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EXPERIMENTAL STUDY

# Modeling brain pathology to study nose-to-brain drug delivery

Altynay TALGATKYZY[1], Talgat KHAIBULLIN[1], Marat SYZDYKBAYEV[1], Maksut KAZYMOV[1], Alua SHARAPIYEVA[1], Kuralay AMRENOVA[1], Shagiyeva DINARA[1], Bakytkul TOKTABAYEVA[1], Meruyert KHAMITOVA[2], Bakytbek APSALIKOV[1], Elvira OZHMUKHAMETOVA[3]

*Medical University Semey, Semey, Republic of Kazakhstan.* **talgatkyzyaltynay@gmail.com**

**ABSTRACT**

OBJECTIVES: To create a new mucoadhesive dosage form based on PluronicF127 followed by transformation into a gel form upon intranasal administration for targeted delivery to brain tissue

METHODS: Citicoline, cytidine diphosphocholine, designated as CDP-choline, was purchased as a white powder with the molecular weight of 510.31 g/mol. The triblock copolymers of polyethylene glycol-block-polypropylene glycol-block-polyethylene glycol (PEG-PPG-PEG), branded as Pluronic F127, was used.

RESULTS: When instilled into the nasal cavity, Pluronic F127 for intranasal administration is transformed into a gel that remains retained for 45-55 minutes, which promotes better penetration of drugs into the brain tissue.

CONCLUSION: The polymer's gelling and adhesive properties performed well, which is crucial for further research at the preclinical stage *(Tab. 1, Fig. 5, Ref. 28).* Text in PDF www.elis.sk

KEY WORDS: pluronic F127, intranasal solution, cytidine diphosphocholine.

## Introduction

Brain disease management is one of the most important problems of modern medicine involving global multidisciplinary groups of scientists in this process. Neuropharmacology covers a number of the present issues inside the remedy of neurological conditions for the reason that pharmacodynamics and kinetics of medicine have disparate characteristics for distinct delivery routes to various parts of the brain. The concept of the blood-brain barrier applies to both intravenous and oral routes of drug administration.

At the same time, the routes of drug administration vary widely and depend on a large number of factors, one of which is high concentration in the tissues of the targeted organ. The expected effects of intranasal administration for the delivery of medication to the brain tissue remain unclear. However, it remains undisputed that the intranasal administration shortens the delivery time owing to the direct transport through the ethmoid bone (1). The mucosal surface area of each nasal passage ranges from 0.15 $m^2$ to 0.22 $m^2$ and has been found to be most effective at low drug dose concentrations, with a simple route of administration and high patient compliance (2).

In neuropharmacology, the drug administration mode plays a significant role and directly determines the delivery route. The global pharmaceutical market is saturated with original and generic drugs that meet the needs of neurologists. However, apart from safety, patients necessitate the risks of side effects to be addressed as well (3). The existing forms of drugs are highly effective and bioavailable, but due to the uniqueness of individual human bodies, the choice of medication form needs to be personalized. The most accessible and simplest administration continues to be the oral route, covering the needs of a wide range of people's social backgrounds (4). The practicality of the tablet form lies in their casual portability, allowing the patients to take them without assistance at home or on the go. In contrast, the parenteral route necessitates the presence of a third party, an antiseptic environment, and the sterility of medical products. Intranasal medications, available as sprays or drops, can be self-administered by patients, with no assistance required.

Global scientific literature holds articles dealing with intranasal drugs such as proteins and peptides (5). But the earliest works are devoted to drugs with local action. The main reason for studying the details of this route of administration is the rapid regeneration of the mucous membranes within nasal passages. The first study of this aspect was carried out in India (6). The main difference from the simple oral route of administration is that intranasal forms bypass gastric juice. As a result, the original pharmacological activity stays preserved. With the parenteral route of administration, the activity of the drug per se does not change, but its delivered volume passing through the blood-brain barrier becomes insufficient (7).

