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ORIGINAL RESEARCH

Nose-to-Brain Drug Delivery and Physico-Chemical Properties of Nanosystems: Analysis and Correlation Studies of Data from Scientific Literature

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Background: In the last few decades, nose-to-brain delivery has been investigated as an alternative route to deliver molecules to the Central Nervous System (CNS), bypassing the Blood–Brain Barrier. The use of nanotechnological carriers to promote drug transfer via this route has been widely explored. The exact mechanisms of transport remain unclear because different pathways (systemic or axonal) may be involved. Despite the large number of studies in this field, various aspects still need to be addressed. For example, what physicochemical properties should a suitable carrier possess in order to achieve this goal? To determine the correlation between carrier features (eg, particle size and surface charge) and drug targeting efficiency percentage (DTE%) and direct transport percentage (DTP%), correlation studies were performed using machine learning.

Methods: Detailed analysis of the literature from 2010 to 2021 was performed on Pubmed in order to build "NANOSE" database. Regression analyses have been applied to exploit machine-learning technology.

Results: A total of 64 research articles were considered for building the NANOSE database (102 formulations). Particle-based formulations were characterized by an average size between 150–200 nm and presented a negative zeta potential (ZP) from −10 to −25 mV. The most general-purpose model for the regression of DTP/DTE values is represented by Decision Tree regression, followed by K-Nearest Neighbors Regressor (KNeighbor regression).

Conclusion: A literature review revealed that nose-to-brain delivery has been widely investigated in neurodegenerative diseases. Correlation studies between the physicochemical properties of nanosystems (mean size and ZP) and DTE/DTP parameters suggest that ZP may be more significant than particle size for DTP/DTE predictability.

Keywords: nanomedicine, intranasal administration, pharmacokinetic, DTE, DTP, machine learning

Introduction

The treatment of Central Nervous System (CNS) diseases remains an urgent challenge because of the presence of the blood-brain-barrier (BBB), which rigorously controls the entry of substances into the brain and, with the same mechanism, limits the passage of most therapeutic molecules.[1]

To overcome the limitations of the BBB and adequately treat CNS diseases, in recent decades, different strategies have been explored by the scientific community, such as the use of nanomedicines, viral vectors, drug delivery by active transporters or receptors, and brain permeability enhancers.[2]

Currently, a widely explored strategy to reach the brain is the intranasal (IN) route, which represents a promising approach for bypassing the BBB.[3] Although the mechanism(s) by which the drug reaches the brain via this route remains unclear, a combination of different pathways appears to be involved. Following IN administration, the drug reaches the vestibular region, characterized by the presence of mucus and hairs/cilia, which limit drug permanence in the nasal cavity

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by mucociliary clearance.[4] As a function of the device used for drug administration, the drug can be located predominantly in the respiratory or olfactory regions. The respiratory region is highly vascularized and innervated by the trigeminal nerve; thus, in this region, the drug can be absorbed into the systemic circulation and undertake an indirect route (the minor pathway) or follow the trigeminal nerve, also known as direct neuronal transport (the major pathway).[5] After systemic absorption, the drug reaches the brain and crosses the BBB. This occurs only if the drug possesses specific physicochemical properties (ie, such as lipophilicity, degree of ionization, small size, molecular weight, and low hydrogen-bonding potential).[6] The olfactory region is located in the upper part of the nasal cavity and is characterized by the presence of olfactory receptors that penetrate the CNS through their axons. Thus, a drug that reaches this region is transported inside the brain.[7] Therefore, both the respiratory and olfactory regions are involved in the direct transfer of molecules from the nose to the brain, owing to the presence of neuronal components (trigeminal and olfactory). Furthermore, the olfactory region is situated below the cribriform plate, a sieved structure that separates the nasal cavity from the CNS and allows the entry of olfactory sensory neurons into the brain. In fact, the drug may cross the cribriform plate and enter the cerebral spinal fluid (CSF) and olfactory bulb.[8]

Although there have been a growing number of studies in this field during the last decades, different aspects need to be addressed and deepened in order to improve translation from preclinical to clinical stages.

Most investigations are currently in preclinical or early clinical stages, where successful claims remain based on the responses of rodent models. Very few works have expanded to human clinical trials have been conducted.

In the field of patents, it is worth mentioning William H. Frey II, the pioneer of nose-to-brain delivery, who in 1989 discovered the IN route as a noninvasive method to bypass the BBB to target therapeutics (including insulin) in the brain for neurodegenerative disorders, such as Alzheimer's disease, stroke, and Parkinson's disease.[9–12]

As mentioned previously, to maximize direct transport into the brain, it is important to use suitable devices that allow drugs to reach the olfactory region.[13] IN administration offers several practical advantages: it is easy to administer, avoids gastrointestinal and hepatic metabolism, enhances drug bioavailability, allows a lower therapeutic drug dose, has fewer systemic side effects, and improves patient adherence to therapy because of its non-invasiveness.[14] Despite all these benefits, it has been shown that the transfer of free molecule to the brain following IN administration into the CNS is less than 1% of the administered dose. Optimization of nasal administration using suitable nanocarriers represents an emerging and extensively investigated strategy to improve efficient drug delivery to the target CNS.[15]

The use of such systems (eg, lipid or polymeric nanoparticles, nanocrystals, and nanogels) improves the therapeutic efficiency of drugs.[16–19] The presence of the nanocarrier temporally masks the physicochemical properties of the payload and protects it from degradation in the nasal cavity, thereby improving its efficacy and reducing off-target effects.[20]

As mentioned, different factors influence the percentage of drugs that can reach the brain; currently, it is difficult to discriminate the involved pathways.

To discriminate the contribution of direct or indirect drug transport to the CNS by nose-to-brain delivery, two critical pharmacokinetic parameters, drug targeting efficiency percentage (DTE%) and drug direct transport percentage (DTP%), were taken into consideration. DTP% is the percentage of drug that enter the brain via direct routes (olfactory and trigeminal nerves), whereas DTE% is a measure of drug accumulation in the brain following IN administration, relative to the systemic contribution.[21]

It is also important to elucidate the mechanism of intranasal drug release. Warm debate remains open among researchers working in this field trying to answer the following question regarding nanomedicine: is the drug released at the site of administration and then transported as a free drug to the CNS, or is the nanocarrier system conveyed by axonal transport to the brain?

Moreover, despite all the benefits of the use of nanomedicine combined with IN administration, to the best of our knowledge, there are relatively few studies describing the suitable characteristics that a carrier designed for this route should to be able to directly reach the brain, reducing the systemic contribution.

During the design of nanotechnological formulations, special attention should be paid to the physicochemical properties of the nanosystem, such as particle size, surface charge, possible surface modifications, and the balance between hydrophilicity/lipophilicity of the loaded drug, which plays an important role in the interaction with biological barriers.

