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RESEARCH ARTICLE

The Role of Amphiphilic Compounds in Nasal Nanoparticles

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Abstract

Nasal medications hold significant importance and are widely utilized due to their numerous advantageous properties, offering a compelling route for both local and systemic therapeutic effects. Nowadays, the development of nasal particles under 1 micrometer is in the focus of much scientific research. In our experiments, the use of innovative nanotechnology to increase the effectiveness of the active substance was of paramount importance. Our aim was to create solid nanoparticles that enable targeted and effective delivery of the active ingredient into the body. The innovation of this experimental series lies not only in highlighting the importance of amphiphilic compounds in enhancing penetration, but also in the fact that while most nasally administered formulations are in liquid form, our formulation is solid. Liquid formulations frequently suffer from the disadvantage of possible leakage during administration, which can reduce the bioavailability of the active ingredient. In our experiments we created novel drug delivery systems of finely divided powders, which, thanks to the penetration enhancers, can be successfully administered. These enhancers facilitate the swift disintegration and penetration of the particles through the membrane. This represents a new direction in nasal drug delivery methods. The results of our trials are promising in the development of innovative pharmaceutical products and outline the role of amphiphilic compounds in more efficient utilization and targeted application of active substances. According to our results it can be concluded that this innovative approach not only addresses the common issues associated with liquid nasal formulations but also paves the way for more stable and effective delivery methods. The use of finely divided powders for nasal delivery, enabled by penetration enhancers, represents a major breakthrough in the field, providing a dependable alternative to conventional liquid formulations and ensuring improved therapeutic results.

Keywords nanoformulations · nasal drug delivery systems · RPMI cells · spray drying

Introduction

The field of nasal medicine has received significant attention in pharmaceutical industry in recent years [1, 2]. Nasal administration has many advantages, among which the direct and effective targeted delivery of drugs to the affected area stands out [3]. This minimizes systemic side effects and makes therapy more effective [4]. Nasal application allows drugs to be quickly absorbed and delivered to the bloodstream, making them an ideal choice for drugs that need to be reached in the body in a short period of time [5]. However, nasal drug administration has several disadvantages as well [6]. These include inconsistencies in drug absorption caused by alterations in nasal physiology due to diseases, the challenge of passing drug molecules through the nasal epithelium, and limited information on factors affecting drug absorption in the nasal cavity. To

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help address these challenges and ensure effective nasal drug delivery, extensive research has been conducted on nasal formulations, innovative drug delivery carriers, and emerging technologies over the past few decades [7]. Among these options, formulation of submicron-sized carriers is proved to be a promising strategy [8]. In innovative nasal drug administration, the use of nanoparticles is of paramount importance [9]. These solid, nanoparticles offer excellent tools for targeted and efficient delivery of active ingredients [10]. The use of nanotechnology allows for a longer and controlled release of active ingredients, which can improve the effectiveness of therapy [11, 12]. Incorporation of penetration-enhancing amphiphilic compounds improves the physical and chemical properties of formulations as well as enhance drug absorption, transepithelial transport, and bioavailability [13, 14]. These excipients play a pivotal role in the field of drug development, especially in those preparations whose effectiveness is sought to increase [15]. As pharmaceutical technology aids, amphiphilic compounds are able to contribute to the success of pharmaceutical products in several respects [16]. First of all, amphiphilic compounds can help improve the solubility and stability of active ingredients [17]. These compounds have properties that allow them to contain both hydrophobic and hydrophilic parts [18]. Taking advantage of this property, they help active pharmaceutical ingredients to dissolve better in water, thus increasing the bioavailability of preparations [19]. Secondly, amphiphilic compounds are extremely useful in targeted delivery of active ingredients. These compounds can help active ingredients penetrate individual tissues or cells more efficiently. To enhance the targeted therapeutic effect of active substances, amphiphilic compounds are often used in preparations that require delivery of the active substance precisely to the target organ or cell [20]. Finally, amphiphilic compounds can also improve the release and effectiveness of active ingredients [21]. These compounds can help with a controlled release of active substances from the composition, which allows prolonged therapy, as well as simplifying dosage [22].

Chlorpromazine serves as an ideal model compound for pharmaceutical experiments due to its classification as a Biopharmaceutical Classification System (BCS) Class IV drug, which is distinguished by its low solubility and low permeability [23, 24]. Due to the low permeability and high first pass metabolism the oral bioavailability of Chlorpromazine is only 30% [25]. Therefore, an alternative administration route should be considered during drug delivery design. As a commonly used antipsychotic, the use of chlorpromazine may demonstrate the benefits and limitations of nasal medication [26, 27]. In addition, the generally known pharmacokinetic properties of chlorpromazine make it possible to conduct a wide study of the behavior of the active substance in various compositions [27].

The aim of the present study was to demonstrate the importance of the development of nasal medicine, the role of nanoparticles in innovative medicine, and the value of using chlorpromazine as an excellent model in this area. For this purpose, different formulations containing chlorpromazine and permeation enhancers were investigated in order to improve the absorption of the active ingredient through the nasal mucosa. The results of experiments with chlorpromazine-based nanoparticles and their significance for the further development of nasal medicine are presented in detail. The novelty of this experimental series is not only in demonstrating the significance of amphiphilic compounds in penetration enhancement but also in the fact that a significant portion of nasally applied formulations are liquid dosage forms, whereas our formulation is solid. Liquid formulations often have the drawback of potentially leaking during application, which can limit the bioavailability of the active substance. Our drug delivery systems consist of finely divided powders, which, thanks to the penetration enhancers, can be successfully administered. These enhancers ensure the rapid disintegration and membrane penetration of the particles. This represents a new direction in nasal drug delivery methods. In our study, the use of innovative nanotechnology to increase the effectiveness of the active substance chlorpromazine was of paramount importance. Our goal was to create solid nanoparticles that enable targeted and effective delivery of the active ingredient into the body. The addition of amphiphilic penetration enhancers to the nanoparticles played a prominent role in the formulation, which effectively increased the absorption and efficiency of the active substance. The effect of these penetration enhancers has been successfully demonstrated in several preliminary experiments. Another important aspect of the innovation was the nanonization of heat-sensitive active ingredients. An effective solution to this problem was also found, which made it possible to deliver the active substances to the nose of rats in the right dose. This new technology allows stable dosing of heat-sensitive active ingredients, contributing to the effectiveness of therapy. The results of our trials are promising in the development of innovative pharmaceutical products and outline the role of amphiphilic compounds in more efficient utilization and targeted application of active substances [28]. The composition-based mechanisms of action and the ability to atomize heat-sensitive active ingredients could inspire further research and drug development in this exciting area [29]. This innovative approach not only addresses the common issues associated with liquid nasal formulations but also paves the way for more

stable and effective delivery methods. The ability to use finely divided powders for nasal delivery, facilitated by penetration enhancers, marks a significant advancement in the field, offering a reliable alternative to traditional liquid forms and ensuring better therapeutic outcomes.

