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In situ Intranasal Delivery Systems: Application Prospects and Main Pharmaceutical Aspects of Development (Review)

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Abstract

Introduction. Intranasal delivery of *in situ* gel-forming systems is a complex but promising direction. Due to the high cost of developing a new chemical object or genetically engineered modification of biological molecules, pharmaceutical companies are focusing on developing technologies for new delivery systems for existing active pharmaceutical ingredients to improve their effectiveness and bioavailability. *In situ* systems for intranasal delivery, due to increased viscosity and mucoadhesion to the nasal mucosa, allow overcoming mucociliary clearance and ensuring complete absorption and prolonged release of drugs.

Text. The article discusses the main advantages of intranasal *in situ* delivery systems shown in preclinical studies, as well as approaches to the technology of obtaining and standardization of these systems. The results of scientific research in this field over the past 15 years are summarized, the most promising polymers for creating thermoreversible and pH-sensitive compositions are identified, and modern methods for evaluating the sol-gel transition *in situ* are analyzed.

Conclusion. The use of *in situ* systems for intranasal administration allows providing a high targeting of the delivery of synthetic and biological molecules to the brain. Currently, numerous pharmacokinetic and pharmacodynamic preclinical studies confirm the effectiveness of such systems, as well as their safety. Thermoreversible commercially available and directionally synthesized polymers (poloxamer 407, PLGA, NIPAAm, etc.), as well as chitosan, remain the most popular for the design of *in situ* delivery systems. *In vitro* and *ex vivo* methods with mucosa and artificial nasal fluid are widely used to assess the parameters of *in situ* gelation, but to increase the reproducibility of the methods and improve the correlation in vitro/in vivo, it is recommended to conduct modeling of the nasal cavity. Developing the technology and methods of screening of intranasal reversible systems will help to get closer to clinical trials and the entry of these delivery systems into the global pharmaceutical market.

Keywords: intranasal delivery, delivery systems, *in situ* gelation, poloxamers, chitosan, mucoadhesion

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

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Интраназальные системы доставки *in situ*: перспективы применения и основные фармацевтические аспекты разработки (обзор)

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Резюме

Введение. Интраназальная доставка *in situ* гелеобразующих систем является сложным, но перспективным направлением. Из-за высокой стоимости разработки нового химического объекта или генно-инженерной модификации биологических молекул фармацевтические компании сосредоточиваются на разработке технологий новых систем доставки для существующих активных фармацевтических ингредиентов с целью улучшения их эффективности и биодоступности. *In situ* системы для интраназальной доставки за счет повышенной вязкости и мукоадгезии к слизистой носа позволяют преодолевать мукоцилиарный клиренс и гарантировать полное всасывание и пролонгированное высвобождение активных фармацевтических ингредиентов.

Текст. В статье рассмотрены виды и основные преимущества интраназальных *in situ* систем доставки, показанные в доклинических исследованиях, а также подходы к технологии получения и стандартизации этих систем. Обобщены данные научных исследований в этой области за последние 15 лет, выделены наиболее перспективные полимеры для создания термообратимых и pH-чувствительных композиций, а также проанализированы современные методы оценки золь-гель перехода *in situ*.
Заключение. Применение *in situ* систем для интраназального введения позволяет обеспечивать высокую таргетность доставки синтетических и биологических молекул в мозг. В настоящее время имеются многочисленные фармакокинетические и фармакодинамические исследования на животных, подтверждающие эффективность таких систем, а также их безопасность. Наиболее востребованными для конструирования систем доставки *in situ* остаются термореверсивные коммерчески доступные и направленно синтезируемые полимеры (полоксамер 407, PLGA, NIPAAm и др.), а также хитозан. Для оценки параметров *in situ* гелеобразования широко используются *in vitro* и *ex vivo* методы со слизистой и искусственной назальной жидкостью, однако для увеличения воспроизводимости методик и улучшения корреляции *in vitro*/*in vivo* рекомендуется проводить моделирование носовой полости. Совершенствование технологии и методов скриннинга интраназальных реверсивных систем поможет приблизиться к проведению клинических исследований и выходу этих систем доставки на мировой фармацевтический рынок.

