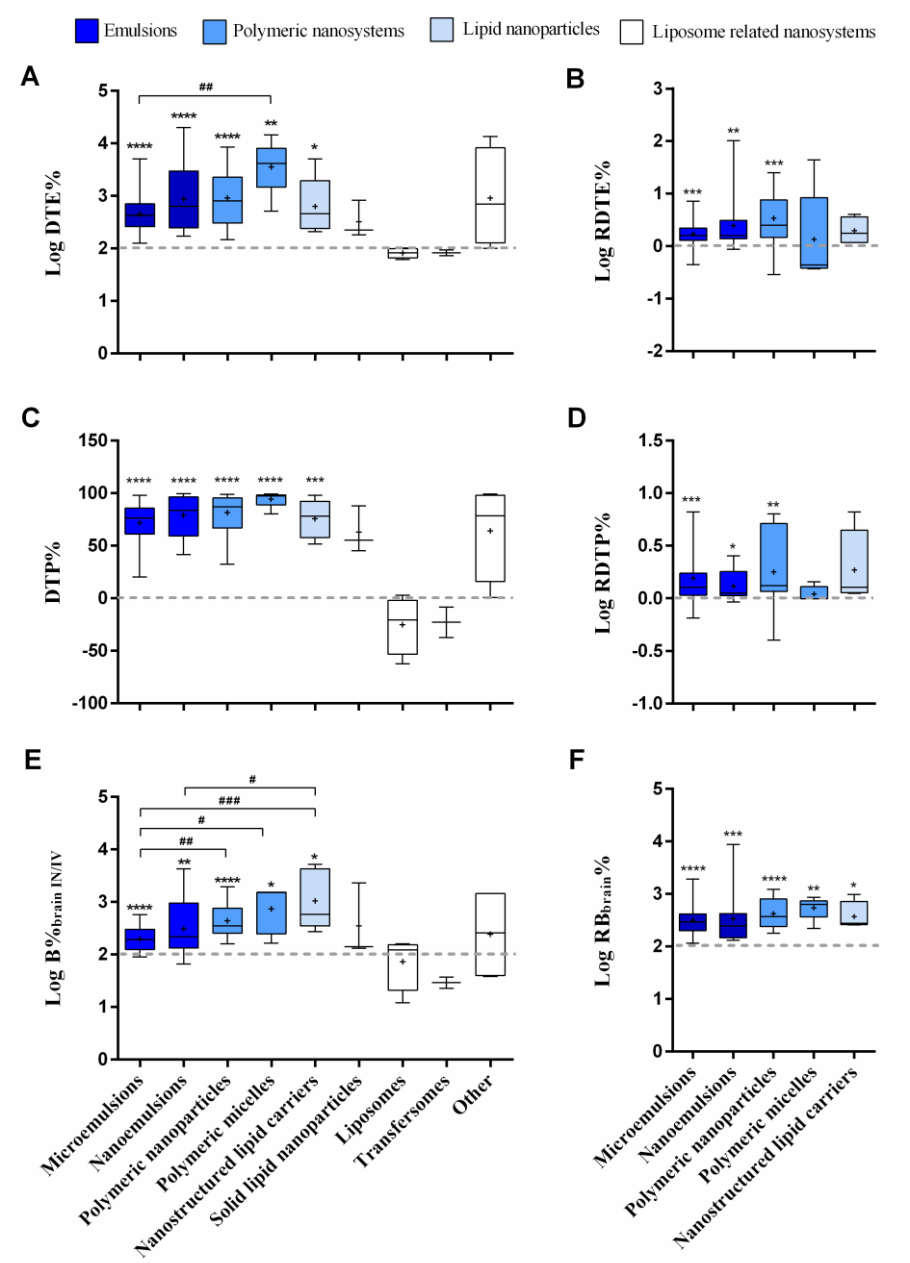

Fig. 6. Formulations brain targeting and bioavailability summary. Log DTE% (A), Log RDTE% (B), DTP% (C), Log RDTP% (D), Log $B\%_{brain\ IN/IV}$ (E) and Log $RB\%_{brain}$ (F) are represented for the different formulation classes. Data correspond to median ± inter-quartile interval and range (box-plot) plus mean indicated by a small "plus" signal (+). Statistical significance of differences between group means evaluated by one-way ANOVA with the Tukey multiple comparisons post-test, $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$; differences between means and reference "no-change" values by one-sample t-test when normal distribution, Wilcoxon signed-rank test when not (medians), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. $B\%_{brain\ IN/IV}$ – comparative brain bioavailability (IN vs. IV); DTE% – drug targeting efficiency; DTP% – direct transport percentage; $RB\%_{brain}$ – relative brain bioavailability; RDTE% – relative drug targeting efficiency; RDTP% – relative direct transport percentage.