Materials and Methods

Materials

Chlorpromazine (CPZ), Kolliphor RH 40, Poloxamer 470, polyvinyl alcohol (PVA), Hexane and bovine serum albumin were obtained from Sigma-Aldrich (St. Louis, MI, USA). 2-hydroxylpropyl-β-cyclodextrin (HPBCD) was purchased from Cyclolab (Budapest, Hungary). Transcultol HP, and Labrasol were kind gifts from Gattefossé (Lyon, France). The human nasal epithelial cell line (RPMI 2650) and the human colon adenocarcinoma Caco-2 cell line were sourced from the American Type Culture Collection (ATCC, Manassas, Virginia, USA). The MTT paint, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, along with buffer solutions such as Hank's Balanced Salt Solution (HBSS) and phosphate-buffered saline (PBS), were procured from Sigma-Aldrich (St. Louis, MI, USA). The RPMI and Caco-2 cell culture maintenance medium, TrypLE™ Express Enzyme, was supplied by Thermo Fisher Scientific (Waltham, MA, USA). Ninety-six-well cell plates and culture flasks were acquired from VWR International (Debrecen, Hungary).

Cell Culturing

RPMI cells were cultured in RPMI medium, while Caco-2 cells were cultured in DMEM in plastic cell culture flasks, supplemented with 2 mM L-glutamine, 100 mg/L gentamycin, and 10% heat-inactiva ted fetal bovine serum at 37 °C in a 5% CO2 atmosphere. The culture medium was replaced twice weekly. Cells were routinely maintained through regular passaging. Before passaging, the flasks were coated with rat-tail collagen. Cells used for cytotoxicity experiments were between passage numbers 10 and 30 [30, 31].

MTT Viability Assay

To assess the cytotoxicity of the excipients selected in the study, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was conducted. The experiments were performed on the cells following this procedure. Cell maintenance happened through weekly passages. For MTT assay, the cells were seeded on a 96-well plate at a density of 10,000 cells per well. Once the cells covered fully the membrane of the wells, the experiment was initiated. First, the culture medium was removed, followed by the application of test solutions, which were incubated with the cells for 60 min. After this incubation period, the tested excipients were removed, and the MTT paint solution at a concentration of 5 mg/mL was added. The cells were then incubated for 3 h. Viable cells converted the water-soluble tetrazolium bromide into a formazan precipitate. After incubation, the formazan precipitate was dissolved using a 25:1 mixture of isopropanol and hydrochloric acid. The absorbance of these solutions was then measured with a spectrophotometer, which is directly proportional to the number of the remaining viable cells. Cell viability was expressed as a percentage of PBS (negative control), with each experiment conducted in five parallel measurements.

Table I Selected Compositions of CPZ for Nano Spray Drying

Compositions	Ingredients	Quantities (mg)	Total quantity (ml)
Composition 1	PVA	100	50
	CPZ	250	
	Labrasol : Kolliphor Rh40 (2:1)	5	
Composition 2	PVA	100	50
	CPZ	250	
	TranscutoL HP : Kolliphor Rh40 (2:1)	5	
Composition 3	PVA	100	50
	CPZ	250	
	HPBCD	250	
Composition 4	PVA	100	50
	CPZ	250	
	Poloxamer 407	5	

Preparation of Solid Submicron-Sized Delivery Systems

Composition 1–4 is created by dissolving the components based on Table I. The final concentration for pray drying was 50 ml in all cases. Solid-phase nanoparticles were produced using a Büchi Nano Spray Dryer B-90 HP system [32]. In this process, the active ingredient is first dissolved in the solvent compositions along with necessary excipients. This prepared solution is then atomized into fine droplets using a piezoelectric nozzle, ensuring uniform droplet size and distribution. These droplets are rapidly dried by a stream of heated gas as they are sprayed into the drying chamber, where the solvent evaporates, leaving behind solid nanoparticles. Finally, the dried nanoparticles are collected using an electrostatic collector, which ensures high recovery efficiency and minimizes product loss. This method allows for precise control over particle size and morphology, which is crucial for consistent drug localization and distribution within the nanoparticles. Blends were injected with previously defined 90% pump rate. The drying gas was heated to 100 °C on the inlet side, and to 33 °C on the outlet side. According to our observations the inlet temperature was decreased to 55 °C due to the API protection. To ensure the required quality of the products, the flow rate was altered from 0.11 m^3/h to 0.16 m^3/h. The actuator was driven at 130 kHz. Physical properties of the prepared carrier were examined by two methods. The particle size distribution was evaluated in the dispersion state using a Malvern ZSP Nano Zetasizer (Malvern Panalytical; Malvern, UK) (Fig. 1).

Determiation of Particle Size and Distribution

Dynamic light-scattering apparatus was applied to evaluate the particle size of the created nanoparticles [33]. In each cases 0.5 g of the samples was dissolved in 20 mL hexane and was exposed to a monochrome light wave. When the light meets a solution containing nanomolecules, the light is scattered in all directions. Each experiment was carried out in five parallel measurements. Each measurement was instantaneous; the analysis of particle size and size distribution took less than one minute per sample.

Thermo Scientific™ Axia™ ChemiSEM™ Scanning Electron Microscope (Auro-Science Consulting, Budapest, Hungary) was used to examine the morphological properties of nanoparticles. The samples were attached to a fixture with a double-sided adhesive tape containing graphite and the excess amount was washed off with argon gas. Any surface pre-treatments and ex-post corrections not used during the measurement. The measurement requires a high vacuum, 20 kV accelerating voltage and 15 µs dwell time. Magnifications were 10000x in all cases.