Ключевые слова: интраназальная доставка, системы доставки, *in situ* гелеобразование, полоксамеры, хитозан, мукоадгезия

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

The intranasal method of administration is one of the most promising non-invasive drug delivery systems into the human body. This route of administration ensures a high adherence of patients to therapy since it does not require the involvement of medical personnel, thereby it being simple to conduct and highly effective, and also not traumatic [1]. The ability of intranasal administration of drugs to provide emergency care has been used for decades by medical and security services of many countries in case of a drug overdose, emergency cardiac arrest, and as an alternative to intravenous injections [2].

Currently, intranasal administration drugs are commercially available for synthetic substances (Naloxone, Dexamethasone, etc.), as well as for some biopreparations [3, 4]. It is worth noting that intranasal delivery of vaccines is successful due to the relatively large absorptive surface and low proteolytic activity of nasal mucosa, as well as due to lower production costs compared to parenteral forms [5].

In recent years, the intranasal route of administration has been used more frequently for targeted delivery of drugs to the brain, as it effectively allows the active substance to cross the blood-brain barrier by transporting it through the olfactory tract and trigeminal nerve [6].

However, despite the high permeability of the nasal mucosa, generally only active pharmaceutical ingredients (APIs) with a small molecular weight (<1000 Da) can be absorbed into the nasal cavity. In addition, mucociliary clearance causes a significant obstacle to intranasal absorption, since the drug dose can be rapidly evacuated from the nasal cavity by cilia movement and also subjected to enzymatic degradation [5, 6]. Classical technological methods of increasing API absorption by adding penetration enhancers and surfactants into the dosage form (DF) are limited for use in intranasal preparations since they can lead to suppression of physiological functions of the olfactory organ [2, 5]. Thus, the problem of intranasal administration should be solved through the development of delivery systems that provide high bioavailability and prolonged ex-

posure of the drug dose on the mucosa. Such systems include intranasal in situ delivery systems models.

"In situ" in Latin means "in the original place" or "in position". The term is used in various fields such as physics, geology, medicine, engineering, etc. In chemistry, *in situ* means "in the reaction mixture," and in biomedical engineering, protein nanogels obtained by *in situ* polymerization provide a multipurpose platform for the storage and release of therapeutically active proteins. In medicine, the term was previously used to describe a method for modeling the structure of internal organs based on cast models made of their surface [7].

Speaking about pharmaceutical development, *in situ* systems are defined as delivery systems that perform a phase transition at a specific site of the body, capable of targeting the release of API, maintaining its relatively constant concentrations due to depot formation, which ensures prolonged action of the system. In a narrow sense, *in situ* systems are considered as reversible systems in which sol-gel transition is performed locally under the action of thermal, chemical, or physicochemical factors determined by the physiological conditions of the application site [3, 5, 6].

In this review, we will explore more detailed techniques aimed at providing evidence of *in situ* gel formation, the applications of these delivery systems, and explore the polymers used in creating *in situ* delivery systems in more detail.

Retrospective of in situ delivery systems

According to the PubMed database of medical publications, the first mention of the term *in situ* in the scientific literature in the aspect of local gel formation dates back to 1984 and describes gelatin gel formation in the stomach of Wistar rats taking a hydrated gelatin diet in animals [8].

The first scientific developments of *in situ* delivery systems date back to the early 2000s. In 2001, L. E. Bromberg published a paper on the use of lightly crosslinked acrylic polymers (carbomers) and a combination of polyacrylic acid with surfactants (pluronics, poloxamers) in intranasal delivery systems with improved properties. Due to the bioadhesive properties, the pharmaceutical composition based on poloxamers demonstrated better retention in the nasal cavity, prolonged exposure in the rat model compared to carbomers [9].

Since the mid-2000s, interest in the development of *in situ* systems began to increase. Since the range of excipients and chemically modified polymers expanded, the opportunities for pharmaceutical development of delivery systems for various routes of administration expanded.

In 2003, researchers from the Korean Institute of Science and Technology (Seoul, South Korea) published a paper describing the use of Thermosensitive Cyclotriphosphazene containing ethylene glycol and amino acid esters as side groups in the technology of targeted delivery of the platinum-based anticancer drug [10].

A study conducted by scientists from the Hacettepe University (Ankara, Turkey) describes for the first time the creation of *in situ* vaginal delivery system for Clotrimazole based on a thermosensitive matrix. They used pluronic 127 (poloxamer 407) as a thermoreversible polymer whose technological properties were improved by the addition of carbomer and a cellulose derivative. A study by E. Bilensoy et al. [11] laid the foundation for a new direction in the technology of thermoreversible *in situ* systems – production and study of polycomplexes of poloxamers with various gelling agents [12].