Inspired by the work of Kozlovskaya and coauthors[22] and in light of the aforementioned considerations, in this study, we sought to perform a detailed analysis of the literature from 2010 to 2021 on PubMed database (https://pubmed.ncbi.nlm.nih.gov/) in order to build a "NANOSE" database that, using machine learning technology, would provide a possible correlation between nanocarriers properties and their ability to deliver the loaded drug following direct transport to the brain considering DTP% and DTE% parameters.

Data preprocessing was performed to scale the features before training the regression models to predict the target variables. Different regression analyses were performed, and key performance metrics, including the R^2 and explained variance scores, were used to assess the performance of these models.

Materials and Methods

Methodology Adopted for Literature Survey

The PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) was used to search for and select scientific publications reporting quantitative data on unknown drugs or unknown drug-loaded drug delivery systems (DDS) to the brain via the nasal route. The search was performed using the following keywords: "intranasal and central nervous system delivery" or "nanoparticles and nose-to-brain" in the temporal range from 2010 to 2021. The search was free of any filters (article type, source, etc). Publications were manually selected based on the title, abstract, and main content.

Specifically, screening of the publications was performed to select the data that were suitable for analysis based on the following criteria: 1) identification and analysis of the publications in the range 2010–2021 with preclinical studies on health or animals model disease (species: rats, mice) that through pharmacokinetics parameters (DTE% and DTP%) quantified the drug transported to the brain as free molecules or following nanomedicine administration via IN vs other routes; 2) collection of the data on nanocarriers (type, size, surface charge) and on drug (type and molecular weight) to build NANOSE database as reported in Supplementary Table 1.

DTP% and DTE%

Two important parameters to quantify the direct transport to the brain after IN administration are DTE% and DTP%.

DTE% was calculated according to the following formula:

$$DTE\% = \frac{(AUC_{brain}/AUC_{blood})_{in}}{(AUC_{brain}/AUC_{blood})_{iv}} \times 100 \quad (1)$$

where AUC_{brain} is the area under the curve of concentration vs time curve of the drug in the brain.

AUC_{blood} is the area under the curve of the concentration vs time curve of the drug in the systemic circulation; $_{in}$ is intranasal, and $_{iv}$ is the intravenous route.

The value of DTE% ranged from 0 to ∞. A DTE% value of ≥ 100 suggests efficient or ineffective brain targeting.

DTP% was calculated according to the following formulas:

$$DTP\% = \frac{B_{in} - B_x}{B_{in}} \times 100 \quad (2)$$

$$B_x \frac{B_{iv}}{P_{iv}} \times P_{in} \quad (3)$$

where B_X is the brain AUC fraction contributed by the systemic circulation through the BBB following IN administration, B_{in} is the brain AUC over time following IN administration, P_{iv} is the blood AUC over time following intravenous administration, and P_{in} is the blood AUC over time following IN administration.

The value of DTP% can range from −∞ to 100%, with values below zero indicating more efficient drug delivery to the brain following systemic administration than following IN administration. The values of the above-described indices are interdependent, and more efficient drug uptake into the brain via direct routes will lead to higher values of both DTE % and DTP%.

Database Building: Data Cleanup, Conversion and Standardization

Data cleanup was performed to make it suitable for the analysis. Data cleanup activities were first conducted, starting from the initial database. All collected data were used in the analysis, and each row was corrected to refer to a single value. Because the particle size and zeta potential (ZP) column values were in the range, we extracted both the extreme and mean values for the particle size and ZP to perform further experiments. Missing values were adjusted by calculating the mean based on the same type/subtype or pharmacological category. It is necessary to convert categorical data into numerical data for certain columns. Data conversion from categorical values to numerical data is required for data analysis and model training because most machine learning algorithms are designed to work with numerical data. The following columns or attributes were converted from categorical to numerical, such as pharmacological categories, active agents, and types/subtypes. Data standardization was performed to bring all variables to a similar scale. Standardization is a process of data transformation that consists of a change in scale to directly compare and interpret the data. Standardizing the data helps machine learning algorithms converge faster to learn the underlying patterns and make accurate predictions. A Z-scoring strategy for data standardization was applied to perform the correct data analysis. Z-score normalization typically refers to rescaling data to a new range, with a mean of 0 and a standard deviation of 1. This technique is useful for optimization algorithms used within machine-learning algorithms that weigh the inputs. Z-score standardization equation (4) was used to transform a given variable into a standard normal distribution. The equation used was as follows:

$$z = \frac{\mathrm{x} - \mu}{\sigma} \tag{4}$$

where z is the standardized value, x is the original value of the variable, μ is the mean of the data, and σ is the standard deviation of the data. The equation shows that, to standardize a variable, the mean is subtracted from the variable, and then the result is divided by the standard deviation.

Results and Discussion

Despite the huge interest of researchers and the growing number of studies in the field of nose-to-brain drug delivery for CNS treatment, there is a lack of clear correlations between the DTE% and DTP% of the therapeutic agent itself or when loaded into nanomedicine following IN administration, and their properties still exist and need to be elucidated. In 2014, Kozlovskaya et al published an interesting review in which they analyzed studies published between 1970 and 2014 that reported the delivery of drugs by different formulations to the brain via IN and parenteral routes to describe brain-targeting efficiency.[22] Specifically, they analyzed publications that studied brain drug delivery and brain-targeting efficiency after IN administration. In this investigation the authors concluded that they were unable to identify correlations between the individual drug physicochemical characteristics, formulation properties, or their combination, and the values of the targeting indices.[22]

At present, this question is open, and starting from this investigation, we aimed to perform elaborate, detailed, and standardized reporting of experimental outcomes by exploiting the use of machine learning technology to find a reliable correlation (if it occurs) that could facilitate the development of nanomedicine designed for this purpose.

Description and Analysis of the Publications Search

A PubMed search using the keywords “intranasal” and “central nervous system” identified overall a total of 2691 publications in the temporal range between 2010–2021. The publication trend in the analyzed field is depicted in Figure 1 (blue line), in which the number of studies (including both reviews and research articles) per year related to the use of IN delivery for CNS targeting can be found. Figure 1 (red line) also shows the trend observed in the PubMed database when the keywords “nanoparticles” and “nose-to-brain” were used in the same temporal range. As shown in Figure 1, the exponential growth of scientific manuscripts published in this field over time is worth noting. Analysis of 2691 publications according to the aforementioned criteria led to the observation that numerous researchers focused on the use of the IN route to achieve CNS, and that about 10% of studies (total n = 286) concerned the use of nanomedicine to achieve this goal, corresponding to the second string of keywords.