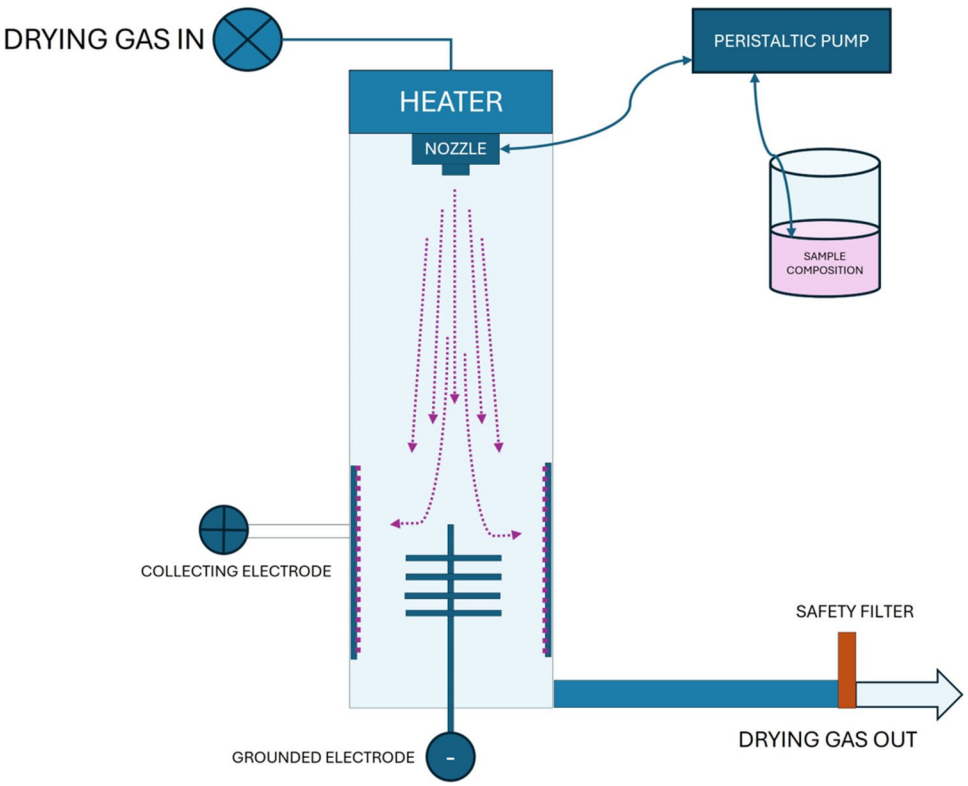

Fig. 1 Schematic figure of Büchi B90 Nano Spray Dryer operation in nanoparticle formulation

Evaluation of Particle Disintegration

Disintegration of nanoparticles was investigated in pH neutral physiological (0.9%) sodium chloride saline [34]. Disintegration time is rounded to the nearest second. Complete disintegration was confirmed by DLS testing. Each experiment was carried out in five parallel measurements [35].

Evaluation of Active Ingredient Dissolution

To determine the dissolution profile of the CPZ, an ERWEKA DT 950 dissolution tester was used. The dissolution medium was the artificial saline solution according to the European Pharmacopoeia's regulation. The determination of the active substance was carried out according to the blow detailed protocol 2.10 with GC-MS [36]. The samples were taken in the 10th and 30th seconds after release into the medium and every 15 s thereafter. During the determination, 5 parallel measurements were carried out.

Stability Tests

Samples were stored under the following conditions to simulate various environmental factors that could influence stability. Samples were stored, for the long term storage at 25 °C ± 2 °C, 60% ± 5% relative humidity (RH), for the accelerated test, at 40 °C ± 2 °C, 75% ± 5% RH and for the refigarated test at 5 °C ± 3 °C representatively. Samples from each batch were stored in tightly sealed containers under each condition for a period of 12 months. Analytical assessments were conducted at specific time points: initial (T0), 1 month, 3 months, 6 months, and 12 months. To determine any changes in the particle size distribution (PSD) over time, nanoparticles were analyzed again using a Malvern ZSP Nano Zetasizer (Malvern Panalytical, UK) with the previosly descibed method. Approximately 1 mg of the nanoparticle sample was dispersed in deionized water under gentle sonication to ensure homogeneity. Measurements were carried out at room temperature in triplicate for each time point. The Z-average particle size and polydispersity index (PDI) were recorded to monitor potential aggregation or changes in particle size over the storage period. Morphological changes in the nanoparticles were assessed using Scanning Electron Microscopy (SEM). During the test previosly apllied test had been repeated. Samples were mounted on aluminum stubs with conductive tape and sputter-coated with gold before imaging. The SEM micrographs were compared over the storage period to detect any alterations in surface texture, shape, or aggregation that could suggest physical instability. To quantify the API within the nanoparticles and detect any degradation, a Gas Chromatograph-Mass Spectrometer (GC-MS) analysis was conducted. The Shimadzu GCMS-QP2010 system, equipped with an SLB-5 ms capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness), was used to determine the API concentration. Samples were injected in triplicate at each time point, and the concentration of the API was determined by comparing the peak areas of the samples to a calibration curve generated from known standards. Any decrease in API concentration beyond 5% of the initial value was considered indicative of chemical instability. The results from the particle size distribution, morphology, and API content analyses were compiled and compared over time. Statistical analysis was performed using ANOVA to detect significant differences between time points and storage conditions. A p-value of < 0.05 was considered significant.

Animals and Sample Collection

All animals received human care, consistent with the national guide (NSMR) and the Laboratory Animals' Guide to Care and Use formulated by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86 – 23, revised 1985). The maintenance and treatment of rats used in this study was also approved by the Institutional Animal Care and Use Committee of the University of Debrecen (2/2021/DEMÁB). The animals were kept under standard conditions. The temperature of the room was 23 ± 2 °C and the lighting was switched to alternating periods of darkness and light for 12 h. The rats were kept on normal rodent chow and ad libitum tap water. Healthy male Sprague-Dawley rats (400 ± 30 g) were anaesthetized with intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). The femoral vein was cannulated for blood collection [37]. 3.0 ± 0,1 mg of CPZ preparations were administered intra nasally with the help of Dry Powder Insufflator (Penn-Century Inc.). Venous blood samples were taken at baseline and 5, 15, 30 and 60 min after CPZ administration; at the end of the experiments rats were terminated by i.v. sodium-pentobarbital injection (200 mg/kg). Venous blood samples were left for 30 min to 1 h at room temperature to coagulate and centrifuged at 10000 x g for 10 min. The resulting supernatant (non-hemolytic serum) was mixed with 2 volumes of acetonitrile, to remove the protein from the serum. After vigorous mixing all samples were centrifuged at 1000 x g for 10 min, and the supernatant were used for GC-MS analysis.