In the global pharmaceutical landscape, sprays and drops are the predominant pharmacological forms employed in most cases for intranasal use, with gels ranking second (8). Notably, when drugs are formulated using nanotechnology, the size of the mol-

[1]Medical University Semey, Semey, Republic of Kazakhstan, [2]NCJSC "Astana Medical University", Astana, Republic of Kazakhstan, and [3]City Multidisciplinary Hospital No. 2 of the Akimat of Astana, Republic of Kazakhstan

**Address for correspondence:** Altynay TALGATKYZY, Medical University Semey, 103 Abaya Str., 071400 Semey, Republic of Kazakhstan. Phone: +77713695265

ecule of active substance is also measured in nanometers. Extensive long-term research dedicated to assessing various dosage forms has proven that penetration from the nasal passage into the brain tissue is possible when the molecule size of the active substance is no more than 200 nm (9). For example, liposomes are designed to encapsulate both hydro- and lipophilic drug molecules. Polymers selected for this delivery must have mucoadhesive, gelling and mucus-penetrating properties (10).

**Features of the structure of the human nasal cavities**

The anatomy of the human nose creates unique properties for drug penetration through the mucous membrane in the treatment of many diseases of the central nervous system (11). The nasal septum not only divides the olfactory organ into 2 vertical halves, but also separates the vestibule region, the respiratory part and olfactory part. The nasal area varies from 150 to 160 $cm^2$ on average, depending on factors such as size, age, presence of congenital or acquired defects. Each section of the nasal interior affects the penetration of molecules differently. The anterior part, lined with squamous epithelium and intertwined with sweat glands, is less significant for drug delivery (12). In contrast, the respiratory part contains ciliated epithelium and connective tissue, with microvilli characteristic of both non-ciliated and ciliated cells. However, the nasal passages, rich in blood vessels and innervated by the first pair of the trigeminal nerve from the pons of the brain stem are of particular interest. The large surface of the olfactory part, lined with basal cells, plays a crucial role in restoring the mucous membrane (13). The supporting cells in the nasal passage also contain microvilli for mechanical protection of the olfactory receptor bulb. Consequently, this section of the nasal passages is of crucial importance in facilitating the delivery of drugs from the nose to the brain tissue. The highest concentration of substances for treating CNS pathologies is located in the bulbs (14). The protection of the mucous membranes of the respiratory organs from various exogenous factors is ensured by the mucociliary system, also known as mucociliary clearance (15) (Fig. 1).

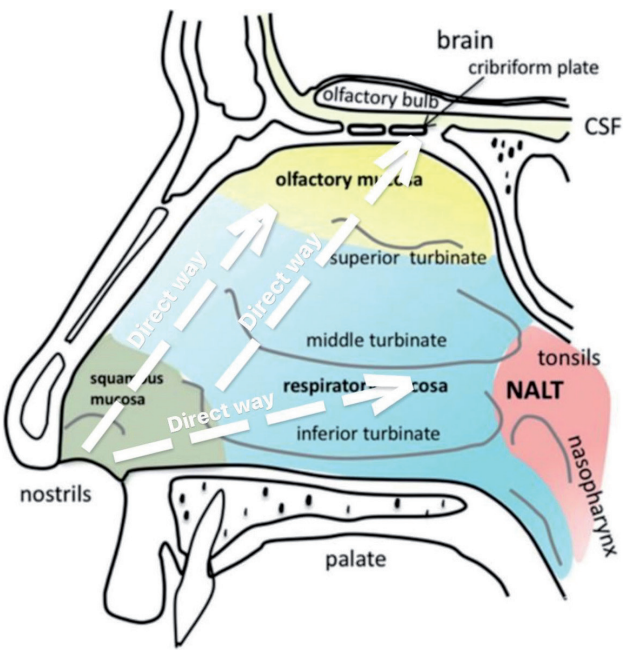



**Fig. 1. Anatomical structure.**

The human body's mucociliary clearance system comprises mucus, ciliated epithelium, and secretion, collectively contributing to an improved internal environment that facilitates oscillatory movements. The cilia within the inner layer, delving deep into the mucosa, propel secretions upward and, during the resting phase, align parallel to the surface of the mucus flow (16). The anatomical structure of the nasal passage, particularly the mucociliary clearance, regulates the duration with which drugs reside on the surface of the mucous membranes. Therefore, incorporating excipients such as gelling and mucoadhesive polymers facilitates creating conditions for better permeability of drugs (17).