Furthermore, the direct comparison of nanosystem behavior to the respective drug solution highlights the advantage of using nanosystems, eliminating the effect of drugs and confounding variables. To do so, we compared DTE% and DTP% values of the IN nanosystems and the respective IN solutions (only studies comparing both were included) by calculating their relative values (RDTE% and RDTP%) plus $RB\%_{brain}$, all Log-transformed (Fig. 6B, 6D and 6F). In the great majority of cases, the IN nanosystem had better brain drug targeting and direct transport than the respective solution. Furthermore, microemulsions, nanoemulsions and PN classes mean/median values were significantly different from zero (Fig. 6B and 6D, one sample t-test when normal distribution, Wilcoxon signed-rank test when not). Relative bioavailability was also improved in all groups (Fig. 6F, same statistical analysis). Nevertheless, we shouldn't consider brain values only, since high blood values can lead to undesirable systemic side effects. Differences between groups were not statistically significant (one-way ANOVA).

Taking this analysis into account, among the groups where the analysis had statistical power, microemulsions were the ones that performed less well (even if still having an improved brain targeting in comparison with the IV formulation). PM seemed to be the most successful when considering Log DTE% values. However, in this last class the evaluation of the relative brain targeting (both Log RDTE% and Log RDTP%, Fig. 5B and 5D) failed to show an evident improvement over the respective IN drug solutions. This was due to the fact that some of the solutions used in these studies performed surprisingly well, and some constituted possible outliers in the solutions data set, as previously mentioned (Fig. 5A). Therefore, the drug itself seems to noticeably influence the brain targeting efficiency. In contrast, the increase of relative brain/blood bioavailability seems less dependent on the drug, and more on the achievement of the nanosystems themselves. In fact, Log DTE% correlated better (and negatively) with $Log(AUCbrain/AUCblood)_{IV}$ than it did (positively) with $Log(AUCbrain/AUCblood)_{IN}$, which indicates that good DTE% (and, consequently, DTP%) values were mostly due to a low IV brain/blood AUC ratio than due to a high IN brain/blood AUC ratio (Table 3 and Supplementary material Fig. S1). That could be attributed to the studied drug's properties, and its poor BBB permeability. Moreover, the same correlations happen with $B\%_{brain\ IN/IV}$, but in a lesser extent, further confirming the influence of IV brain AUC values (that are not included in this ratio's calculation).

Table 3. Spearman and Pearson correlations of Log DTE%, Log $B\%_{brain\ IN/IV}$ and logarithm values of relative bioavailability through the IN route.

	$Log(AUC_{brain}/AUC_{blood})_{IN}$				**$Log(AUC_{brain}/AUC_{blood})_{IV}$**				**Log $B\%_{brain\ IN/IV}$**			
	r_S	**Spearman *p* value**	**r_{xy}**	**Pearson *p* value**	**r_S**	**Spearman *p* value**	**r_{xy}**	**Pearson *p* value**	**r_S**	**Spearman *p* value**	**r_{xy}**	**Pearson *p* value**
Log DTE%	0.24	0.023	0.29	0.023	-0.76	< 0.0001	-0.73	< 0.0001	0.68	< 0.0001	0.68	< 0.0001
Log AB_{brain}%	0.23	0.022	0.13	0.022	-0.44	< 0.0001	-0.47	< 0.0001	-	-	-	-

$B\%_{brain\ IN/IV}$ – comparative brain bioavailability (IN vs. IV); DTE% – drug targeting efficiency; r_S – Spearman's correlation coefficient; r_{xy} – Pearson's correlation coefficient.

Tmax values' distribution of each nanosystem class was not able to discriminate significant differences among nanosystems (ANOVA, supplementary material Fig. S2).

In what concerns relative values, most mean/median values of log transformed relative Tmax in the brain (Log RTmax brain) of the IN nanosystems compared to the IV route (Fig. 7A), and of the IN nanosystems

compared to the respective IN solutions (Fig. 7B), are equal to or below zero, with the small gains only reaching statistical significance in the most represented class, microemulsions. This indicates that brain delivery through the IN route can be at least as fast as the IV route, which is in accordance with the conceived idea that the IN route is a fast route to the brain (when compared with oral administration, for example).