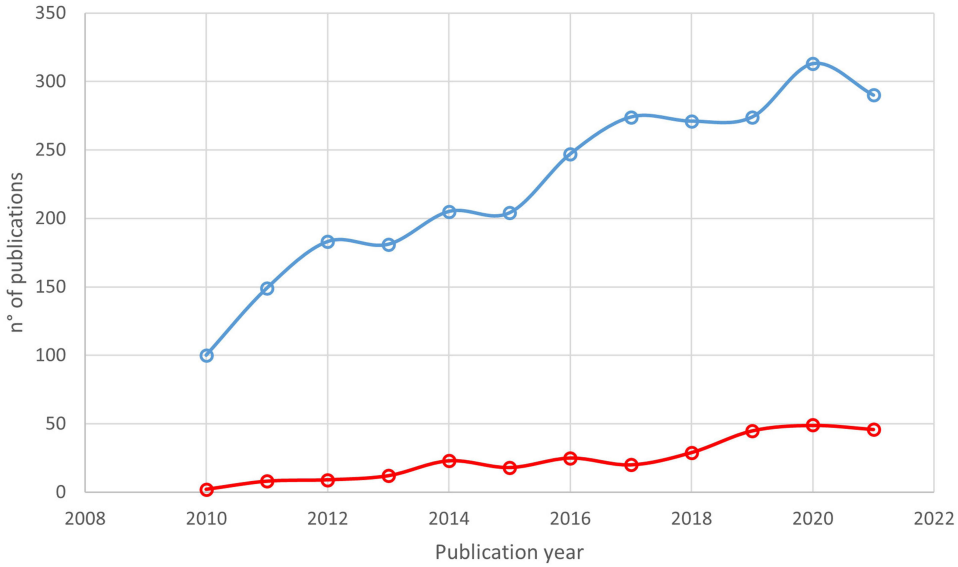

Figure 1 The publication trends observed adopting the keywords "intranasal" and "central nervous system" (blue line) and "nanoparticles" and "nose-to-brain delivery" (red line) as a function of number of publications per year in the range between 2010–2021. Search performed on Pubmed database.

Based on the obtained results, publications that matched all the criteria mentioned in the Materials and Methods section (methodology adopted for literature survey) were considered to build the NANOSE database, which consisted of 64 research articles. If a manuscript reported more than one type of nanomedicine, the database contained the results for each product with related DTP and DTE data (a total of 102 formulations).

Despite the large number of studies published in the temporal range adopted, most publications were not suitable for our goal and were excluded because they were scientific reviews or articles in which only preliminary in vitro biological investigations were considered. However, publications in which in vivo experiments that reported pharmacokinetic parameters were collected (Supplementary Table 1), including DTP and DTE values reported in the article, or calculated using equations (1) and (2) when they were not explicitly expressed.

Pharmacological Application of the Drug/Nanomedicine in the Collected Publications (64 Articles)

A critical analysis of the literature has revealed that the use of nose-to-brain delivery is currently under investigation to treat different CNS pathological conditions.

As depicted in Figure 2, most of the identified publications were conceived for epilepsy treatment, followed by psychiatric disorders, such as schizophrenia and neurodegenerative diseases, among which Parkinson's and Alzheimer's were the most relevant. In addition, drugs belonging to other pharmacological categories have been investigated for nose-to-brain delivery, such as anti-tumor, anti-migraine, and anti-infection, such as meningitis, cerebral malaria, or neurocysticercosis. Approximately 10% of the identified publications were not conceived for specific neurological conditions and merely investigated the use of model drug/dye/nanomedicine for potential CNS targeting. Specifically, the majority of the investigated compounds had small molecular weights (Figure 3) in the range of 250–400 g/mol, constituting small natural or synthetic molecules. Biomacromolecules, such as monoclonal antibodies or proteins, were not considered in this study.

Nanomedicine Categories Investigated in the Collected Publications

All collected publications assessed the delivery of molecules after nasal administration by nanomedicine. Emulsions and particulate systems were the most commonly used, as compared to the other types of drug delivery systems. The main formulations investigated were emulsion or nanoparticulate systems (Figure 4), followed by in situ gelling systems,

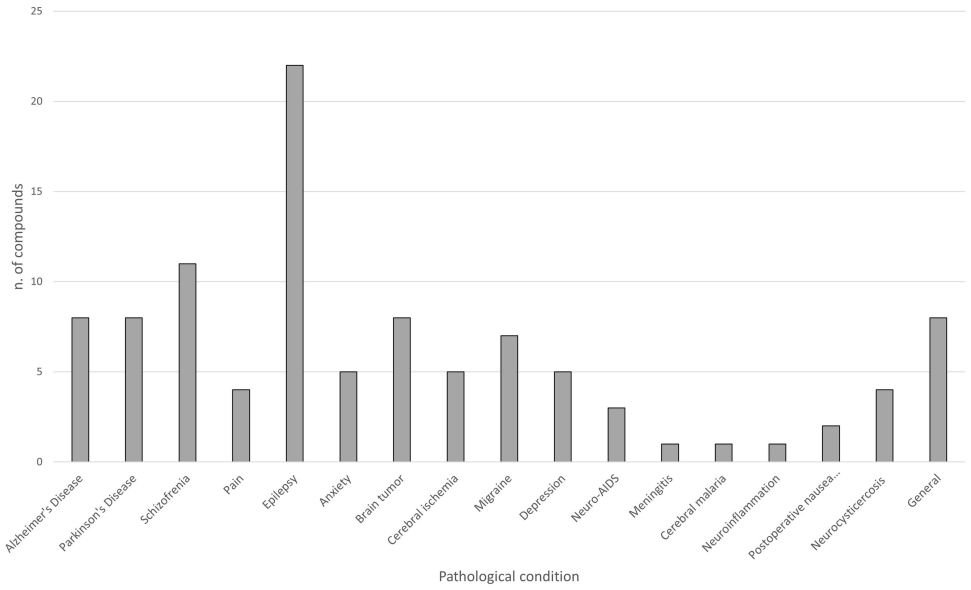

Figure 2 Classification of the therapeutic application of the drugs that were used in the identified publications according to their pharmacological effects.

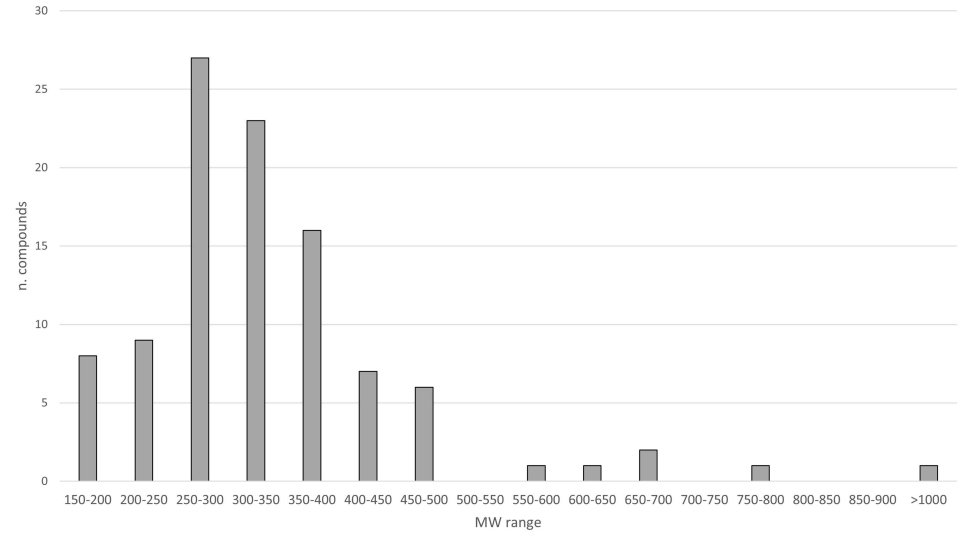

Figure 3 Classification of the drugs that were used in the collected publications based on molecular weight range.

nanomicelles, liquid crystals, hydrogels, and phospholipid nanovesicular carriers. Among emulsions, it is possible to distinguish between micro- and nano-emulsions based on their mean size below or above 100 nm.