Despite numerous advantages of the intranasal route of administration, practical use is still far from flawless. The efficacy of the concentration of active substance in the brain tissue via the trigeminal and olfactory nerves, is a prerequisite for the development of a highly effective system. The ultimate success of this system depends on factors such as residence time in the nasal cavity, solubility, and stability, while the use of polymers with gelling properties can serve as a platform for creating dosage forms (18). Not all polymers in medicine meet the requirements for intranasal administration. However, previously studied substances, Chitosan and *PluronicF127*, have mucoadhesive properties, enhancing the drug penetration. The ability to form a gel at body temperature eliminates side effects for patients such as preventing the flow into the nasopharynx, mitigating any burning sensation, and adverting the spread of the unpleasant taste associated with the medicine. Despite the diversity of polymers' properties, the correct ratio during manufacturing holds the key to establishing the quality parameters for intranasal drug delivery systems, especially in overcoming the hematoencephalic barrier (19).

The goal of our research is to create a new mucoadhesive dosage form based on *PluronicF127* that can be transformed into a gel form upon intranasal administration for targeted delivery to brain tissue.

Thermosensitive polymer solutions are highly potential, along with the development of high-quality delivery systems, facilitating easy scalability of production and allowing these solutions to be in the form of nasal drops or sprays ( 20). In our work, we investigated a gelling solution based on *PluronicF127* in healthy experimental rats (following the Ethics Committee Protocol of the NC JSC "SMU" #2, dated October 28, 2020, and in accordance with the Statement on the Use of Animals for Neuroscience Research).

There are scientific papers dedicated to the use of *PluronicF127*, as a platform for creating dosage forms. However, in these experiments, the gelling polymer was used in the treatment of ocular pathologies and was applied directly under the conjunctiva. Pluronic gelling polymer F127 has been proven to have the potential to increase the concentration of the antibacterial drug ciprofloxacin in the cornea of the eye. Building on the

experience gained from using this polymer, we systematically conducted a series of experiments on rats to study the residence time on the nasal mucosa of a polymer gel-forming solution containing citicoline powder. Citicoline is chemically composed of cytidine-5'-(trihydrogen phosphate) mono (2-(triethylammonio)ethyl) (ether) hydroxide (internal salt) and is presented as sodium salt in certain forms of preparations.

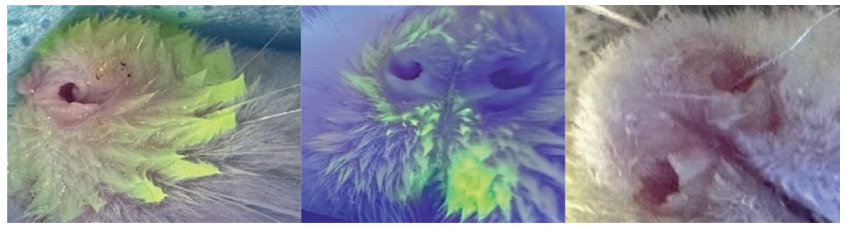


**Fig. 2. Photographs of instillation of Pluronic-based gelling solution F127 into the nasal cavity of experimental rats for 35, 45, and 55 minutes.**

## Materials and methods

Citicoline, *also* known as cytidine diphosphocholine and designated as CDP-choline, was purchased as a white powder with a molecular weight of 510.31 g/mol. The triblock copolymers, specifically polyethylene glycol-block-polypropylene glycol-block-polyethylene glycol (PEG-PPG-PEG), branded as Pluronic F127, were sourced from BASF (Germany).

Intranasal solutions were prepared by dissolving 20% *PluronicF127* in a 1 mg/ml solution of citicoline and 1 mg/ml fluorescein. All experiments were carried out on experimental rats in the vivarium of the Scientific and Educational Laboratory of the NC JSC "SMU". The weight of rats varied in the range of 210–270 grams. During the experiments, a volume of 50 µl of each solution was instilled into both nasal passages of rats. In the first series, the bright color of fluorescein facilitated the assessment of retention on the nasal mucosa by visual inspection aided by UV light and photography using the iSlim 2020 AF device (KYE Systems Corp., Taiwan). Nasal fluid samples were collected by wetting swabs (OlaSilk Sense, Russia). Subsequently, the samples were placed in test tubes with alcohol for complete extraction in 2 ml of 90% ethanol (Dosfarm LLP, Kazakhstan) for 1 hour, followed by analysis of the concentration on a high-performance liquid chromatograph. Each series of experiments was repeated on 5 different rats.