Despite the study of various possible routes and modes of administration of reversible smart systems, intranasal delivery *in situ* remains the most popular direction of research, the number of publications concerning which increases every year (Figure 1).

The growing research interest in the development of intranasal in situ systems is associated with their advantages, which have been repeatedly noted in numerous works. Reversible systems provide sustained and prolonged drug action, improve patient compliance, and reduce the frequency of drug administration compared to conventional drug delivery systems [1, 3, 5, 6].

Intranasal *in situ* systems can demonstrate greater bioavailability due to high mucoadhesion and local sol-gel transition, which contribute to prolonged exposure even under conditions of mucociliary clearance. In their study, K-.L. Hu et al. [13] evaluated the effect of mucociliary clearance on the bioavailability of lidocaine hydrochloride administered by intranasal spray and gel systems. The absolute bioavailability of lidocaine nasal gel was approximately 1.5 times greater than that of the nasal spray, and the APIs targetability to the olfactory canal/lateral ventricles after nasal gel administration was 1.2 times greater than that of

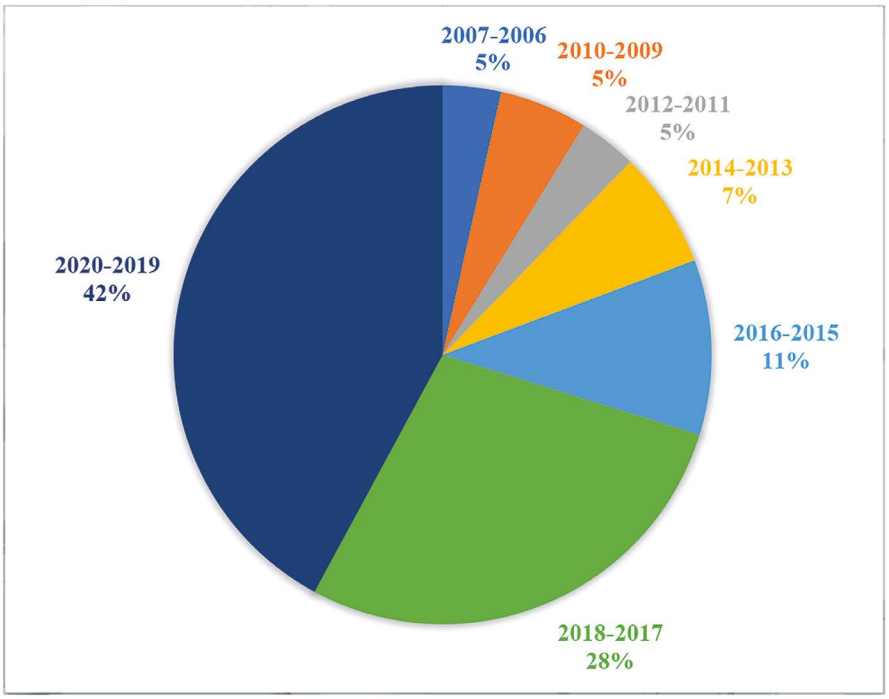

Figure 1. **Diagram of changes in research interest for *in situ* intranasal delivery systems (according to PubMed; keywords: "in-situ intranasal", "intranasal forms for system delivery")**

the spray. It was concluded that lidocaine delivery to the brain was enhanced when the gel was used as a delivery system.

Screening of polymers for in situ gel formation

Depending on the type of stimulus potentiating the phase transition, the matrices of *in situ* systems can be divided into thermoreversible, pH-dependent, ion-sensitive, moisture-activated, etc. [1].

Selecting a proper polymer with desirable potentiating stimuli, mucoadhesive properties, viscosity, and drug release is essential for the development of an effective in situ gel.

Both natural (chitosan, gellan gum, pectin, etc.) and synthetic (poloxamer (P407 and P188), carbopol, etc.) stimulation-sensitive polymers are used in modern studies [14–31].

By the results of scientific publication analysis in the PubMed database using the keywords "intranasal forms for system delivery" and "in-situ intranasal", the ten most used polymers for in situ systems were selected and ranked in descending order of frequency of their use (Table 1).

Thus, at present most *in situ* systems function regarding the type of thermoreversible (poloxamers) or pH-sensitive (chitosan, carbomers, etc.) gel formation.