They can be grouped as polymeric nanoparticles, representing approximately 72% of the particulate carriers investigated in the collected publications, and lipid nanoparticles are classified as solid lipid nanoparticles (17%) and nanostructured lipid carriers (11%).

Figures 5A and 6A reported classification of the nanomedicine investigated in the collected publications based on their mean size and ZP respectively. Particle-based formulations were in the nanosize range, with a mean size below 550 nm, and were mainly characterized by an average size between 150–200 nm (Figure 5A).

Specifically, 75% of the carriers investigated showed a mean size of <50–200 nm; 16.8% of carriers were between 200–350 nm; and 7.4% of the formulations designed for nose-to-brain delivery consisted of systems with size between 350–550 nm. These data indicate that the mean size of the carrier plays a crucial role, affecting its transport through direct or indirect pathways.

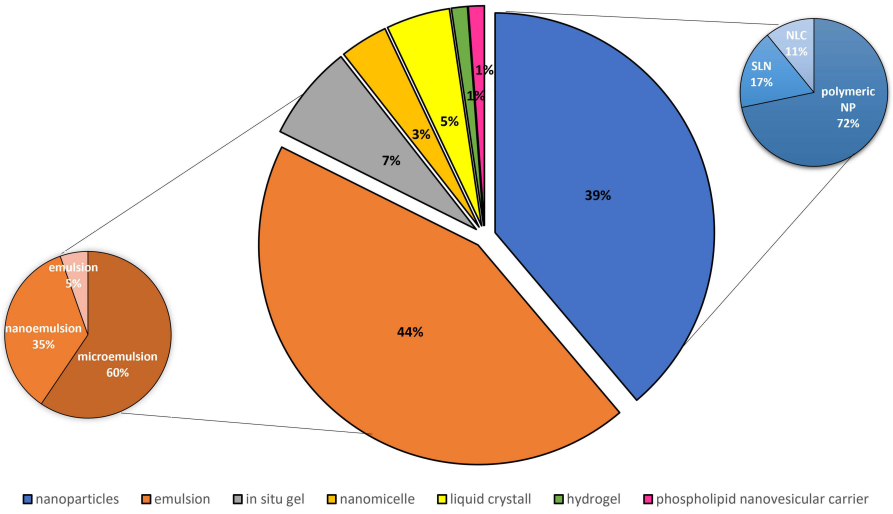

Figure 4 Summary of the type of formulations investigated in the collected publications.

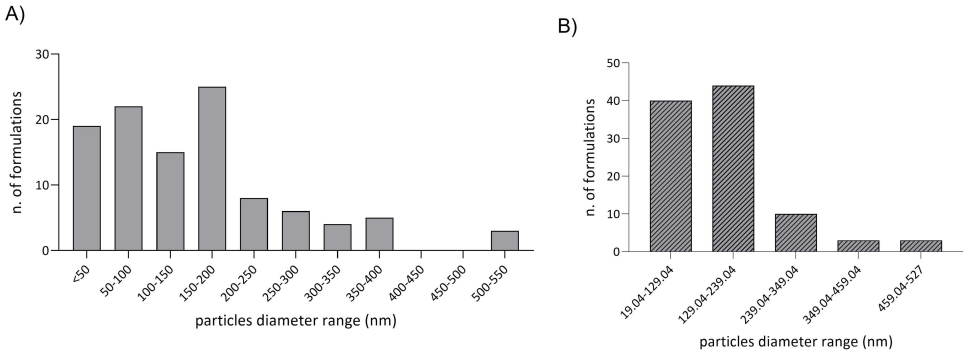

Figure 5 Classification of the nanomedicine investigated in the collected publications based on their mean size and grouped in different diameter range (**A**) and in the range studied by mathematical correlation (**B**).

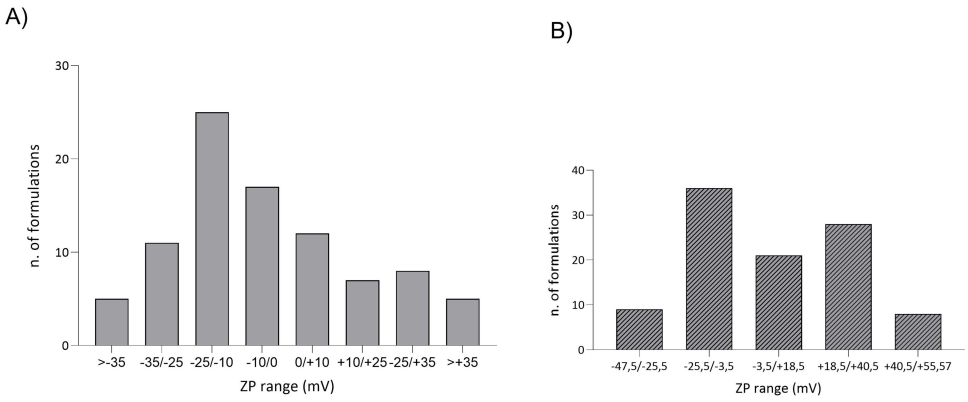

Figure 6 Classification of the nanomedicine investigated in the collected publications based on their surface charge and grouped in different zeta potential range (**A**) and in the range studied by mathematical correlation (**B**).

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In particular, nose-to-brain delivery may occur via the olfactory neuroepithelium via paracellular, transcellular or neuronal transport.

Specifically, as reported in the literature, the paracellular pathway allows the passage of hydrophilic and small molecules through tight junctions between sustentacular cells or clefts between sustentacular cells and olfactory neurons via a slow and passive mechanism.[23]

Highly lipophilic and < 500 Da molecular weight drugs can be transported through transcellular processes across sustentacular cells, most likely by receptor-mediated endocytosis, fluid phase endocytosis, or passive diffusion. Finally, the neuronal pathway consists of drug transport into neuronal cells by endocytosis or pinocytosis, and intracellular axonal transport to the olfactory bulb.[24] Olfactory ensheathing cells (OECs) are a special type of glia that ensheath bundles of olfactory axons that connect the olfactory epithelium to the olfactory bulb and play a key role in drug transport via the retrograde axonal route. A possible hypothesis (that has to be proven) is that, considering the location and role of OECs, these cells could phagocytize drugs or nanomedicines administered intranasally and could switch the engulfed material with olfactory neural cells.[17,24] As reported in the literature, to achieve direct transport to the CNS, the particle diameter should be below that of olfactory axons, which, in the case of humans, is comprised between 100–700 nm.[25] The involvement of the respiratory regions (trigeminal nerve and nasal mucosa) and the systemic pathway in particle transport to the brain could promote the passage of carriers with sizes above those mentioned. Figure 5B revised the groups that are described in the range studied by mathematical correlation.