## Results and discussion

*Preliminary stage*

Before starting the main stage of the study, we conducted a preparatory stage. Preparation included: assessment of the level of consciousness of rats under sedation and without sedation, selection of the volume of solution for intranasal administration from 40 to 70 µl, and gelation time for a given route of administration.

Exclusion criteria were weight of rats below 205 grams, long gelation time, and administration of a gelling solution with a volume of less than 50 µl.

*Study design*

The experimental design involving rats at the scientific and educational laboratory of Semey Medical University, the Republic of Kazakhstan provides a valuable advantage for tailoring the chemical properties of new polymer-based mucoadhesive dosage forms for subsequent preclinical research. The critical factor in the experiment was the method of administration into the nasal passages. When the rats were positioned on their backs, the solution completely penetrated the nasal passages, with the possibility of partial ingestion. When positioned laying on the side, there was a partial reverse flow of the solution, evident through fluorescence at the entrance to the nasal passages. Only when the administration carried out while the rats were lying on the back was followed by repositioning them on the side was it possible to fully adapt the method minimizing the loss of solution and maximizing the gelation effect (Fig. 2). According to various sources, the temperature of rats' nasal passages in rats varies from 35.5ºC to 37.1ºC. Therefore, the gelation of the entire injected solution was not feasible when instilled on the side. Table 1 shows the gelation time for various routes of administration.

Nasal solutions were prepared according to the method previously proposed by Khutoryanskiy et al for the production of ophthalmic drug forms based on polymers of nonionic nature (22).

Figure 2 depicts the flushing process of fluorescein and allows retention to be observed visually and with a camera. We found that the retention time is directly determined by the method of instillation. Aligning these rules with the physiological structure of the internal nasal cavity contributes to extending the retention duration. So, for example, lying on the side lowers

**Tab. 1. Methods of introducing the solution into the nasal passages and retention time.**

| Rat No. | Horizontal position with head tilted back, (50 µl injected), min | Horizontal position on the side, (50 µl injected), min | Horizontal position on the back followed by repositioning on the side (50 µl injected) |
|---|---|---|---|
| 1 | 60 | 35 | 60 |
| 2 | 50 | 40 | 65 |
| 3 | 55 | 30 | 60 |
| 4 | 50 | 35 | 58 |
| 5 | 48 | 42 | 62 |

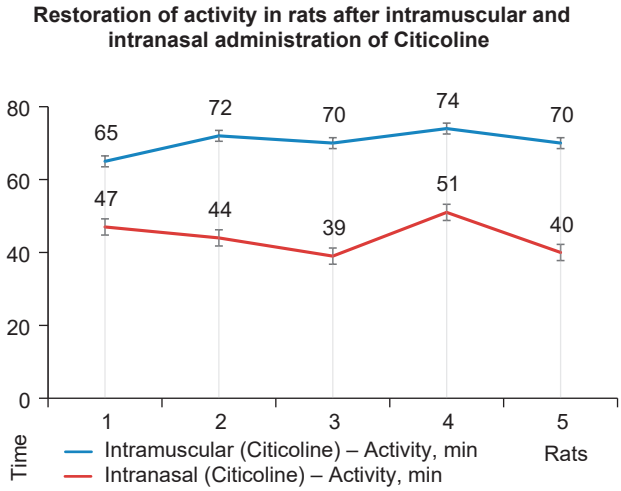



**Fig. 3. Dynamics of citicoline release over time.**

the temperature at the entrance to the nasal passages, prolonging the gel transformation time by 15 minutes compared to tilting the head back. Failing to immediately position the subject the side causes most of the solution to flow into the nasopharynx, reducing fluorescein concentration and consequently implying a reduced dose of the administered drug. In addition, at the initial stage, maintaining a fixed head position for approximately 12.5 minutes is necessary for enhancing the gel adherence to the mucous membrane.