Sample Preparation

The collected supernatants were dried under a gentle stream of N2 at 60 °C using a Turbovap LV concentrator. The samples were re-dissolved in 100 µl CH_3CN and 1 µl was injected into a GC-MS equipment.

Gas Chromatograph-Mass Spectrometric (GC-MS) Analysis

Gas chromatograph-mass spectrometer (Shimadzu GCMS-QP2010, Kyoto, Japan) was applied to determine the CPZ content. At this setup the GC was equipped with an SLB-5 ms capillary column (Supelco, Bellefonte, PA, USA) (30 m x 0.25 mm i.d.; 0.25 µm film thickness). The operating conditions were set as follows: carrier gas was helium (He) with a flow rate of 32 cm/sec. The column temperature program was as follows: held at 150 °C for 0.25 min, ramped from 150 °C to 300 °C at 40 °C/min, and finally maintained at 300 °C for 4 min. The injection port temperature was set at 280 °C, while the interface temperature was 300 °C. A 1 µL sample was injected into the GC-MS using a micro-syringe with a split ratio of 1:50. The mass spectrometer was equipped with an electron ionization (EI) source, with ionization energy set to 70 eV, ion source temperature at 200 °C, and a solvent cut time of 2.5 min. Measurements were conducted in Selected Ion Monitoring (SIM) mode, with registered ions at 318 m/z and 58 m/z, chosen based on the EI spectrum obtained in SCAN mode.

Statistical Analysis

To perform statistical analysis, Microsoft Excel 2021 and SigmaStat 4.0 (version 3.1; 2019, SPSS, Chicago, IL, USA,) were used. Viability studies on cell cultures and *in vitro* dissolution tests were carried out by one-way ANOVA and repeated measures ANOVA method, followed by Tukey or Dunnett follow-up testing. The difference between the averages was considered significant in those cases when p found to be smaller than 0.005. Each experiment was repeated at least five times to process the data.

Results

Selection of Core Materials and Excipients for Nasal Particles

During the selection of excipients, common pharmaceutical technology regulations were followed. The excipients used are all pharma grade, so their purity and quality meet the highest standards. Drawing from our previous research experience and results, and considering findings available in international literature, we selected the components with great care. During the composition formulations, possible incompatibilities between the components were examined. After standard and forced stability tests, we can state that the performed systems are stable. The formulations did not change between storage conditions suitable for the active substance (UV protection) after 4 weeks. Based on our screening studies on *in vitro* cell cultures, safe, non-toxic excipients and composition were selected. For the investigations, we used standardized methods from the European Pharmacopoeia. These selected formulations were then used for further investigations.

In Vitro Toxicity Screening of Core Materials and Excipients for Nasal Particles

In our toxicity studies, applied materials were tested under *in vitro* conditions. RPMI 2650 cell line was used as a model of the nasal epithelium. Evaluation of safety aspects has been performed by MTT cell viability tests.

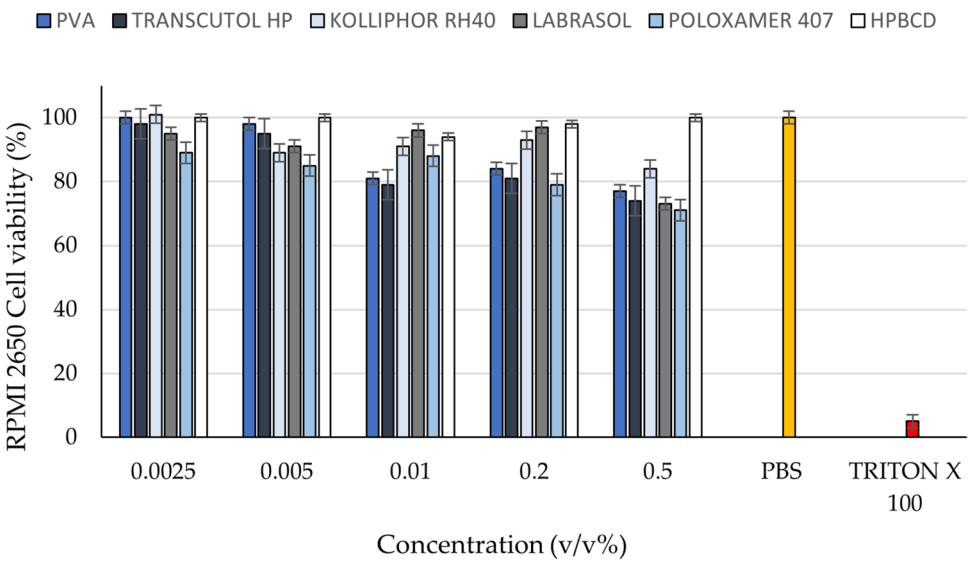

Fig. 2 Effect of PVA and other excipients on RPMI cell viability, evaluated with MTT cell viability assay. Positive control was Triton X-100, negative control was phosphate-buffered solution (PBS). Each data point represents the mean ± SD, $n = 5$

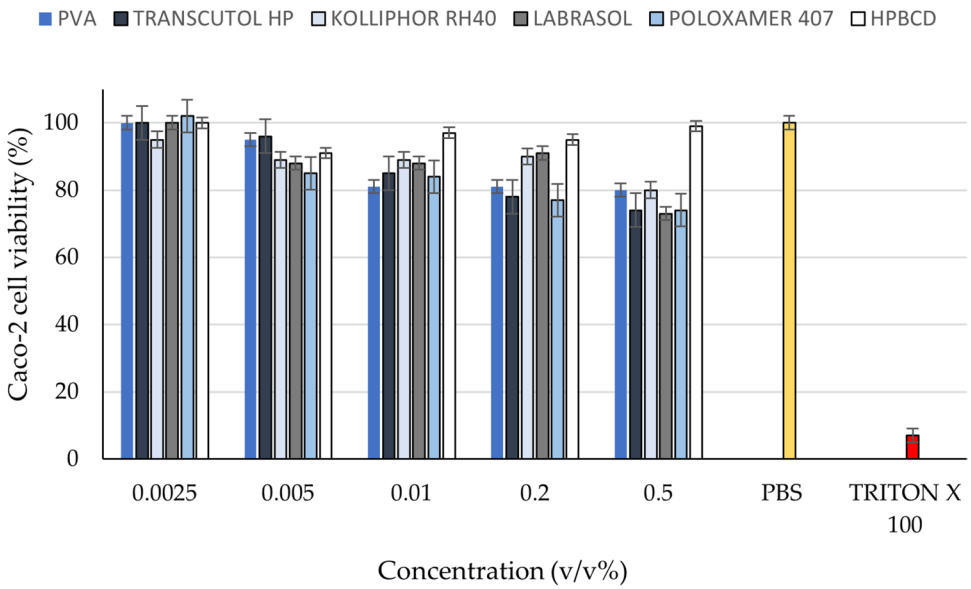

Fig. 3 Effect of PVA and other excipients on Caco-2 cell viability, evaluated with MTT cell viability assay. Positive control was Triton X-100, negative control was phosphate-buffered solution (PBS). Each data point represents the mean ± SD, $n = 5$