Table 1. **Ranking of smart polymers used for *in situ* intranasal systems**

Rank	Polymer/Polymer Group	Percentage (%)
1	Poloxamers	23,00
2	Chitosan	22,00
3	Hydroxypropyl methylcellulose	7,70
4	Polylactic-glycolic Acid (PLGA)	6,70
5	Gums (Gellan and konjac)	5,70
6	Carbomers	5,70
7	Pectin	2,80
8	Alginates	2,80
9	Gelatin	1,90
10	Sodium hyaluronate	0,96

To correct and improve the properties of finished pharmaceutical compositions, not single-component matrices, but polymer mixtures with identical or different in situ gel formation mechanisms are often used [12]. The technology of obtaining polycomplexes based on poloxamer 407 is widely known, in which

mucoadhesive polymers with a molecular weight above 100 kDa, such as lightly crosslinked acrylic polymers (carbopols), chitosan, and various cellulose derivatives, are added to the poloxamer gel matrix to improve the bioadhesion properties: sodium carboxymethyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl cellulose, and methyl cellulose [1].

Along with the use of commercially available polymers, directed synthesis of polymers that meet the specific needs of the researcher is also in demand to create *in situ* systems.

A research team at the University of Arizona (USA), headed by Bae Hoon Lee, conducted a directed synthesis of a polymer to produce *in situ* intranasal system. The resulting polymer of N-isopropylacrylamide, 2-Hydroxyethyl methacrylate, and acrylic acid (NIPAAm) was thermoreversible and mucoadhesive and had no acute and chronic toxicity [32–34]. The gelation temperature of NIPAAm was 37 °C, which is higher than that of the best known thermoreversible polymer, poloxamer 407 (about 26 °C).

In vivo studies of intranasal in situ systems

A growing number of published preclinical studies in recent years have demonstrated the efficacy and safety of intranasal in situ delivery systems [16, 20, 21, 35–38].

One of the first studies was conducted in 2014 by two major medical centers of China Harbin University and Fudan University. A group of researchers headed by Ch. Li succeeded in creating a stable thermoreversible composition based on poloxamer 407 and carrageenan for targeted delivery of ketorolac tromethamine [35]. A pharmacokinetic study of the intranasal delivery system in rats demonstrated an increased absolute bioavailability (68.8 ± 23.3 %) and an increased mean exposure time (8.8 ± 3.5 hours) in situ gel compared to the intranasal spray (24.8 ± 13.8 %, 3.9 ± 0.6 hours).

A comprehensive *in situ* safety study of an intranasal dexamethasone delivery system was conducted at the University of Queensland (Australia) [36]. By conducting systematic cytotoxicity studies using lactate dehydrogenase (LDH) detection, no increase was detected compared to the baseline LDH level, while the integrity of the tissue of the explanted human nasal mucosa was preserved, which was further confirmed by histopathological examination of the tissue. The results obtained by P. Pandey et al. indicate the safety of the delivery system and prolonged effect due to the depositing of the drug in the human nasal mucosa.

N. Ahmad et al. [37] evaluated the pharmacokinetic characteristics of a nasal *in situ* gel containing naringenin nanoemulsion created based on a thermoreversible polycomplex of poloxamer 407 and chitosan. The study compared intranasal administration of the developed delivery system with intramuscular administration of the reference drug. In situ gel increased the bioavailability of naringenin within the central nervous system (CNS). Neurobehavioral activity (motor activity and grasping power) was significantly improved in rats with cerebral ischemia, followed by antioxidant activity. Toxicity studies performed have established a safe profile of the intranasal delivery system.

A collaborative international study performed by the Delhi Institute of Pharmaceutical Sciences and Research (New Delhi, India) and the King Saud University (Riyadh, Saudi Arabia) on raloxifene hydrochloride in intranasal *in situ* gel form for the treatment of osteoporosis demonstrated an improvement in 7.4 times in the bioavailability of APIs compared to the registered oral tablet form of raloxifene hydrochloride. In vivo pharmacodynamic studies also showed that bone density after the intranasal delivery system was increased by 162 % and biochemical markers were significantly improved compared to oral administration of raloxifene hydrochloride pills [38].