Studies focused on concentrated aqueous solutions (20%) of triblock copolymers PEG-PPG-PEG, commercially known as *PluronicF127*, have proven their unique propensity for being transformed into a gel at temperatures above 37ºC (23). The solutions prepared for our experiment contained pharmacologically active drugs and at storage temperatures below –5ºC, they were transparent with low viscosity. In this regard, this solution is easily applicable in the form of drops or nasal spray. When introduced into the physiological environment, the body temperature inside the nose triggers an immediate gelation process.

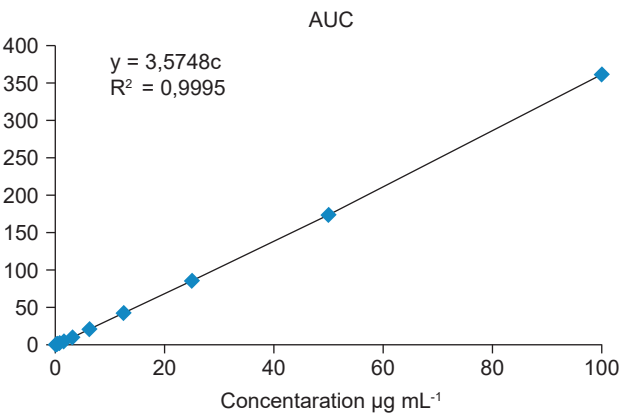



**Fig. 4. Citicoline calibration curve graph for high-performance liquid chromatography.**

The drug citicoline, known for its neuroprotective activity in treating cerebral circulatory disorders, was loaded into the nanoparticles of the polymer through ionic gelation. This type of dosage form was then further investigated for its potential of being delivered to the brain tissue via the intranasal route (24). Over the past decade, research has accumulated experience in studying drug delivery through similar gelling systems proven to be effective both *ex vivo* and *in vivo*. To enhance the mucoadhesive properties of polymers, the methods of conjugation and production of thiomers are used. Conjugation involves the use of thiols and catechols, while thiomers are formed by adding thiol groups to polymers. Pharmacokinetic studies on rats showed a significantly higher concentration of the growth hormone drug in the blood plasma, particularly when administered via the intranasal route and using carbofilcysteine/glutathione 114.8 mIU/ml as compared to saline 17.5 mIU/ml. Pharmacological studies in rats demonstrated an earlier onset of the "active motor phase" effect with intranasal administration of citicoline compared to traditional intramuscular administration. The "zero activity" state after intramuscular administration was 1.5 times longer than after intranasal administration. The average time for activity to occur after intramuscular administration was 70.2 minutes, whereas after intranasal administration, it was 44.2 minutes (Fig. 3). These results were likely influenced by both, the route of administration and the prolonged exposure of the solution due to the gelling system, which allowed for a more gradual drug release.

Each series of experiments was carried out on rats under standard dietary, housing, and nutrition conditions. The vivarium is located within the accredited research laboratory of the NC JSC "SMU". The evidence-based study of the polymer was conducted on experimental animals, and the testing of the intranasal solution was carried out directly *in vivo* without a preliminary assessment of biocompatibility in rats. Visual control aided by a camera and UV lamp continued until the fluorescence under the fluorescein lamp in the nasal passages completely ceased.

For the convenience of instilling the solution, a special clamp was used, where the nasal part of the rat was placed into an oval hole with soft edges, thereby immobilizing the animal and ensuring the introduction of the full volume correctly. Before each use, the solution was stored in a medicine storage refrigerator at a temperature of minus 4–5ºC. A volume of 50 µl was then taken with a dispenser and instilled into each right nasal passage of the rat. According to early retention study protocols for mucoadhesive dosage forms, the average gelation time is 12.5±1.2 minutes (25). Collection from the nasal passages was carried out 5 minutes after instillation and subsequently at intervals of 10 minutes up to 60 minutes. The obtained samples were analyzed on a Stayer high-performance liquid chromatograph from Acvilon, using a reverse-phase analysis option.