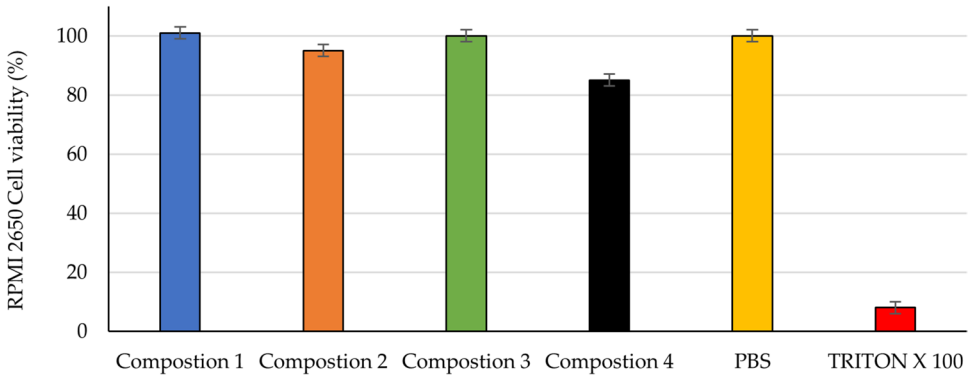

Fig. 4 Effect of dissoluted compositions on RPMI 2650 cell viability, evaluated with MTT cell viability assay. Positive control was Triton X-100 (0.5 v/v%), negative control was phosphate-buffered solution (PBS). Each data point represents the mean ± SD, $n = 5$

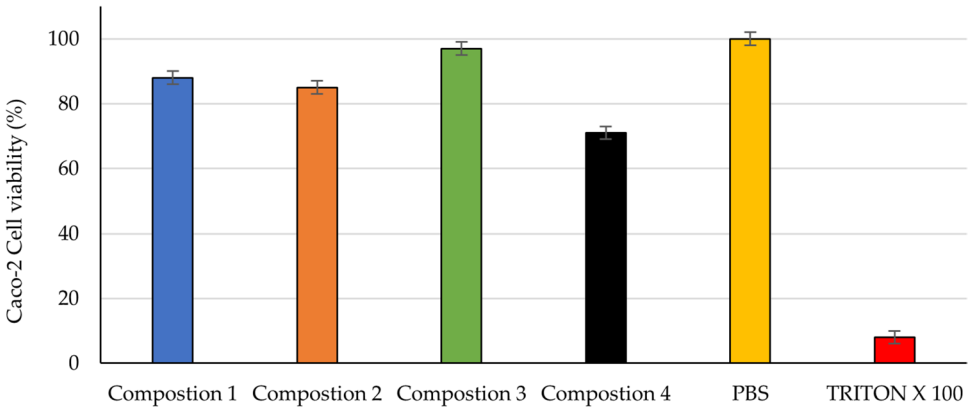

Fig. 5 Effect of dissoluted compositions on Caco-2 cell viability, evaluated with MTT cell viability assay. Positive control was Triton X-100, negative control was phosphate-buffered solution (PBS). Each data point represents the mean ± SD, $n = 5$

Our measurements were supplemented with MTT cell viability test on Caco-2 cell line as well. Results had been demonstrated at Figs. 2 and 3. Our experiments have shown that the selected substances are non-toxic either at the applied concentrations or at a possibly higher concentration. The experiments were also carried out with the compositions. The results are demonstrated in Figs. 4 and 5. The results confirmed that the compositions did not

Table II Evaluated Parameters for Nano Spray Drying Process

Parameter	Standard values	Evaluated values
Inlet gas temperature	100 °C	55 °C
Output gas temperature	35 °C	30 °C
Feed pump rate	90%	90%
Nebulizer rate	80%	90%
Nebulizer voltage	125 kHz	130 kHz
Chamber pressure	30 mbar	55 mbar
Flow rate	110 dm³/h	160 dm³/h

cause any significant change in cell viability. Composition 3 changed the cell viability the least, and Composition 4 proved to be the less tolerable composition.

Formulation of Solid nano Carriers

Due to the high sensitivity of the active substance to high temperatures and direct light, several experiments had to be performed to adjust the parameters of the usual nano spray drying operation and standardize the ideal conditions. Based on our measurements, the ideal production conditions are summarized in Table II.

Determination of Particle size and Distribution

According to the performed determinations, it was demonstrated that the formulated solid particles are in the submicron size range. The measurement results are summarized in Figs. 6 and 7. Based on the size distribution studies, small, insignificant size differences were found in parallel measurements. Spray drying procedure of composition 1 and 2 resulted in particles with similar sizes around 450 nm. The formulation process of composition 4 resulted in larger particles around 750 nm, while the spay drying of composition 3 resulted in extremely small particles around 250 nm.

Evaluation of Particle Disintegration

The prepared compositions showed convincing, rapid disintegration during disintegration tests. The most rapid disintegration was clearly measured in case of HPCD-containing Composition 3, while the slowest disintegration was measured with Poloxamer-containing Composition 4. The measurement results are illustrated in Fig. 8.

Evaluation of Active Ingredient Dissolution

The dissolution profile of the compositions was summarized in Fig. 7. During the dissolution tests, an average of 75% of the incorporated active substance could be detected. No significant differences could be evaluated between dissolution profiles in the studies. According to the results of the dissolution test, we can conclude that approximately 70–75% of the initial 250 mg of the active ingredient was encapsulated within the drug delivery systems during formulation. The difference in the kinetics of dissolution can be explained by the results presented in Fig. 9. Upon examination of the kinetic profiles, determining factors of similarity and dissimilarity among the curves revealed no significant differences in kinetics. The curves and the dissolution of the systems were identified as similar.