S. S. Bachhav et al. [16] studied the possibility of creating a targeted sympathetic-adrenal-medullary system based on in situ Diazepam intranasal form, which passes into a mucoadhesive gel upon contact with small amounts of water. The drug was developed as an alternative to invasive administration for epileptic seizures. In pharmacokinetic studies in a rat model using Diazepam spray as the reference drug, it was shown that APIs release from the delivery system (pH 6.4) reached 50 % in 10 minutes and lasted for 1 hour. The absolute bioavailability was 50 % for both intranasal administration of the reference drug and the in situ delivery system. Intranasal administration of Diazepam showed immediate absorption with rapid and high APIs concentration in the brain extracellular fluid compared to intravenous administration of Diazepam solution. The targeting potential of the in situ delivery system was 2 times greater than that of intranasal administration of Diazepam spray, which the researchers explained by the mucoadhesive and microemulsion properties of the delivery system.

Y. Sun et al. [20] described a method for in situ medullary targeting delivery of Paeonol, a phenolic compound isolated from plants of the genus Peony, used in Chinese medicine. The mechanism of in situ gel formation was based on the use of ion-sensitive hydroxypropyl methylcellulose. To study the movement and distribution of APIs in rats, fluorescent tags were used. The geometric mean fluorescence intensity of the cells at 1 h, 4 h, and 6 h was 1841 ± 24, 2261 ± 27, and 2757 ± 22, respectively. In situ gel loaded with solid lipid particles with fluorescent tags effectively accumulated in the brain region after administration through the olfactory region, and a fluorescent response was observed in the olfactory bulb, cerebellum, and striatum.

Intranasal in situ forms not only with synthetic APIs but also with immunobiological as well as peptide substances show superiority in pharmacokinetic studies. In a more recent study conducted by M. J. Majcher et al. [21] in situ hydrogel delivery systems based on modified chitosan loaded with a positive allosteric modulator of the Dopamine receptor D_2 showed a prolonged action when administered intranasally (up to 72 hours) on an MK-801 induced preclinical schizophrenia model in rats even at low doses of the drug (0.5 mg/kg). In comparison, conventional peptide drug intraperitoneal administration requires twice the dose of APIs to achieve a therapeutic effect that lasts only a few hours.

Unfortunately, despite numerous preclinical pharmacokinetic studies, no full-scale clinical studies of *in situ* intranasal delivery systems have been conducted to date [5]. Some of the first steps in this direction are being taken by the international team of scientists from India and Saudi Arabia [38].

Evidence of in situ gel formation

A critical parameter of in situ systems that must be evaluated during development and used to screen experimental compositions is proof of the occurrence of the target *in situ* gel formation.

In vitro and *ex vivo* methods are most commonly used to estimate this index [13, 18, 24, 39–45].

Ex vivo methods are used to determine the mucoadhesive component of *in situ* forms since it has been shown that the mucoadhesion value tends to increase sharply during the phase transition [13]. The main indicators of mucoadhesion include strength and mucoadhesion time [39, 40].

The study conducted by H. F. Salem et al. [18] used sheep's intact nasal mucosa obtained within 1 h after slaughtering as an *ex vivo* model. The intact mucosa was cleaned in 0.9 % sodium chloride solution. To study mucoadhesion strength, nasal mucosa sections selected for the study (2.5×1 cm^2 in size) were fixed on a slide with cyanoacrylate glue and gravimetrically determined the force of tissue detachment with *in situ* gel sample (weight 1.0 g) evenly distributed between them from each other [18].

Mucoadhesion time is also determined using the nasal mucosa of freshly slaughtered cattle *ex vivo*, pretreated with a solution simulating nasal fluid. Sections of the mucosa are fixed on a glass beaker with the mucosal surface facing outward. A small amount of nasal *in situ* gel was placed on the mucosal surface, after which the resulting model was placed in a cell containing 100 ml of nasal fluid simulation solution and subjected to slow stirring at 10 rpm. The time required for *in situ* gel erosion was noted visually and considered as *in vitro* mucoadhesion time [41].

The nasal fluid used in the experiments of P. Asha et al. is widely used to study other indicators of in situ intranasal systems. Its composition is described in detail in H. S. Mahajan and S. Gattani works [42] An aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$ per liter, pH6 ± 0.1 is used as a nasal fluid simulation.

In vitro methods applied to study the *in situ* behavior of intranasal systems are used to evaluate both thermoreversible matrices and systems that provide a phase transition by another method.

In the artificial nasal fluid medium, thermostated at different temperatures (from 33 °C to 37 °C in various studies [18, 43, 44, 45]), the sol-gel transition required for *in situ* gel formation is recorded visually or by instrumental methods (digital viscometry) [43–45].