The chromatograph was equipped with a fluorimetric detector using a quartz halogen lamp (26). Control over the chromatography process and analysis of the obtained chromatogram data was carried out using the Multichrome computer program. The analysis used a separation column, Phenomenexluna 5u C18, with a protective cartridge filled with sorbent, bidistilled water, acetonitrile

(27), and acetate buffer. The volume of the injected sample was 20 µl; the flow rate was 1 ml/min.

The analysis of nasal fluid samples was carried out as follows: 0.5 ml of double-distilled water was added to a cotton swab moistened with nasal fluid, and extraction was performed in ultrasonic bath for 2 hours. The resulting solution was filtered through a 6-µm filter and analyzed.

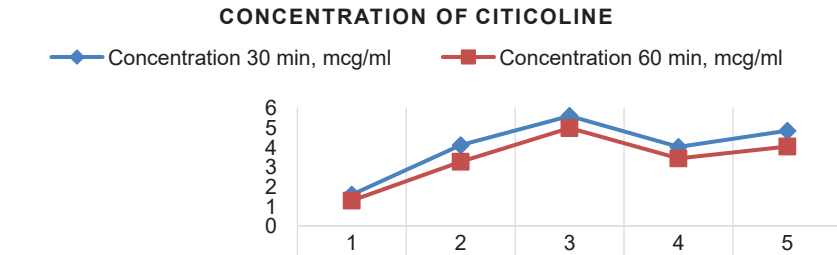
### Reagents used in this study

Double-distilled water was used to prepare solutions for all chemical reagents, both primary and secondary. The citicoline medication contains a minimum of 98% of the active ingredient. For the citicoline solution, 0.1 mg of citicoline was dissolved in 50 ml of distilled water from 0.01 grams of powder, ensuring a 98% concentration of the active ingredient. Additionally, an aqueous solution of γ-cyclodextrin (CD) (Fluka) containing at least 98% of the primary ingredient was utilized. By dissolving 0.0324 g of dry matter that contained at least 98% of the primary component in 25 ml of distilled water, a solution of γ -cyclodextrin with a concentration of $110^{-2}$ M was created. To construct a calibration curve, 1 ml of ammonium acetate buffer solution was added to each of the 8 test tubes. A volume of 0.25 ml of $5{\times}10^{-5}$ M γ-cyclodextrin, then various volumes of the original citicoline solution in the concentration ranging from 0.1to100 µg/ml were diluted with a buffer solution to a total volume of 5 ml, and an aliquot of 50 µl was taken from each tube and introduced into the chromatograph. To prepare the mobile phase, we used acetonitrile ("Panteac") with the main substance content of 99.9% (28).

In this series of experiments, the study was conducted on five laboratory rats using a single type of dosage form. The rats were administered a gelling solution containing citicoline, which was based on *Pluronic F127.*

To establish the citicoline calibration curve, 3,000 mg of the drug was dissolved in 3,000 mg of ultrapure distilled water, resulting in a concentration of 1 mg per 1 ml. Subsequently, a range of solutions was prepared by diluting this standard solution to concentrations ranging from 100 to 0.1 ng $ml^{-1}$ (Fig. 4).

We compared the concentrations of five different nasal samples containing citicoline in the nasal passages 30 minutes after instillation. The results showed that in one animal, the average concentration of the drug was 1.58 mcg/ml, which was significantly 5 times lower than that observed at later time points after the instillation of Pluronic F127 (mean=7.91 µg/ml, p=0.001). This difference remained statistically significant when compared to the concentrations observed in animals 4 and 5, which were 3 times lower (mean=4.0 µg/ml and 4.82 µg/ml, respectively, p=0.001). Animal 3 exhibited the second highest concentration of citicoline in the nasal passages (mean=5.57 µg/ml, p=0.001). It is worth noting that in this part of the experiment, the sample size was less than n=5, leading us to consider the distribution abnormal.




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Concentration 30 min, mcg/ml | 1,58 | 4,09 | 5,57 | 4 | 4,82 |
| Concentration 60 min, mcg/ml | 1,28 | 3,25 | 4,96 | 3,42 | 4,02 |

**Fig. 5. Changes in the concentration of citicoline in experimental animals depending on the time of instillation into the nasal passages.**

After 60 minutes, the pattern of citicoline concentration differed from that observed at 30 minutes, and this difference was statistically significant (p=0.001). Since the number of involved rats remained constant, the distribution was also considered abnormal. The lowest average concentration of citicoline was observed in rat 1, specifically at 1.28 µg/ml, which was 0.3 µg/ml lower than the concentration observed at 30 minutes, and 5.9 times lower than the concentration in animal 2 (mean=7.56 µg/ml, p=0.001) (Fig. 5).