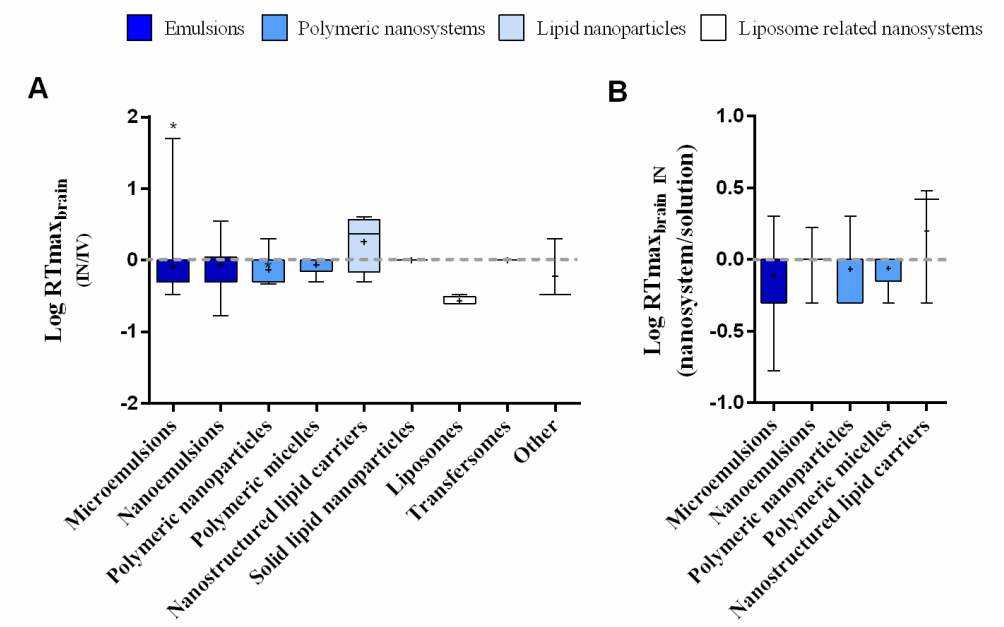

Fig. 7. Representation of the Log of IN/IV (A) and nanosystem/solution (B) ratios of Tmax brain values for all nanosystem groups. Data correspond to median ± inter-quartile interval and range (box-plot) plus mean indicated by a small "plus" signal (+). Statistical significance of differences between means and reference "no-change" values by one-sample t-test when in presence of normal distribution, Wilcoxon signed-rank test when not (medians), * $p < 0.05$; IN – intranasal; IV – intravenous; RTmax – quotient of the time required to reach maximum brain concentration between IN and IV administration, and IN administration of the nanosystem or the respective solution.

4. Conclusions and final remarks

Pre-clinical brain targeting studies have generally put in evidence some of the advantages of IN brain drug delivery and have showed utility in comparing different carrier nanosystems among themselves and with drug solutions. However, this review showed that there is a high heterogeneity on how these assays have been conducted, analyzed and reported in scientific literature.

A grater uniformity of future reports of IN brain-targeting studies would help with the interpretation of the relative value of newly developed formulations. Some recommendations: a plain drug solution should be used IV instead or in addition to the developed nanosystem whenever possible, since the nanosystem itself can markedly alter the intrinsic PK and biodistribution of the drug; the validation parameters of analytical methods should be described; PK ratios calculation (formulas and definition terms) should always be reported; when comparing different formulations, DTE% and B% should preferably be compared after

logarithmic transformation; the animal model could be harder to standardize, due to variable resources and administration techniques, but given the elevated number of animals required to characterize drug AUC in the brain, the use of mice, and a minimum study duration of 8 h, might be good compromises.

Regarding the characterization of the formulations, in order to promote the progressive understanding of the factors that influence brain targeting, it should be as complete as possible. Formulations' characterization should include the nanosystem itself (mean hydrodynamic size, PDI, and zeta potential, detailing the conditions of its determination), and the final preparation (osmolality, pH, and viscosity, including temperature and velocity dependence), given the relevance of all these factors in nasal delivery. Drug release kinetics and the interaction with cells in the nasal mucosa are other important factors that can be informative, and further work to promote *in vitro* tests standardization is still required.

Nevertheless, the existing non-clinical brain targeting studies of IN delivery of small drugs, although likely naturally biased for success cases, confirmed the expectation regarding the advantage of the IN route and the use of carrier nanosystems. Almost all (reported) nanosystems had favorable targeting ratios, and these were higher than the comparative IN drug solution. Moreover, success has been obtained for a large group of drugs already.

Regarding nanosystem classes, microemulsions (the most represented class, with the lowest mean particle size and lowest value range) and PN (the second most represented, with the highest mean particle size and highest value range) were only significantly different regarding Log $B\%_{brain\ IN/IV}$, which was higher for PN, as it was for PM and NLC (also in comparison with microemulsions). These differences were lost when considering the comparison of nanosystems with the respective IN drug solution, especially in the case of PM, where the number of reports is still small. This happened because some drugs reached the brain so efficiently, even as drug solutions, that further benefit from nanosystems became less evident. That being said, it was not possible, from the current global analysis, to clearly discriminate the overall superiority of a nanosystem class in relation to another with respect to brain targeting and bioavailability. We are only able to conclude that, in the reported works, nanosystems do seem to be better than the respective drug solutions, particularity regarding brain bioavailability.

It is important to mention that, given the restrictive nature of the applied inclusion/exclusion criteria, it is not possible to generalize our results. In fact, only low molecular weight entities were included (protein and gene derived drugs were left out). Moreover, some nanosystem classes are underrepresented or have not been represented at all, because many studies describing IN nanosystem development do not design *in vivo* studies for DTE% or DTP% calculation.