Nanomedicines were also grouped based on their surface charge (Figure 6A), revealing that the majority of carriers studied in the collected publications presented a negative ZP from −10 to −25 mV. Specifically, 45.5% of the carriers showed a ZP below −35 to −10 mV, 32.2% of the systems were characterized by ZP values in the range of −10 to +10 mV, and 22.2% exerted a positive charge ranging from +10 to > +35 mV.

An open debate still exists regarding the ideal surface properties of a carrier conceived for nose-to-brain delivery. Some researchers have suggested that a carrier with a positive surface charge is needed when nasal administration is considered to improve the interaction with nasal mucin, overcoming the rapid mucociliary clearance in the nasal cavity and promoting a higher residence time after administration.[26] However, other theories support the idea that strong interactions can enhance particle absorption in the nasal mucosa, promoting particle passage into the systemic pathway. Thus, the role of surface charge of carriers in nose-to-brain transport remains unclear.[16]

Furthermore, the carrier surface charge seems to influence particle brain distribution mediated by the olfactory or trigeminal nerve pathways.[26] The involvement of olfactory or trigeminal transport affects particle localization in caudal or rostral brain regions in a time-dependent manner.[16] We previously demonstrated that the transfer of positive polymeric nanoparticles to the brain parenchyma was slower than that of negative polymeric nanoparticles because of the involvement of intra- or extra-neuronal pathways. The intra-neuronal pathway (trigeminal nerve) involves axonal transport and requires hours to days for drugs (and assumed nanoparticles) to reach brain regions. The extra-neuronal pathway, which probably relies on bulk flow transport through peri-neural channels, delivers drugs and particles directly to the brain parenchymal tissue and/or CSF.[27]

As shown in Figures 5B and 6B, nanomedicines were further classified according to different mean sizes and ZP ranges to collect a higher number of formulations for each group useful for the mathematical correlation.

Machine Learning Evaluation: ZP and DTE/DTP

Data collected (Supplementary Table 1) were analyzed exploiting Machine Learning approach.

As previously mentioned, data preprocessing was performed to scale the features before training the regression models to predict the target variables. This process ensures that each feature contributes proportionally to the model's performance, preventing any feature from dominating the others owing to differences in their magnitudes. Standardization is particularly beneficial when working with regression models that are sensitive to the scale of input features. Standardized features are more interpretable and make it easier to understand the impact of feature values on a model's predictions.

Given the nature of the data, the experiments were conducted considering three different data splittings. Specifically, we performed a regression analysis considering the DTE/DTP and NPvsDRUG SOLUTION, DTE/DTP, and NPvsNP data, and a third setting in which we included both types of data. For each of the above data splittings, we evaluated the relationship

between the predictors (ie, particle size and ZP) and target value. The latter can be a DTE or DTP. Considering the aforementioned three data splittings and two target variables, we evaluated six different experimental settings.

Regression analysis offers a wide range of predictors in Machine Learning. However, considering the aforementioned settings, we selected the most suitable setting for this goal. This task considered only two predictors and one target, with a dataset of 100 samples. Therefore, the number and dimensionality of the data suggest that high-dimensional methods (eg, artificial neural networks) are unsuitable for this task. Therefore, we evaluated the following methods:

Linear Regression[28]
Decision Tree Regression[29]
Random Forest Regression[30]
Support Vector Regression (SVR)[31]
Nu Support Vector Regression (NuSVR)[32]
Gaussian Process Regressor[33]
K-Nearest Neighbors Regressor[34] (KNeighbors)
Ridge Regression[35]
Lasso Regression[36]

To assess the performance of these models, we utilized key performance metrics, including R^2 and explained variance scores. The R^2 score measures the extent to which the model captures variance in the target variable. The explained Variance quantifies the proportion of the variance in the target variable explained by the model. The difference between R^2 and Explained Variance is that the latter does not account for biased variance, that is, if the error of the predictor is unbiased, the two values are the same. Consequently, the large difference between the two scores revealed a bias in the model. After detecting the most suitable model for each setting, we fine-tuned the parameters and evaluated their generalization capability using k-fold cross-validation.

Analysis on the DTP Predictability

Table 1 shows the best models for DTP predictability considering a random split of 90% of the data for training and 10% for testing. Such splitting has been defined to keep as many training samples as possible, owing to the limited amount of data. The entire dataset was composed of 100 samples, of which 46 were NPvsNP and 54 were type NPvs DRUG SOLUTION. Therefore, the number of training samples is further limited, particularly when analyzing the two types individually.

When considering all the data (ie, "both" in Table 1), we obtained good results with a KNeighbors Regressor. The Decision Tree Regressor achieved even better results for the NPvsNP type, with an R^2 score of 0.85 and an explained variance of 0.91. For NPvsDRUG SOLUTION splitting, the best results were achieved by Linear Regression; however, we observed that the quantitative performances were not satisfactory. These results suggest that data splitting may significantly affect the results as well as the choice of the model. In particular, the results decreased when we included the NPvsDRUG SOLUTION samples in the data. However, the high performance of Decision Tree Regression indicates that this model may be a good choice for a general approach.

For the Decision Tree model, we further evaluated the feature importance of the two inputs. Results show that ZP has a 0.58 of importance in the DTP prediction for the Decision Tree regressor, whereas particle size has a 0.41 of importance (see Figure 7). Similar results were obtained in all experiments, suggesting that ZP provides a higher contribution to the estimation of DTP.