### Conclusion

A transparent liquid solution based on *Pluronic F127* polymer has been prepared for intranasal administration as a polymer solution. This solution is stored in a refrigerator and upon instillation into the nasal cavity, it transforms into a gel that is retained for 45-55 minutes. This property of the polymer solution promotes a better penetration of drugs into the brain tissue, indicating its good adhesive properties. The studied dosage form has been found to be devoid of any side effects, as evidenced by the absence of any animals dropping out of the experiment, while their behavior, general condition, and appearance remained unaffected. The use of an intranasal dosage form based on *the Pluronic F127* polymer resulted in a slight decrease in the concentration of citicoline 60 minutes after instillation compared to 30 minutes, confirming the drug's extended release and high pharmacological activity.

### References

**1. Porfiryeva NN, Semina II, Moustafine RI, Khutoryanskiy VV.** Intranasal Administration as a Route to Deliver Drugs to the Brain (Review). Drug development & registration 2021; 10 (4): 117–127. https://doi.org/10.33380/2305-2066-2021-10-4-117-127.

**2. Hua Yang, Dong Sun, Yuanyuan,Tang, Xiuzhen Sun.** Biomechanical study of particle deposition in the nasal and nasopharyngeal cavities of healthy subjects. 2022. DOI: 10.21203/rs.3.rs-2320566/v1 LicenseCC BY 4.0.

**3. Dolenina OE, Perevalova VD.** German companies in the global pharmaceutical market. 2023. DOI: 10.24891/fa.16.3.266.

**4. Sam Orubu et al.** WHO essential medicines for children 2011–2019: age-appropriateness of enteral formulations. Arch Dise Childhood 2021; 107 (4): archdischild-2021-32183.

**5. Nose-to-Brain Delivery of Peptides and Proteins**. 2023. DOI: 10.1007/978-3-031-23112-4_10 In book: Nasal Drug Delivery.

**6. Jeehye Maeng, Kyunglim Lee.** Systemic and brain delivery of antidiabetic peptides through nasal administration using cell-penetrating peptides Literature Review. Frontiers Pharmacol 2022.

**7. De Martini LB, Sulmona C, Brambilla L.** Cell-Penetrating Peptides as Valuable Tools for Nose-to-Brain Delivery of Biological Drugs. Cells 2023; 12 (12): 1643. DOI: 10.3390/cells12121643.

**8. Imaeva AE, Balanova Y, Kontsevaya A.** Availability and Affordability of Medicines for the Treatment of Cardiovascular Diseases in Pharmacies in Six Regions of the Russian Federation. 2018. Rational Pharmacotherapy in Cardiology. DOI: 10.20996/1819-6446-2018-14-6-804-815.

**9. Kolev J, Grigorov E, Petrova G.** Review of the Strengths of New Generic Medicinal Product Appearance at the Market October 2019.

**10. Imam ME, Hornof M, Valenta C, Bernkop-Schnürch A.** Evidence for the interpenetration of mucoadhesive polymers into the mucus gel layer. S.T.P. Pharma Sciences 2023; 13 (3): 171–176.

**11. Singh M, Kanoujia J, Parashar P, Arya M, Tripathi CB, Sinha VR et al.**Assessment of improved buccal permeation and bioavailabilityoffelodipine-microemulsion-based cross-linked polycarbophilgel. Drug DelivTransl Res (Internet) 2018; 8 (3): 591–601.

**12. European pharmacopoeia** 10.4. 2.9.4. Dissolution test fortransdermal patches. (https://pheur.edqm.eu/app/10-4/content/10-4/20904E.htm?highlight=on&terms=dissolution).Revised April 2021.

**13. Aneman R, Prat A.** The blood–brain barrier. Cold Spring Harborperspectives Biol 2015; 7 (1): a020412.

**14. Bourganis V, Kammona O et al.** Recent advances in carrier mediated-nose-to-brain delivery of pharmaceutics. Eur J Pharm Biopharm 2018; 128: 337–362.