Other factors, such as drug and nanosystem properties, are variables that might strongly influence the relative efficacy of a nanosystem. Furthermore, bias could derive from substantial differences in study design. Study duration, IV formulation, animal model, analytical method and drug dosage are all factors that could influence the results in a great extent. In any case, we, as others have before us [6], would like to recognize that the extrapolation of results from animal models to humans carries a risk, since anatomy and physiology and, consequently, nanosystem performance is likely to be substantially different. Furthermore, performance

in both animals and humans might also be influenced by the delivery device itself, a variable that is mostly overlooked [3].

Future work will explore the presented data further, trying to identify factors in study design, formulation composition and properties that associate with pharmacokinetic results, and how they interact or exert their influence, in order to guide the design of future experiments and formulations.

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6. References

[1] WHO | The top 10 causes of death, WHO. (n.d.). http://www.who.int/mediacentre/factsheets/fs310/en/ (accessed April 1, 2017).

[2] Y. Kim, K. Na, Neuroprotection in schizophrenia and its therapeutic implications, Psychiatry Investig. 14 (2017) 383–391.

[3] H. Gao, Progress and perspectives on targeting nanoparticles for brain drug delivery, Acta Pharm. Sin. B. 6 (2016) 268–286. doi:10.1016/j.apsb.2016.05.013.

[4] S. Md, G. Mustafa, S. Baboota, J. Ali, Nanoneurotherapeutics approach intended for direct nose to brain delivery, Drug Dev. Ind. Pharm. (2015) 1–13.

[5] M. Kapoor, J.C. Cloyd, R.A. Siegel, A review of intranasal formulations for the treatment of seizure emergencies, J. Controlled Release. 237 (2016) 147–159. doi:10.1016/j.jconrel.2016.07.001.

[6] D. Sharma, R.K. Sharma, N. Sharma, R. Gabrani, S.K. Sharma, J. Ali, S. Dang, Nose-to-brain delivery of PLGA-diazepam nanoparticles, AAPS PharmSciTech. 16 (2015) 1108–1121. doi:10.1208/s12249-015-0294-0.

[7] Y.K. Katare, J.E. Piazza, J. Bhandari, R.P. Daya, K. Akilan, M.J. Simpson, T. Hoare, R.K. Mishra, Intranasal delivery of antipsychotic drugs, Schizophr. Res. (2016) 1–12. doi:10.1016/j.schres.2016.11.027.

[8] L. Kozlovskaya, M. Abou-Kaoud, D. Stepensky, Quantitative analysis of drug delivery to the brain via nasal route, J. Controlled Release. 189 (2014) 133–140. doi:10.1016/j.jconrel.2014.06.053.

[9] A. Kumar, A.N. Pandey, S.K. Jain, A. Kumar, A.N. Pandey, S.K. Jain, Nasal-nanotechnology: revolution for efficient therapeutics delivery, Drug Deliv. 23 (2016) 671–683. doi:10.3109/10717544.2014.920431.

[10] T.K. Vyas, A.K. Babbar, R.K. Sharma, S. Singh, A. Misra, Preliminary brain-targeting studies on intranasal mucoadhesive microemulsions of sumatriptan, AAPS PharmSciTech. 7 (2006) 1–9.

[11] R.M.A. Abd-Elal, R.N. Shamma, H.M. Rashed, E.R. Bendas, Trans-nasal zolmitriptan novasomes: in-vitro preparation, optimization and in-vivo evaluation of brain targeting efficiency, Drug Deliv. 23 (2016) 3374–3386. doi:10.1080/10717544.2016.1183721.

[12] S.A. Nour, N.S. Abdelmalak, M.J. Naguib, H.M. Rashed, A.B. Ibrahim, Intranasal brain-targeted clonazepam polymeric micelles for immediate control of status epilepticus: in vitro optimization , ex vivo determination of cytotoxicity , in vivo biodistribution and pharmacodynamics studies, Drug Deliv. 23 (2016) 3681–3695. doi:10.1080/10717544.2016.1223216.

[13] N.K. Bari, M. Fazil, Q.H. Md, M.R. Haider, B. Gaba, J.K. Narang, S. Baboota, J. Ali, Brain delivery of buspirone hydrochloride chitosan nanoparticles for the treatment of general anxiety disorder, Int. J. Biol. Macromol. 81 (2015) 49–59. doi:10.1016/j.ijbiomac.2015.07.041.

[14] N. Ahmad, I. Ahmad, S. Umar, Z. Iqbal, M. Samim, F.J. Ahmad, PNIPAM nanoparticles for targeted and enhanced nose-to-brain delivery of curcuminoids : UPLC / ESI-Q-ToF-MS / MS-based pharmacokinetics and pharmacodynamic evaluation in cerebral ischemia model, Drug Deliv. 23 (2016) 2095–2114. doi:10.3109/10717544.2014.941076.

[15] R.B. Patel, M.R. Patel, K.K. Bhatt, B.G. Patel, R. V Gaikwad, Microemulsion-based drug delivery system for transnasal delivery of carbamazepine: preliminary brain-targeting study, Drug Deliv. 23 (2016) 207–213. doi:10.3109/10717544.2014.908980.