Table 1 Regression Algorithms Comparison for DTP Estimation

Regressor	Target	Data Split Type	R^2 Score	Explained Variance
KNighbors	DTP	Both	0.38	0.39
Decision Tree	DTP	NPvsNP	0.85	0.91
KNighbors	DTP	NPvsDRUG SOLUTION	0.06	0.11

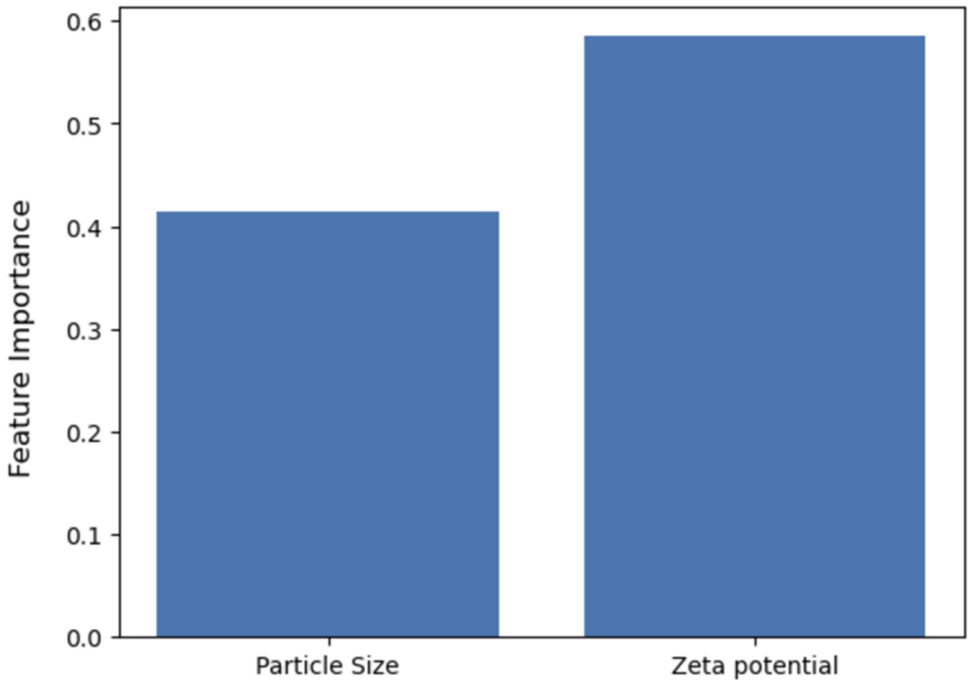

Figure 7 Input feature importance scores for DTP prediction.

The results in Table 1 were obtained by considering only one random split between the training and test datasets. To better evaluate the models, we evaluated ten random folds. Table 2 lists the best results for the 10 runs.

The results in Table 2 confirm that performance is significantly affected by the data split. Indeed, the data where NPvsDRUG SOLUTION samples were introduced reduced the performance. Moreover, by changing the random selection of train/test samples, the results changed significantly for the K-neighbor regressor but remained stable for the Decision Tree. This result further encourages the adoption of Decision Trees for general DTP regression. It is worth mentioning that the 10 folds was created using only training data, whereas the performances were measured using the test data. This further explains the slight decrease as the number of samples with which the models were trained was 90% of the training set.

Additional detailed observations can be made by considering the results obtained with 10 random splits. Figure 8 shows the Mean Absolute Errors within each split for the evaluation of the Decision Tree Regressor with the split containing only the NPvsNP samples.

Some splittings (x-axis) have very low errors (eg, numbers 1, 5, 7, and 9), whereas others have acceptable errors with narrow distributions (eg, numbers 3 and 10). However, the other four runs exhibited very large ranges. For instance, run number 6 has errors close to zero, but also close to 50, with a very large distribution. The last experiment further suggests that a predictive model can be built; however, the data selected for training are crucial. To further confirm this

Table 2 10-Folds Evaluation for DTP Prediction

Regressor	Target	Inputs	Data split Type	R^2 Score	Explained Variance	Finetuned R^2 Score	Finetuned Explained Variance
KNighbors	DTP	Particle Size + ZP	Both	0.38	0.39	0.27	0.27
Decision Tree	DTP	Particle Size + ZP	NPvsNP	0.85	0.91	0.88	0.89
KNighbors	DTP	Particle Size + ZP	NPvsDRUG SOLUTION	0.06	0.10	−0.09	−0.05

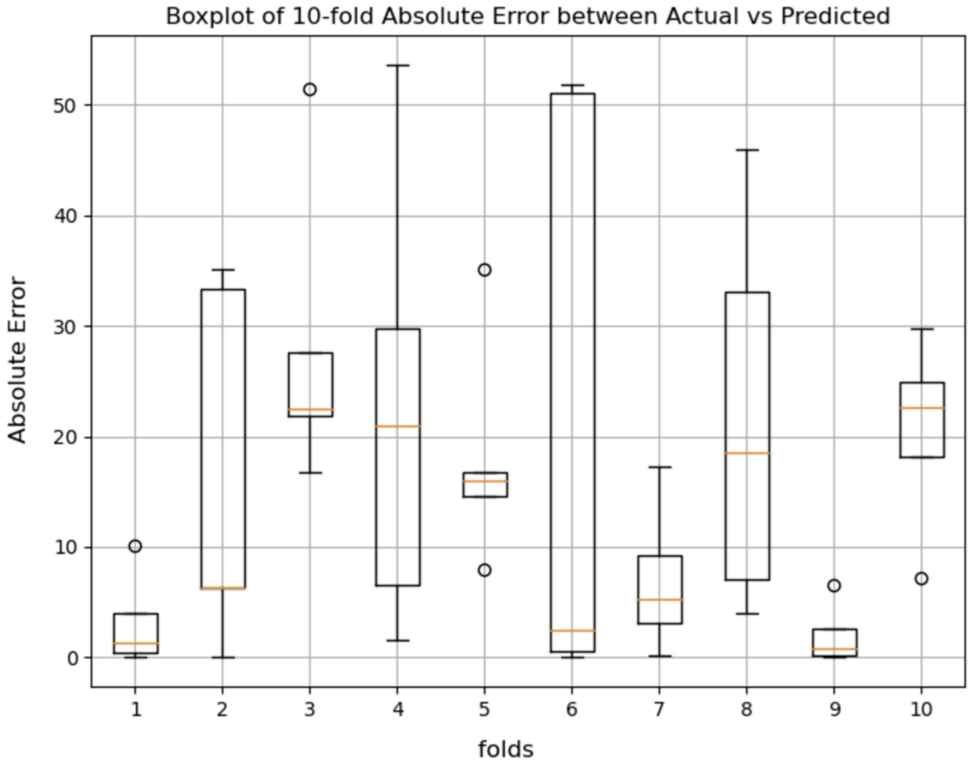

Figure 8 Absolute Errors distribution for the 10 folds (Decision Tree on NPvsNP samples).

assumption, we observed how the training and test data randomly selected for the 10 runs were distributed over the ZP and particle size ranges.

Figure 9 shows the distribution of feature values (ie, the input of the models) considering the data used for training and the data used for the test. We selected an example related to very good results and an example of very bad results, namely, a good split and a bad split. Subsequently, we compared the distributions of the training and test features. From this figure, it is evident that, for the bad split (ie, the second row), the training feature distribution is significantly different from that of the test. This implies that the model is trained (ie, learns) from a set of data that is significantly different from the set of data on which it is evaluated (ie, the test data). Conversely, in the case of a good split, the two distributions are similar and the results are very high.

Analysis on the DTE Predictability

In the case of DTE prediction, the Decision Tree regressor outperformed with an R^2 score of 0.47 and explained a variance value of 0.57 with data type both. For the other two splittings, the R^2 score was very low for all the evaluated regressors (Table 3).