**15. Bonferoni MC, Riva F, Invernizzi A et al.** Alpha tocopherolloadedchitosanoleatenanoemulsions for wound healing. Evaluation on cell linesand ex vivo human biopsies, and stabilization in spray dried Trojanmicroparticles. Eur J Pharm Biopharm 2018; 123: 31–41.

**16. Awad R, Avital A, Sosnik A.** Polymeric nanocarriers for nose-to--brain drug delivery in neurodegenerative diseases and neurode-velopmental disorders. Acta Pharm Sin B 2022 (in Press).

**17. Keller LA, Merkel O, Popp A.** Intranasal drug delivery: opportu-nities and toxicologic challenges during drug development. Drug Deliv Transl Res 2022; 12 (4): 735–757.

**18. Belgamwar AV, Khan SA, Yeole PG.** Intranasal dolutegravirsodium-loaded nanoparticles of hydroxypropyl-beta-cyclodextrinforbrain delivery in Neuro-AIDS. J Drug DelivSci Technol 2019; 52: 1008–1020.

**19. Al Khateb K, Ozhmukhametova EK, Mussin MN, Seilkhanov SK, Rakhypbekov TK, Lau WM, Khutoryanskiy VV.** In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery. Internat J Pharmaceutics 2016; 502 (1–2): 70–79.

**20. AbilovaGK, KaldybekovDB, OzhmukhametovaEK, Saimova AZ, Kazybayeva DS, Irmukhametova GS, Khutoryanskiy VV.** Chitosan/poly (2-ethyl-2-oxazoline) films for ocular drug delivery: Formulation, miscibility, in vitro and in vivo studies. Eur Polymer J 2019; 116: 31–320.

**21. Alabdly A, Kassab H.** Formulation Variables Effect on Gelation Temperature of Nefopam Hydrochloride intranasal in Situ Gel. Iraqi J Pharm Sci 2023; 31 (Suppl 2022): 32–44.

**22. Khutoryanskaya OV, Morrison PWJ, Seilkhanov SK, Mussin MN, Ozhmukhametova EK, Rakhypbekov TK, Khutoryanskiy VV.** Hydrogen–Bonded Complexes and Blends of Poly (acrylic acid) and Methylcellulose: Nanoparticles and Mucoadhesive Films for Ocular Delivery of Riboflavin. Macromol Biosci 2014; 14 (2): 225–234.

**23. Agibayeva LE, Kaldybekov DB, Porfiryeva NN, Garipova VR.** Gellan gum and its methacrylated derivatives as in situ gelling mucoadhesive formulations of pilocarpine: In vitro and in vivo studies. Internat J Pharmaceutics 577, 119093.

**24. Kolawole OM, Lau WM, Khutoryanskiy VV.** Chitosan/β-glycerophosphate in situ gelling mucoadhesive systems for intravesical delivery of mitomycin-C. Internat J Pharmaceutics: X 1, 100007.

**25. Kolawole OM, Lau WM, Khutoryanskiy VV.** Synthesis and evaluation of boronated chitosan as a mucoadhesive polymer for intravesical drug delivery. J Pharm Scie 108 (9): 3046–3053.

**26. Cook MT, Schmidt SA, Lee E, Samprasit W, Opanasopit P.** Synthesis of mucoadhesivethiol-bearing microgels from 2-(acetylthio) ethylacrylate and 2-hydroxyethylmethacrylate: novel drug delivery systems for chemotherapeutic agents. J Materials Chem B 3 (32): 6599–6604.

**27. Taihyun Chang.** Chromatographic Separation of Polymers. DOI: 10.1021/bk-2018-1281.ch001 October 29, 2018 Recent Progress in Separation of Macromolecules and Particulates Chapter 1pp 1–17.

**28. Jiménez-Skrzypek G, Ortega-Zamora C, González-Sálamo J, Hernández-Sánchez C, Hernández-Borges J.** The current role of chromatography in microplastic research: Plastics chemical characterization and sorption of contaminants. https://doi.org/10.1016/j.jcoa.2021.100001.

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