[16] S. Haque, S. Md, J.K. Sahni, J. Ali, S. Baboota, Development and evaluation of brain targeted intranasal alginate nanoparticles for treatment of depression, J. Psychiatr. Res. 48 (2014) 1–12. doi:10.1016/j.jpsychires.2013.10.011.

[17] S. Haque, S. Md, M. Fazil, M. Kumar, J.K. Sahni, J. Ali, S. Baboota, Venlafaxine loaded chitosan NPs for brain targeting: pharmacokinetic and pharmacodynamic evaluation, Carbohydr. Polym. 89 (2012) 72–79. doi:10.1016/j.carbpol.2012.02.051.

[18] G.M. El-zaafarany, M.E. Soliman, S. Mansour, G.A.S. Awad, Identifying lipidic emulsomes for improved oxcarbazepine brain targeting : in vitro and rat in vivo studies, Int. J. Pharm. 503 (2016) 127–140. doi:10.1016/j.ijpharm.2016.02.038.

[19] A. Khan, S.S. Imam, M. Aqil, A. Ahad, Y. Sultana, A. Ali, K. Khan, Brain targeting of temozolomide via the intranasal route using lipid based nanoparticle: brain pharmacokinetic and scintigraphic analysis, Mol. Pharm. (2016) 1–32. doi:10.1021/acs.molpharmaceut.6b00586.

[20] R. Narayan, M. Singh, O. Ranjan, Y. Nayak, S. Garg, G. V Shavi, U.Y. Nayak, Development of risperidone liposomes for brain targeting through intranasal route, Life Sci. 163 (2016) 38–45. doi:10.1016/j.lfs.2016.08.033.

[21] D.A. El-setouhy, A.B. Ibrahim, M.M. Amin, O.M. Khowessah, E.S. Elzanfaly, Intranasal haloperidol-loaded miniemulsions for brain targeting : evaluation of locomotor suppression and in-vivo biodistribution, Eur. J. Pharm. Sci. 92 (2016) 244–254. doi:10.1016/j.ejps.2016.05.002.

[22] S.K. Singh, P. Dadhania, P.R. Vuddanda, A. Jain, S. Velaga, S. Singh, Intranasal delivery of asenapine loaded nanostructured lipid carriers: formulation, characterization, pharmacokinetic and behavioural assessment, RSC Adv. 6 (2016) 2032–2045. doi:10.1039/C5RA19793G.

[23] G. Mustafa, A. Ahuja, A. H, A. Rohaimi, S. Muslim, A.A. Hassan, S. Baboota, J. Ali, Nano-ropinirole for the management of Parkinsonism: blood-brain pharmacokinetics and carrier localization, Expert Rev. Neurother. (2015) 1–16. doi:10.1586/14737175.2015.1036743.

[24] O. Dale, R. Hjortkjaer, E. Kharasch, Nasal administration of opioids for pain management in adults, Acta Anaesthesiol. Scand. 46 (2002) 759–770.

[25] K. Moksnes, O.M. Fredheim, P. Klepstad, S. Kaasa, A. Angelsen, T. Nilsen, O. Dale, Early pharmacokinetics of nasal fentanyl: is there a significant arterio-venous difference?, Eur. J. Clin. Pharmacol. 64 (2008) 497–502. doi:10.1007/s00228-007-0444-8.

[26] M.R. Patel, R.B. Patel, K.K. Bhatt, B.G. Patel, V. Gaikwad, M.R. Patel, R.B. Patel, K.K. Bhatt, B.G. Patel, R. V Gaikwad, Paliperidone microemulsion for nose-to-brain targeted drug delivery system: pharmacodynamic and pharmacokinetic evaluation, Drug Deliv. 23 (2016) 346–354. doi:10.3109/10717544.2014.914602.

[27] A.P. Singh, S.K. Saraf, S.A. Saraf, SLN approach for nose-to-brain delivery of alprazolam, Drug Deliv. Transl. Res. 2 (2012) 498–507. doi:10.1007/s13346-012-0110-2.

[28] B. Shah, D. Khunt, M. Misra, H. Padh, Application of box-behnken design for optimization and development of quetiapine fumarate loaded chitosan nanoparticles for brain delivery via intranasal route, Int. J. Biol. Macromol. 89 (2016) 206–218. doi:10.1016/j.ijbiomac.2016.04.076.

[29] B. Shah, D. Khunt, M. Misra, H. Padh, Non-invasive intranasal delivery of quetiapine fumarate loaded microemulsion for brain targeting: formulation, physicochemical and pharmacokinetic consideration, Eur. J. Pharm. Sci. 91 (2016) 196–207. doi:10.1016/j.ejps.2016.05.008.

[30] R. Wen, Q. Zhang, P. Xu, J. Bai, P. Li, S. Du, Y. Lu, Xingnaojing mPEG2000-PLA modified microemulsion for transnasal delivery: pharmacokinetic and brain-targeting evaluation, Drug Dev. Ind. Pharm. (2015) 1–10. doi:10.3109/03639045.2015.1091471.