S. Mohamed et al. [24] proposed to introduce a criterion for the evaluation to optimize the visual assessment of gel formation. The in situ gel was scored as (–) if gel formation did not occur, and scored as (+) if the gel formed after a few minutes and dissolved rapidly. A grade of (++) was recorded if the gel formed immediately and remained for several hours, and (+++) if the gel formed immediately and remained for an extended period.

Additional evaluation parameters are the gel time and the strength of the gel formed.

As a parameter of gel time in the study of H. F. Salem et al. [18] was taken the time in which *in situ* system reached the viscosity stopping the rotation of a magnetic

rod in the environment of artificial nasal fluid placed in the measuring vessel when placed on a magnetic stirrer with heating (stirring speed 30 rpm, temperature 34 °C).

H. Gholizadeh et al. study [44] proposed to estimate the time of gel formation visually when incubating tubes with gel in a water bath (temperature was 37 °C). The gel formation time was recorded when no flow was observed in the tilted tube. The optimal time of gel formation in this study is referred to as an interval of up to 15 minutes.

Gel strength is an additional screening parameter evaluating the ability of *in situ* systems to resist mucociliary clearance. In the study [18] to assess this parameter, 25 g of mucoadhesive gel was placed in a 50 ml measuring cylinder and incubated at 34 °C. Then, a weight of 17 g was placed on the surface of the gel. The time required for this weight to penetrate 5 cm into the mucoadhesive gel was determined as the gel strength.

Gel formation temperature is an obligatory parameter when selecting compositions of thermoreversible systems. In various works it is suggested to estimate this characteristic by any available methods (heating in a water bath, magnetic stirrer, or thermostatting in a climatic chamber), using a thermometer or a thermosensor for temperature fixation. The completion of the phase transition, as in the case of determining the gel time, is fixed by visual or instrumental methods, as well as by stopping the stirrer, in the case of using a magnetic stirrer [18].

Despite the variety of *in situ* gel-forming investigation methods described in the scientific literature, all of them, nevertheless, are not reproducible and imitate physiological features of the nasal cavity with varying degrees of reliability.

As an alternative or addition to the existing *ex vivo/in vitro* methods, *in vitro* modeling of the nasal cavity is currently in progress. Such models will help to standardize research techniques and take into account more physiological conditions: not only ionic composition, mucosa, and temperature, but also the geometry, surface area, and angle of the nasal cavity mucosa [46–55].

In vitro modeling of nasal cavity

Modern *in vitro* models of the nasal cavity are used to study the distribution of conventional liquid intranasal formulations, but they also have special potential for modeling the *in situ* gel formation process of the delivery systems under consideration [46–55].

Three-dimensional (3D) models are created based on data from computed tomography, acoustic rhinometry, and magnetic resonance imaging of healthy patients [47–53]. The models developed are used both to illustrate particle distribution processes of intranasally administered APIs [52], to measure the surface area of nasal sprays [54], and to assess the effect of mucociliary clearance on intranasal medications [48].

In vitro 3D models of nasal cavity are often made using 3D printing technology [55] or silicone molds correlating with the physiological structure of the nasal cavity [54]. To simulate certain processes, artificial nasal fluid, mucin layer or directed airflow, and constant temperature can be added to the model [49-55].

A visualization technique can be suggested for the evaluation of measurable parameters. Thus, in the study of V. Kundoor and R. N. Dalby [54], after using a silicone model of the human nasal cavity (Koken Co., Ltd.) to determine the distribution of nasal spray, the data were processed in Adobe Photoshop program, and the results were interpreted by calculating the distribution surface area.

Thus, we suppose that the creation of a standardized nasal cavity model for use as a screening method for determining *in situ* gel formation is very promising in our opinion.

CONCLUSIONS

The use of *in situ* systems for intranasal administration makes highly targeted delivery of synthetic and biological molecules to the brain possible. Currently, there are numerous pharmacokinetic and pharmacodynamic studies on animals confirming the effectiveness of such systems, as well as their safety. Thermoreversible commercially available and directionally synthesized polymers (poloxamer 407, PLGA, NIPAAm, etc.), as well as chitosan, remain the most demanded the design of *in situ* delivery systems. *In vitro* and *ex vivo* methods with mucosal and artificial nasal fluid are widely used to estimate *in situ* gel formation parameters. However, nasal cavity modeling is recommended to increase the reproducibility of the techniques and increase *in vitro/in vivo* correlation. Improving the technology and screening methods for intranasal reversible systems will help to lead them to clinical trials and the launch of these delivery systems on the global pharmaceutical market.

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