Animal Studies of CPZ Utilization

The actual CPZ content of the preparations was determined prior to the experiments. These results were submitted in Fig. 10. Our results showed that the CPZ content of the

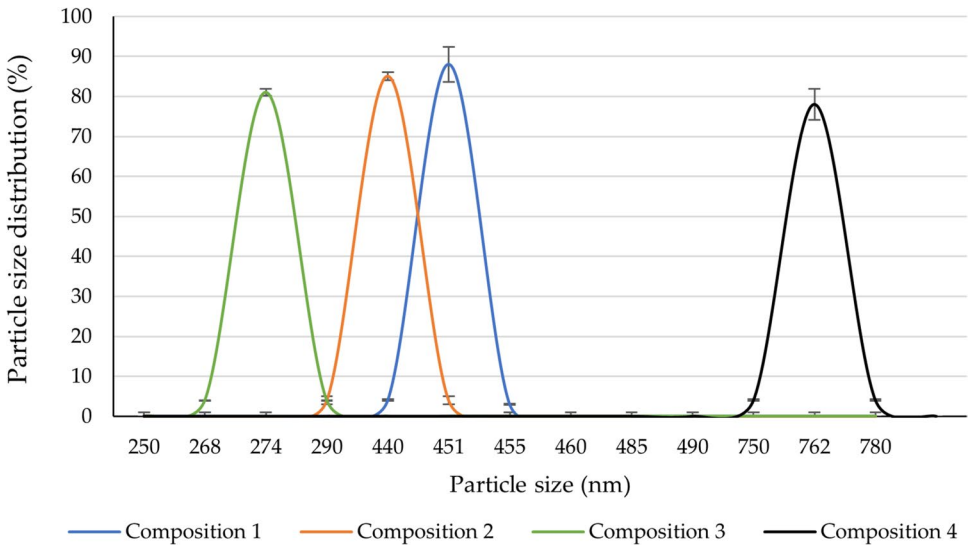

Fig. 6 Size and size distribution of the dispersed Compositions [1–4] evaluated using Malvern ZSP nano zetasizer. Each data point represents the mean ± SD, $n = 5$

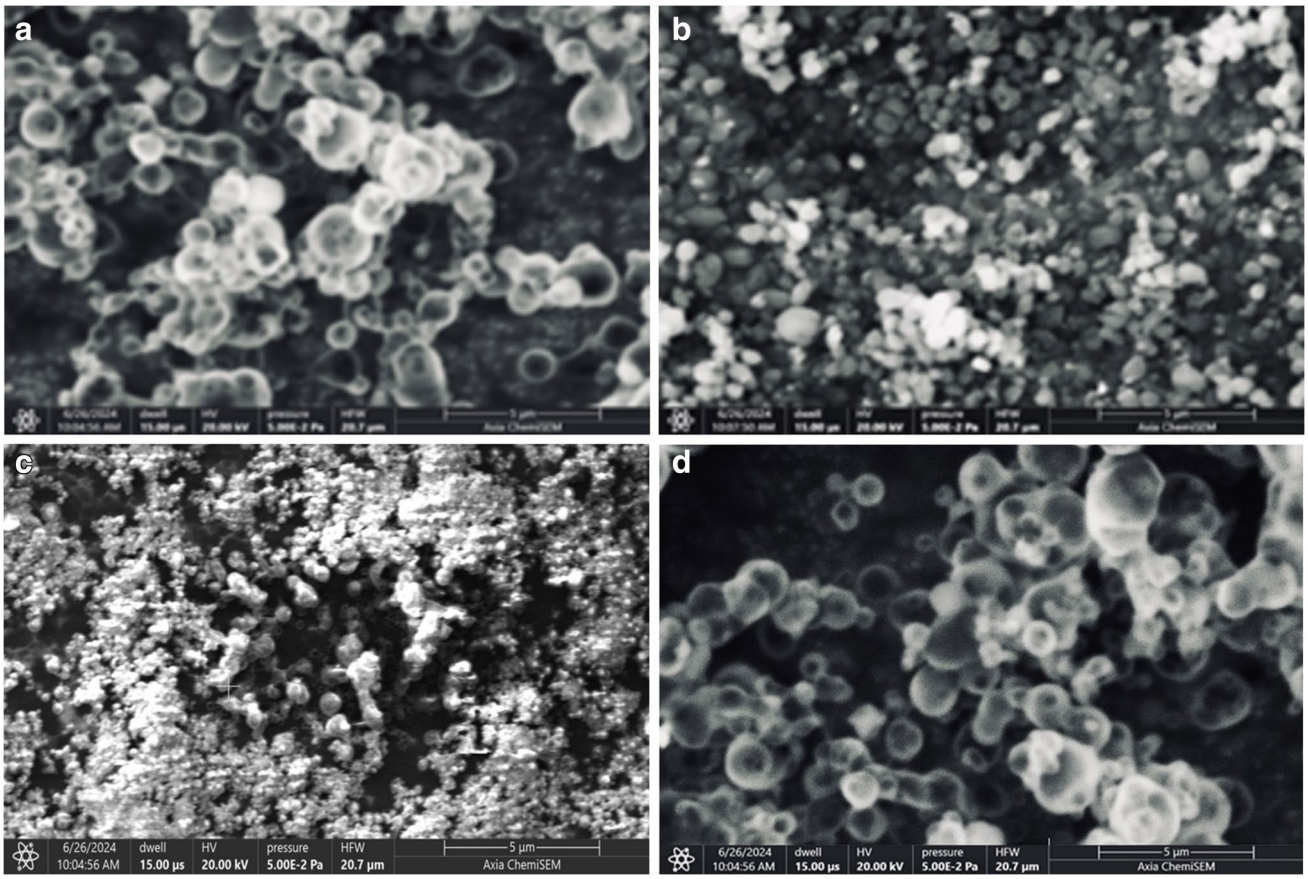

Fig. 7 Scanning electron microscopic images of formulated particles form Composition 1–4