[31] G.A. Abdelbary, M.I. Tadros, Brain targeting of olanzapine via intranasal delivery of core-shell difunctional block copolymer mixed nanomicellar carriers : in vitro characterization , ex vivo estimation of nasal toxicity and in vivo biodistribution studies, Int. J. Pharm. 452 (2013) 300–310. doi:10.1016/j.ijpharm.2013.04.084.

[32] S. Md, R.A. Khan, G. Mustafa, K. Chuttani, S. Baboota, J.K. Sahni, J. Ali, Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: pharmacodynamic, pharmacokinetic and scintigraphy study in mice model, Eur. J. Pharm. Sci. 48 (2013) 393–405. doi:10.1016/j.ejps.2012.12.007.

[33] M. Fazil, M. Shadab, S. Haque, M. Kumar, S. Baboota, J.K. Sahni, J. Ali, Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting, Eur. J. Pharm. Sci. 47 (2012) 6–15. doi:10.1016/j.ejps.2012.04.013.

[34] K. Florence, L. Manisha, B.A. Kumar, K. Ankur, M.A. Kumar, M. Ambikanandan, Intranasal clobazam delivery in the treatment of status epilepticus, Pharm. Nanotechnol. 100 (2011) 692–704. doi:10.1002/jps.

[35] R. Pangeni, S. Sharma, G. Mustafa, J. Ali, S. Baboota, Vitamin E loaded resveratrol nanoemulsion for brain targeting for the treatment of Parkinson's disease by reducing oxidative stress, Nanotechnology. 25 (2014) 1–13. doi:10.1088/0957-4484/25/48/485102.

[36] D. Patel, S. Naik, K. Chuttani, R. Mathur, A.K. Mishra, A. Misra, Intranasal delivery of cyclobenzaprine hydrochloride-loaded thiolated chitosan nanoparticles for pain relief, J. Drug Target. 21 (2013) 759–769. doi:10.3109/1061186X.2013.818676.

[37] C. Yu, P. Gu, W. Zhang, C. Cai, H. He, X. Tang, Evaluation of submicron emulsion as vehicles for rapid-onset intranasal delivery and improvement in brain targeting of zolmitriptan, Drug Deliv. 18 (2011) 578–585. doi:10.3109/10717544.2011.600784.

[38] M. Kumar, A. Misra, A.K. Mishra, P. Mishra, K. Pathak, Mucoadhesive nanoemulsion-based intranasal drug delivery system of olanzapine for brain targeting, J. Drug Target. 16 (2008) 806–814. doi:10.1080/10611860802476504.

[39] H.M. Abdel-bar, A.Y. Abdel-reheem, G.A.S. Awad, N.D. Mortada, Evaluation of brain targeting and mucosal integrity of nasally administrated nanostructured carriers of a CNS active drug, clonazepam, J. Pharm. Pharm. Sci. 16 (2013) 456–469.

[40] R.B. Patel, M.R. Patel, K.K. Bhatt, B.G. Patel, R. V Gaikwad, Evaluation of brain targeting efficiency of intranasal microemulsion containing olanzapine: pharmacodynamic and pharmacokinetic consideration, Drug Deliv. 23 (2016) 307–315. doi:10.3109/10717544.2014.912694.

[41] H.A. Salama, A.A. Mahmoud, A.O. Kamel, M.A. Hady, G.A.S. Awad, Phospholipid based colloidal poloxamer-nanocubic vesicles for brain targeting via the nasal route, Colloids Surf. B Biointerfaces. 100 (2012) 146–154.

[42] H.A. Salama, A.A. Mahmoud, A.O. Kamel, M.A. Hady, G.A.S. Awad, Brain delivery of olanzapine by intranasal administration of transfersomal vesicles, J. Liposome Res. 22 (2012) 336–345. doi:10.3109/08982104.2012.700460.

[43] T.B. Devkar, A.R. Tekade, K.R. Khandelwal, Surface engineered nanostructured lipid carriers for efficient nose to brain delivery of ondansetron HCl using Delonix regia gum as a natural mucoadhesive polymer, Colloids Surf. B Biointerfaces. 122 (2014) 143–150. doi:10.1016/j.colsurfb.2014.06.037.

[44] C. Yu, J. Meng, J. Chen, X. Tang, Preparation of ergoloid mesylate submicron emulsions for enhancing nasal absorption and reducing nasal ciliotoxicity, Int. J. Pharm. 375 (2009) 16–21. doi:10.1016/j.ijpharm.2009.03.006.

[45] N. Ahmad, R. Ahmad, A.A. Naqvi, A.A. Md, M. Ashafaq, M. Samim, Z. Iqbal, F.J. Ahmad, Rutin-encapsulated chitosan nanoparticles targeted to the brain in the treatment of cerebral ischemia, Int. J. Biol. Macromol. 91 (2016) 640–655. doi:10.1016/j.ijbiomac.2016.06.001.