To investigate these results, we analyzed the DTE data distribution, as shown in Figure 10 (ie, boxplot data distribution). The statistical indices of DTE are summarized in Table 4.

The first observation was that the mean and median values were significantly different by an order of magnitude. This is mainly caused by the outliers observed in the boxplot (ie, circles), whose maximum is close to 300.000. Further analysis revealed that the two higher values are 299.126,61 and 19.545,16, and they belong to samples with different Type DTE/DTP values. Moreover, these two specific samples have the same particle size and ZP, equal to 89.36±11,18 nm and −9.83± 0.12 mV respectively. Such pairs have the same inputs and very different outputs, which is a contradiction, in addition to their divergence from the statistical distribution.

These troubled results could be related to the fact that there is too much skewness in the data, and thus any statistical model cannot work effectively. Indeed, in skewed data, the tail regions may act as outliers for statistical models, and we

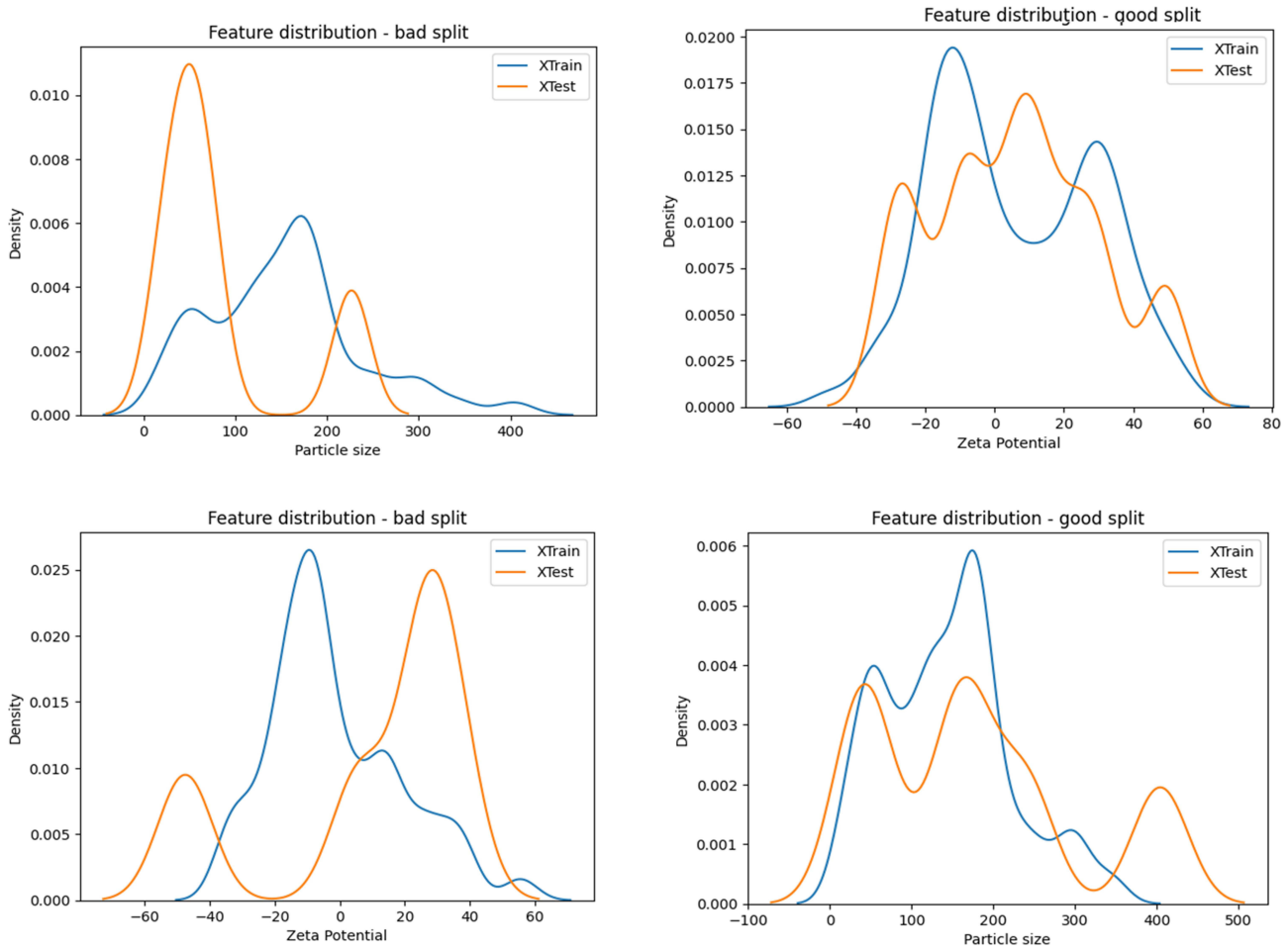

Figure 9 Distribution of input features considering a good (top) and a bad split (bottom).

know that outliers adversely affect a model's performance, especially in regression-based models. In such cases, removing outliers and normalizing the data may allow the evaluation of the regression models. Therefore, based on the statistical analysis of the DTE distribution, we repeated the experiments by considering only samples with a DTE lower than 1.000, resulting in a dataset of 78 samples.

In the case of the filtered DTE, Decision Tree regressor outperformed the other models in the case of data types NPvsDRUG SOLUTION and with all data. Linear regression analysis showed acceptable results for the NPvsNP data (Table 5). However, based on the same considerations done for the DTP analysis about the limitation of few data distribution and the high skewness of DTE data that forced us to further reduce the number of samples to be analyzed, we believe that any further investigation would not bring to significant results in the case of DTE estimation.

Table 3 DTE Regression Models' Results

Regressor	Target	Inputs	Data split Type	R^2 Score	Explained Variance	Finetuned R^2 Score	Finetuned Explained Variance
Decision Tree	DTE (<1000)	Particle Size + ZP	both	0.47	0.57	0.48	0.60
Decision Tree	DTE (<1000)	Particle Size + ZP	NPvsNP	−0.19	−0.17	−0.19	−0.17
KNighbors	DTE (<1000)	Particle Size + ZP	NPvsDRUG SOLUTION	−0.20	−0.0001	−0.20	−0.001