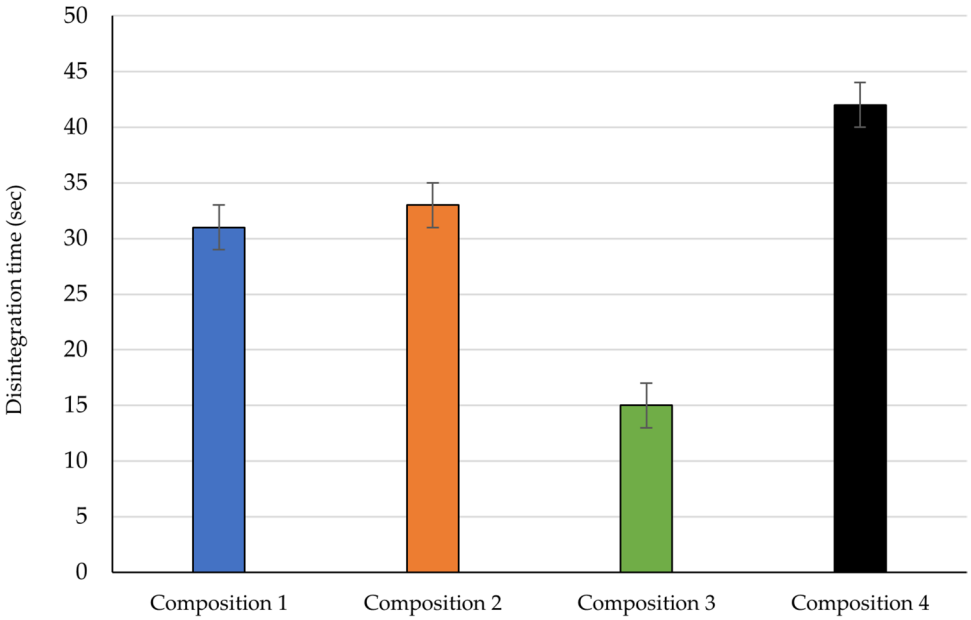

Fig. 8 Complete disintegration time of Compositions in sec. Each data point represents the mean ± SD, $n = 5$

formulations was higher than the expected 50%. The CPZ content of the different preparations was as follows: Composition 1: 57.7; 2: 55.5; 3: 56.6 and for Composition 4: 62.9%.

Based on our animal experiments demonstrated in Fig. 11, we found Composition 3 and 4 to be the most suitable for further development. Following the administration

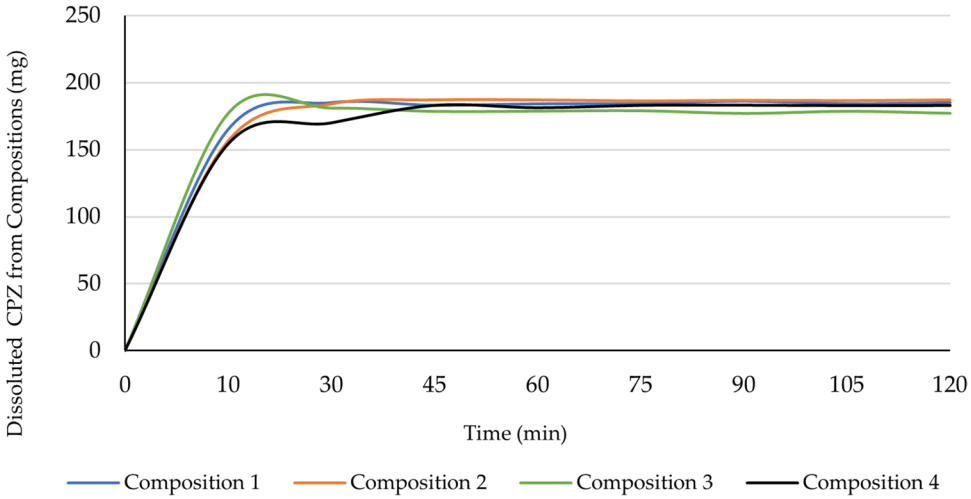

Fig. 9 Dissolution profiles of CPZ from the formulated compositions [1–4]. Each data point represents the mean ± SD, $n = 5$

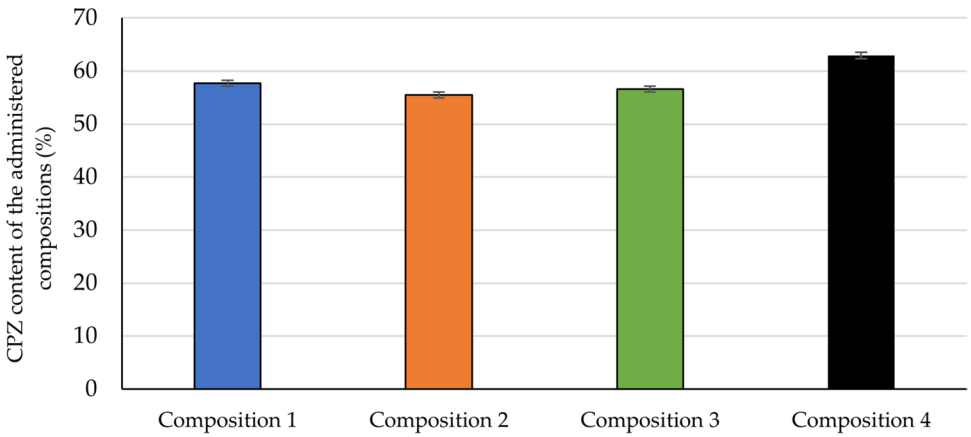

Fig. 10 CPZ content of administered compositions [1–4]. Each data point represents the mean ± SD, $n = 5$

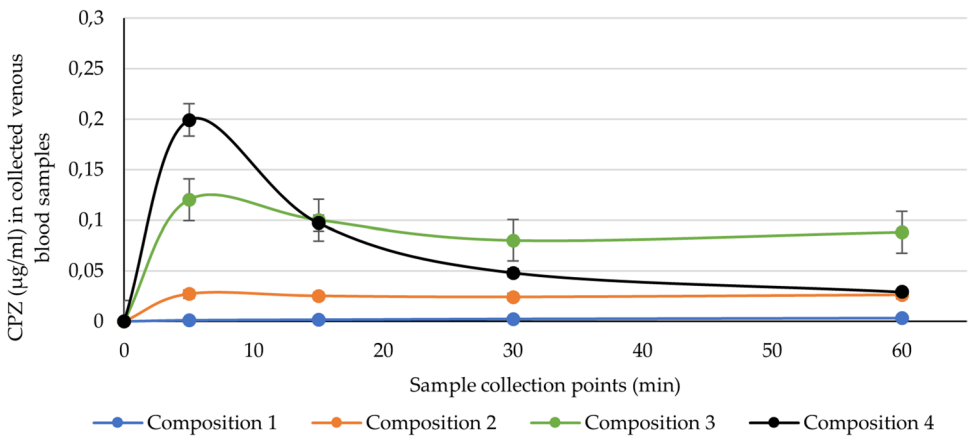

Fig. 11 Measured CPZ (µg/ml) in collected venous blood samples after 5, 15, 30 and 60 min of composition 1–4 administration. Each data point represents the mean ± SD, $n = 5$

of Composition 4, the blood level of the active ingredient CPZ increased rapidly, reaching maximum concentration quickly, followed by a marked decrease in the drug's levels. In the case of Composition 3, the measured blood levels were also significantly higher compared to Composition 1 and 2. Additionally, Composition 3 exhibited a rapid increase in blood levels followed by a relevant plateau phase, which could offer further advantages. This phenomenon in connection with the nanoparticles with CD excipients can be attributed to several factors. HPBCD is known to form inclusion complexes with drugs, which can significantly enhance their solubility and bioavailability. After disintegration, the