[46] Q. Zhang, X. Jiang, W. Jiang, W. Lu, L. Su, Z. Shi, Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain, Int. J. Pharm. 275 (2004) 85–96. doi:10.1016/j.ijpharm.2004.01.039.

[47] H.M. Rashed, R.N. Shamma, E.B. Basalious, Contribution of both olfactory and systemic pathways for brain targeting of nimodipine-loaded lipo-pluronics micelles: in vitro characterization and in vivo biodistribution study after intranasal and intravenous delivery, Drug Deliv. 24 (2017) 181–187. doi:10.1080/10717544.2016.1236848.

[48] X. Wang, N. Chi, X. Tang, Preparation of estradiol chitosan nanoparticles for improving nasal absorption and brain targeting, Eur. J. Pharm. Biopharm. 70 (2008) 735–740. doi:10.1016/j.ejpb.2008.07.005.

[49] H.S. Mahajan, M.S. Mahajan, P.P. Nerkar, A. Agrawal, Nanoemulsion-based intranasal drug delivery system of saquinavir mesylate for brain targeting, Drug Deliv. 21 (2014) 148–154. doi:10.3109/10717544.2013.838014.

[50] D. Patel, S. Naik, A. Misra, Improved transnasal transport and brain uptake of tizanidine HCl-loaded thiolated chitosan nanoparticles for alleviation of pain, J. Pharm. Sci. 101 (2012) 690–707. doi:10.1002/jps.

[51] J. Lalani, D. Baradia, R. Lalani, A. Misra, Brain targeted intranasal delivery of tramadol: comparative study of microemulsion and nanoemulsion, Pharm. Dev. Technol. (2014) 1–10. doi:10.3109/10837450.2014.959177.

[52] T.K. Vyas, A.K. Babbar, R.K. Sharma, A. Misra, Intranasal mucoadhesive microemulsions of zolmitriptan: preliminary studies on brain-targeting, J. Drug Target. 13 (2005) 317–324. doi:10.1080/10611860500246217.

[53] T.K. Vyas, A.K. Babbar, R.K. Sharma, S. Singh, A. Misra, Intranasal mucoadhesive microemulsions of clonazepam : preliminary studies on brain targeting, J. Pharm. Sci. 95 (2006) 570–581. doi:10.1002/jps.

[54] D. Mittal, S. Md, Q. Hasan, M. Fazil, A. Ali, S. Baboota, J. Ali, Brain targeted nanoparticulate drug delivery system of rasagiline via intranasal route, Drug Deliv. 23 (2016) 130–139. doi:10.3109/10717544.2014.907372.

[55] S. Md, S. Haque, M. Fazil, M. Kumar, S. Baboota, J.K. Sahni, J. Ali, Optimised nanoformulation of bromocriptine for direct nose-to-brain delivery : biodistribution, pharmacokinetic and dopamine estimation by ultra-HPLC / mass spectrometry method, Expert Opin. Drug Deliv. 11 (2014) 827 – 842. doi:10.1517/17425247.2014.894504.

[56] G. Sharma, A.K. Mishra, P. Mishra, A. Misra, Intranasal cabergoline: pharmacokinetic and pharmacodynamic studies, AAPS PharmSciTech. 10 (2009) 1321–1330. doi:10.1208/s12249-009-9329-8.

[57] S. Patel, S. Chavhan, H. Soni, A.K. Babbar, R. Mathur, A.K. Mishra, K. Sawant, Brain targeting of risperidone-loaded solid lipid nanoparticles by intranasal route, J. Drug Target. 19 (2011) 468–474. doi:10.3109/1061186X.2010.523787.

[58] M. Kumar, A. Misra, A.K. Babbar, A.K. Mishra, P. Mishra, K. Pathak, Intranasal nanoemulsion based brain targeting drug delivery system of risperidone, Int. J. Pharm. 358 (2008) 285–291. doi:10.1016/j.ijpharm.2008.03.029.

[59] H. Bshara, R. Osman, S. Mansour, A.E.A. El-shamy, Chitosan and cyclodextrin in intranasal microemulsion for improved brain buspirone hydrochloride pharmacokinetics in rats, Carbohydr. Polym. 99 (2014) 297–305. doi:10.1016/j.carbpol.2013.08.027.

[60] M.I. Alam, S. Baboota, A. Ahuja, M. Ali, J. Ali, J.K. Sahni, A. Bhatnagar, Pharmacoscintigraphic evaluation of potential of lipid nanocarriers for nose-to-brain delivery of antidepressant drug, Int. J. Pharm. 470 (2014) 99–106. doi:10.1016/j.ijpharm.2014.05.004.

[61] V. V Jogani, P.J. Shah, P. Mishra, A.K. Mishra, A.R. Misra, Intranasal mucoadhesive microemulsion of tacrine to improve brain targeting, Alzheimer Dis. Assoc. Disord. 22 (2008) 116–124.

[62] K. Jain, S. Sood, K. Gowthamarajan, Optimization of artemether-loaded NLC for intranasal delivery using central composite design, Drug Deliv. 22 (2015) 940–954. doi:10.3109/10717544.2014.885999.