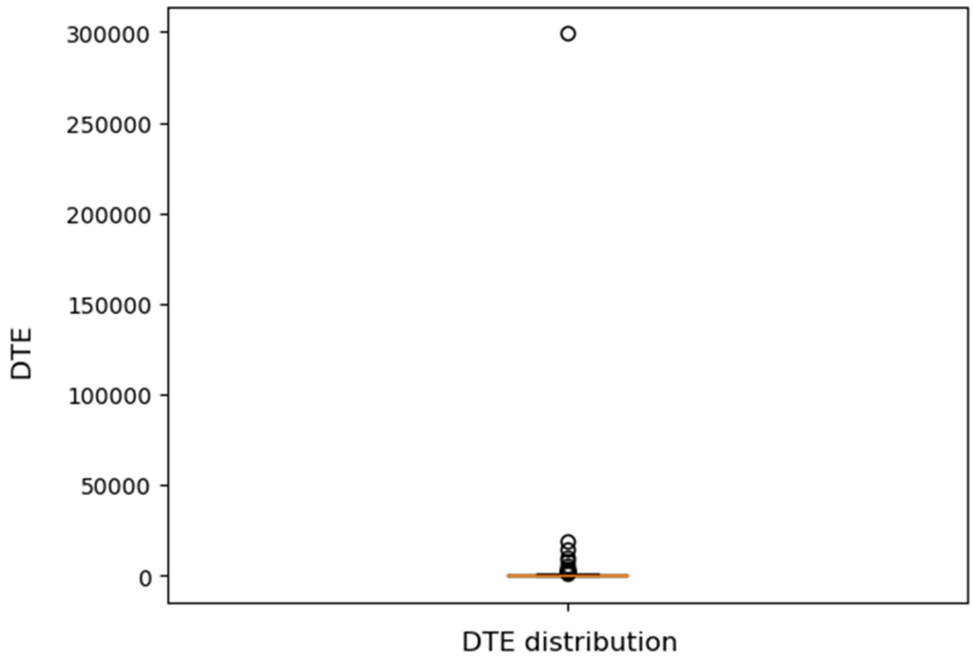

Figure 10 DTE data distribution Box plot.

Data Augmentation

A common solution is to use data augmentation to address the problem of a few data samples. It considers real data and creates similar samples by adding or removing a random statistical perturbation such that the augmented data belong to

Table 4 Statistical Indices of DTE Values in the Dataset

DTE Statistic	Value
Count	100.00
Mean	4.227,79
Std	29.918,47
Min	0.00
25%	250,66
50%	409,98
75%	759,93
Max	299.126,61

Table 5 DTE Regression with Filtered Data

Regressor	Target	Inputs	Data split Type	R^2 Score	Explained Variance
Decision Tree	DTE (<1000)	Particle Size + ZP	Both	0.37	0.38
Linear Regression	DTE (<1000)	Particle Size + ZP	NPvsNP	0.17	0.26
Decision Tree	DTE (<1000)	Particle Size + ZP	NPvsDRUG SOLUTION	0.39	0.51

Table 6 Regressors 10-Folds Results with Hyperparameters Optimization

Regressor	Target	Inputs	Data split Type	Mean R^2 Score	Mean Explained Variance	Median MAE
Decision Tree	DTP	Particle Size + ZP	NPvsNP	0.47	0.49	14.28
KNighbors	DTP	Particle Size + ZP	NPvsDRUG SOLUTION	0.66	0.67	13.86
KNighbors	DTP	Particle Size + ZP	Both	0.50	0.51	13.94
Decision Tree	DTE	Particle Size + ZP	NPvsNP	0.61	0.66	1005.70
KNighbors	DTE	Particle Size + ZP	NPvsDRUG SOLUTION	0.48	0.49	426.40
Decision Tree	DTE	Particle Size + ZP	Both	0.43	0.43	844.63

the same statistical distribution. This is a common preprocessing step in all deep-learning pipelines to facilitate model generalization. Specifically, new samples were generated for each sample by perturbing the original data with a noise of 10% of the original value.

The resulting dataset contained 500 samples. In the case of DTE, samples with only two values are omitted (ie, DTE = 29.9126,61, 19.545,16, which are contradictory). The results for the augmented data are listed in Table 6. As expected, data augmentation allowed the training and test samples to lie on the same statistical distributions, and the models were able to better learn the relationships between features and target variables. In this case, we also computed the median of the Mean Absolute Errors over ten runs to determine the mean displacement between the predicted and actual values. Again, it is possible to predict the DTP within a certain margin; however, the DTE results are challenging. The last experiment opens room for new and deeper investigations with more and better data from real experiments.

Overall, our results suggest that to build a well-performing machine learning model, it is essential to train the model on and test it against data that come from the same distribution. However, when only a limited amount of data from the target distribution are available, building a robust model may not be sufficient. Moreover, we observed different model behaviors for different values of Type DTP/DTE. The results show that poor performance occurs only when the training and test distributions are very different, whereas high results can be obtained when the two distributions are similar. In conclusion, the availability of more samples (eg, orders of thousands) would provide more robust predictive models and statements. Nevertheless, we can affirm that a Decision Tree regressor model can be successfully designed to estimate the value of DTP, especially for NPvsNP-type samples. In addition to the abovementioned issues, DTE estimation further adds to the difficulty of modeling such a large range of target variables in an extremely skewed distribution. This did not allow the building of a strong prediction model for the DTE values.

Conclusion

Literature published in the temporal range between 2010–2021 in the PubMed database was studied in relation to the use of IN delivery for CNS targeting. Analysis of 2691 publications has led to the observation that 10% of the studies (n = 286) concerned the use of nanomedicine to achieve this goal with proof of concept. Sixty-four research articles (102 formulations) were collected and used to build the NANOSE database matching specific criteria such as: i) the presence of preclinical studies on health or animal model disease (rats and mice) through the evaluation of pharmacokinetic parameters (DTE% and DTP%) to quantify the drug transported to the brain; and ii) data on nanocarrier features (type, size, surface charge).

Most of the identified publications were conceived for epilepsy treatment; the majority of the compounds studied had a low molecular weight in the range of 250–400 g/mol, consisting of small natural or synthetic molecules. Emulsions and particulate systems are more commonly used than other types of drug delivery are. Particle-based formulations were in the nanosized range, with a mean size below 550 nm, and were mainly characterized by an average size of 150–200 nm. The majority of carriers studied in the collected publications presented a negative ZP from −10 to −25 mV.

Machine learning was used to perform correlation studies between the physicochemical properties of the nanomedicine (size and ZP) and %DTE or %DTP parameters. The proposed data considered in vivo studies that considered nanomedicine given for IN/IV vs free drug or nanomedicine IN vs nanomedicine IV. The study suggested that it would be possible to estimate the value of the DTP with sufficient data for training such that it completely expresses the distribution of the phenomena. The prediction of DTE was less confident with respect to DTP, resulting in a less reliable DTE prediction model. The most general-purpose model for the regression of DTP/DTE values is represented by Decision Tree regression, followed by KNeighbor regression. The results suggest that there was a difference between the NPvsNP and NPvsDRUG SOLUTION data types in terms of DTP/DTE predictability. Specifically, the NPvsNP data were more predictable Experiments also suggest that ZP may be more significant than particle size for DTP/DTE predictability; however, this assumption requires more accurate investigation.

In light of the aforementioned findings, it can be concluded that surface charge (through ZP evaluation) more than the mean size parameter could affect the direct transport of drugs into the brain after IN administration, probably owing to its mucoadhesion properties. Furthermore, other important features such as functionalization, shape, density, coating material of nanomedicine, and viscosity of the formulations should be considered to better understand the fate of nanocarriers.

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Disclosure

The authors report no conflicts of interest in this work.

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