HPBCD-drug complex can readily dissolve, facilitating quicker absorption across biological membranes. Additionally, the rapid disintegration of the nanoparticles containing HPBCD likely leads to a swift release of the drug in a form that is more readily absorbed. This can result in a faster onset of action and sustained blood levels due to the efficient initial absorption phase. Moreover, cyclodextrins such as HPBCD can enhance the permeability of the drug through mucosal membranes. This improved membrane penetration can lead to higher initial absorption rates, maintaining prolonged drug levels in the bloodstream. Furthermore, HPBCD may stabilize the active ingredient, preventing its degradation and ensuring a more consistent release and absorption profile over time. In summary, the combination of enhanced solubility, efficient absorption, and improved membrane penetration provided by HPBCD likely accounts for the prolonged blood levels of the active ingredient observed in the animal experiments, despite the rapid disintegration of the nanoparticles. We found this observation very useful and will definitely emphasize investigating this phenomenon in our upcoming studies.

Discussion

In the present study, different nasal formulations were developed containing chlorpromazine and permeation enhancers were investigated in order to improve the absorption of the active ingredient through the nasal mucosa. Amphiphilic compounds contribute to the formation of particles of stable and uniform size, which can increase the consistency of therapy [38]. According to our results, amphiphilic compounds have a pivotal role in controlling the stability and size distribution of [39, 40]. The role of amphiphilic compounds was prominent in the preparation of nanoparticles and the regulation of their stability [41]. These compounds allow you to create solid nanoparticles that efficiently transport the active substance to the body. This is particularly important as stability directly affects the quality and efficacy of the medicinal product [42].

As safety is an important aspect of pharmaceutical formulations, cytotoxicity of the applied permeation enhancers and the compositions themselves was investigated using MTT assay on RPMI 2650 cell line. Based on the results, Composition 3 -which contained HPBCD- changed the cell viability the least. Burga-Sánchez *et al*. have also observed that HPBCD was able to improve the safety of articaine [43]. Composition 4 proved to be the least tolerable composition presumably due to its Poloxamer 407 content. This is consistent with the observation of Dumortier *et al*.; they also found that Poloxamer 407 has cytotoxic effect [44]. Viability studies in cell cultures demonstrated that amphiphilic compounds might have different effects on cell viability, however, it is important to note that in our experiments the applied concentrations can be used safely in the formulated product. Based on these observations, the selection of amphiphilic compounds as active ingredient supplements does not compromise the safety of these [45, 46].

According to the results of *in vitro* dissolution studies, approximately 75% of the incorporated active substance was detected. Between the dissolution profiles of the compositions no significant difference was evaluated. During the disintegration test, the quickest disintegration was measured in case of HPCD-containing composition 3, while the slowest disintegration was measured in the case of Poloxamer 407 containing Composition 4. The active substance is released from the nanoparticles quickly, which might be an important factor in ensuring the rapid effect of therapy. In addition, nanoparticles are characterized by complete dissolution of the components in an aqueous medium, which also supports the effectiveness of the active ingredients [47, 48].

Our animal studies demonstrate that amphiphilic compounds have a significant influence on the penetration of the active substance. Composition 3 has proven to be particularly the most tolerable according to the biocompatibility and in disintegration tests. In animal experiments also Composition 3 and Composition 4 demonstrated the best results. HPBCD has been extensively studied in numerous research endeavors as an excipient. It possesses several advantageous properties in pharmaceutical formulations [49]. One of these properties is its significant role as an absorption enhancer, as demonstrated in various studies. Our results have highlighted that this beneficial characteristic of HPBCD can also be effectively utilized in the formulations developed by our research team [50]. Composition 4 presumably due to its Poloxamer 407 content demonstrated promising results in animal experiments, however, it must be emphasized that this composition should be used with care, since in our measurements, higher concentrations of excipients might cause irritation of the nasal mucosa.

We can conclude that our results might be useful in the development of future pilot products and in the formulation process of other sensitive active ingredients. These data lay the foundation for an innovative approach to nasal medicine and allow for a more effective and targeted use of active substances in practice [51]. Further research directions include further study of the optimal concentration and combinations of other amphiphilic compounds, as well as the development of novel innovative pharmaceutical formulations.

Conclusions

According to our experiments, it can be concluded that amphiphilic compounds might play a prominent role in the preparation of nanoparticles and nasal cavity medication [52]. During these experiments, we have achieved results that touch on many important aspects and can significantly contribute to the development of innovative pharmaceutical products. Based on our results, the presence of amphiphilic compounds has a serious impact on the size distribution and aggregation properties of particles. This is critical for the stability and efficacy of the medicinal product. It is important to note that amphiphilic compounds not only affect the physical properties of nanoparticles, but also affect cell viability [53]. *In vitro* dissolution studies show that the active substance is released extremely quickly from nanoparticles, which is ideal for nasal application, as it allows for a rapid therapeutic effect [54]. In addition, our data on the dissolution of active substances of nanoparticles show that the components are completely soluble in an aqueous medium, which contributes to the effectiveness of the active ingredients. *In vivo* animal studies demonstrated that amphiphilic compounds significantly affect the penetration of the active substance. Based on our results, there are many opportunities in future research directions and drug development. In addition to further investigating the optimal concentration and combination of amphiphilic compounds, we also plan to develop innovative pharmaceutical products. The results achieved are promising in this exciting area and could contribute to a more efficient and targeted application of active substances in practice.

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Data Availability The data that support the findings of this study are available from the corresponding author (zoltan.ujhelyi@pharm.unideb.hu) with the permission of the head of the department, upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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