[63] E. Muntimadugu, R. Dhommati, A. Jain, V.G.S. Challa, S. M, W. Khan, Intranasal delivery of nanoparticle encapsulated tarenflurbil : a potential brain targeting strategy for Alzheimer's disease, Eur. J. Pharm. Sci. 92 (2016) 224–234. doi:10.1016/j.ejps.2016.05.012.

[64] X. Xiao, Y. Zhu, J. Bu, G. Li, J. Zhou, S. Zhou, Evaluation of neuroprotective effect of thymoquinone nanoformulation in the rodent cerebral ischemia-reperfusion model, BioMed Res. Int. (2016) 1–12.

[65] S. Wang, P. Chen, L. Zhang, C. Yang, G. Zhai, Formulation and evaluation of microemulsion-based in situ ion-sensitive gelling systems for intranasal administration of curcumin, J. Drug Target. 20 (2012) 831–840. doi:10.3109/1061186X.2012.719230.

[66] N. Ahmad, R. Ahmad, A.A. Md, M. Samim, Z. Iqbal, F.J. Ahmad, Quantification and evaluation of thymoquinone loaded mucoadhesive nanoemulsion for treatment of cerebral ischemia, Int. J. Biol. Macromol. 88 (2016) 320–332. doi:10.1016/j.ijbiomac.2016.03.019.

[67] V. Law, C. Knox, D. Wishart, E. Al, DrugBank Version 5.0, (2017).

[68] Y.M. Gabal, A.O. Kamel, O.A. Sammour, A.H. Elshafeey, Effect of surface charge on the brain delivery of nanostructured lipid carriers in situ gels via the nasal route, Int. J. Pharm. 473 (2014) 442–457. doi:10.1016/j.ijpharm.2014.07.025.

[69] Y.C. Hou, S.Y. Tsai, P.Y. Lai, Y.S. Chen, P.D.L. Chao, Metabolism and pharmacokinetics of genipin and geniposide in rats, Food Chem. Toxicol. 46 (2008) 2764–2769. doi:10.1016/j.fct.2008.04.033.

[70] F. Wang, J. Cao, J. Hao, K. Liu, Pharmacokinetics, bioavailability and tissue distribution of geniposide following intravenous and peroral administration to rats, Biopharm. Drug Dispos. 35 (2014) 97–103. doi:10.1002/bdd.

Supplementary material

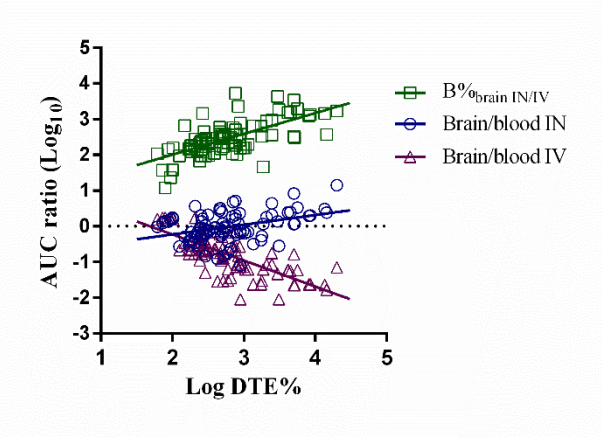

Fig. S1. Graphical representation of the correlation of Log DTE% with Log $B\%_{brain\ IN/IV}$ and Log AUC ratios.

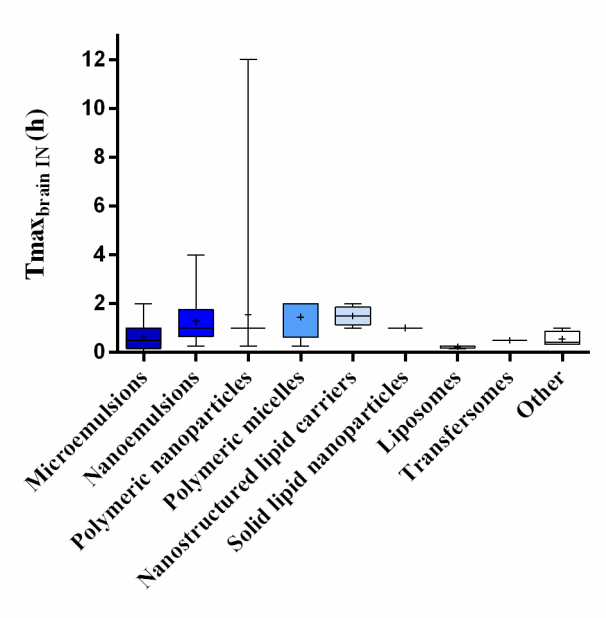

Fig. S2. Representation of $Tmax_{brain\ IN}$ values for all nanosystem groups. Data correspond to median ± inter-quartile interval and range (box-plot) and mean indicated by a small "plus" signal (+). IN – intranasal; Tmax – time required to reach maximum concentration.