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Development of a Physiologically Relevant Simulated Nasal Fluid for In Vitro Dissolution Studies

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ABSTRACT

Introduction: Nasal drug delivery systems offer unique advantages, including rapid absorption, bypassing first-pass metabolism, and effectively targeting the central nervous system. However, the complex composition of natural nasal mucus poses challenges in replicating its properties for in vitro studies. Existing simulated nasal fluid (SNF) often lacks critical components, limiting the effectiveness in mimicking the natural nasal mucus under a physiological environment. **Methods:** Three novel SNF solutions were developed with a composition that includes key components (sodium, potassium, calcium, chloride, lipids, and mucin) at concentrations like those found in human nasal fluid. This study compared the developed SNFs with commercial products and human nasal fluids to assess its ability to simulate ionic strength, lipid, and protein content, all of which affect drug solubility and mucosal adhesion. Additionally, this study examined the impact of lipids and mucin on SNF properties including pH, conductivity, density, viscosity, surface tension, and zeta potential, as well as the solubility of model drugs (i.e., melatonin and triamcinolone acetonide) in different SNF solutions. **Results:** The developed SNFs more accurately replicated the composition of human nasal fluid compared to commercial products. The inclusion of lipids and mucin in the SNF improved its ability to simulate mucosal adhesion and drug solubility, evaluating the potential to enhance the accuracy of nasal drug delivery system assessments. Cost analysis demonstrated that developed SNF could be produced at a significantly lower cost than current commercial options, providing a cost-effective and adjustable solution for research applications. **Conclusion:** The developed SNFs provide a physiologically relevant and standardized medium for in vitro nasal dissolution studies. Accurate replication of human nasal mucus enhances the reliability of drug formulation and evaluation, offering a cost-effective alternative to current commercial products.

KEYWORDS: Nasal fluid, simulated nasal fluid, nasal drug development, nasal drug delivery, solubility, dissolution

INTRODUCTION

Nasal drug delivery systems offer significant advantages over traditional routes by providing faster systemic absorption, bypassing the gastrointestinal tract and first-pass metabolism, and offering noninvasive administration, which can increase patient compliance. These systems are particularly beneficial for targeting central nervous system disorders due to the direct connection between the nasal cavity and brain, bypassing the blood-brain barrier. Despite these advantages, the development and evaluation of nasal delivery systems are often constrained by the complexity of the nasal environment, particularly the nasal mucus.

Human nasal fluid (HNF) is a complex mixture, predominantly consisting of water (≈ 95% w/w), mucins (≈ 0.2–5.0% w/v), globular proteins (≈ 0.5% w/v), salts (≈ 0.5–1.0% w/w), lipids (≈ 1–2% w/w), and other components (*1*). The physicochemical properties of nasal mucus, such as pH, typically ranges from 5.5–6.5 in adults, making it slightly acidic compared to the pH of plasma (7.4) (2–5). Baseline pH of HNF is approximately 6.3, with a slightly higher pH of 6.4 in the anterior part, which is more easily influenced by buffer solutions compared with the posterior pH (3). Nasal pH does not differ significantly between patients with and without cystic fibrosis or chronic sinusitis when technical factors are considered, though it shows slight acidity (pH 5.7) after endoscopic sinus surgery (4, 5). Under normal physiological conditions, the nasal mucus layer has a thickness of approximately 10–15 µm and is continuously

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refreshed by mucociliary clearance, which propels about 2 L of mucus per day from the nasal cavity towards the nasopharynx (*6*). This process ensures the effective removal of particulate matter and pathogens, providing protection for the respiratory tract. The anatomical structure of the nasal cavity and nasal epithelium are illustrated in Figure 1. The unique composition of mucins and lipids in nasal mucus affects its protective and rheological properties, which significantly influence drug solubility, retention, and absorption, posing unique challenges for drug formulation and delivery studies.

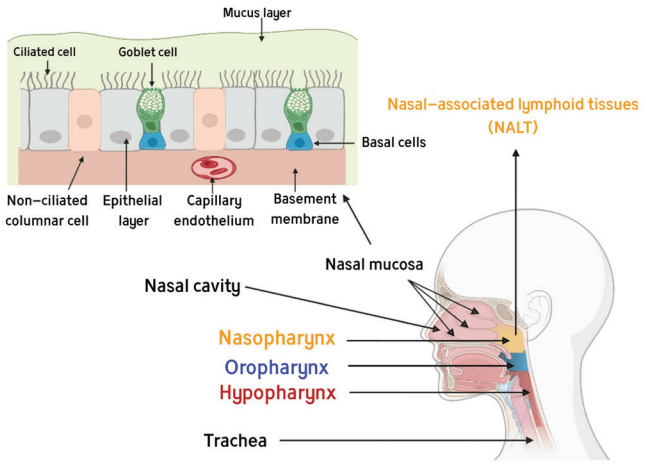

Figure 1. Anatomical structure and function of nasal mucus secretion in the nasal cavity.
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The variability, scarcity, presence of pathogens, and ethical concerns associated with collecting HNF restrict its use in research. Current compositions of nasal fluid often lack crucial biochemical components or do not accurately replicate the ionic strength and pH of natural nasal fluids. The aim of this study was to develop a new biorelevant SNF composition that more accurately mimics the physicochemical and rheological properties of human nasal mucus, integrating robust literature data with practical composition considerations. This approach not only enhances the physiological relevance of in vitro studies but also supports more accurate assessment and development of nasal drug delivery systems.

Composition Analysis of Human Nasal Fluid (HNF)

The limited volume of mucus available in the nasal cavity presents a significant obstacle for compositional analysis. Typically, natural mucus secretion does not yield enough for such studies, necessitating the adoption of specialized collection techniques. Various methods have been documented in the literature for effectively collecting nasal mucus. These include filter paper adsorption, which directly absorbs mucus from the nasal cavity, and the use of small spoons to carefully extract the mucus (*7–9*). Techniques such as induced sneezing and cryo-stimulation are employed to enhance mucus production, facilitating collection. Additionally, x-ray microanalysis has been utilized to analyze the distribution and quantity of mucus, offering a noninvasive approach to study its composition (*10*, *11*). The considerable variation in ion concentrations observed in mucus samples obtained by different collection techniques may be due to the inherent variations introduced by the distinct collection techniques employed.

The ion composition of HNF has been comprehensively studied (Table 1) (*6–12*). Reported ion concentrations exhibit considerable variability, highlighting the need for a standardized composition of SNF that can accurately reflect the nasal environment. To simulate diverse conditions within the nasal cavity, the composition of a newly developed SNF should be adjustable to encompass extreme ion concentrations that may occur. In the current study, ions with a pivotal influence on drug delivery were identified and prioritized to determine their concentrations, followed by establishing the concentrations of other ions.

Table 1. Ion Concentrations in Nasal Fluid (mmol/L)

Source	Sodium	Potassium	Calcium	Magnesium	Chloride	Phosphate
(*6*)	128–150	17–41	4	5	139	ND
(*7*)	98–225	23–68	3–14	ND	ND	3–7
(*8*)	≈ 110	≈ 30	ND	ND	≈ 125	ND
(*9*)	138–189	31–40	1.0–1.85	ND	156–217	0.72–1.31
(*10*)	127 ± 6	27 ± 3	5 ± 1	ND	140 ± 7	ND
(*10*)	142 ± 28	43 ± 10	ND	ND	150 ± 36	ND
(*11*)	141 ± 8	61 ± 8	ND	ND	170 ± 12	ND
(*12*)	85 ± 10	ND	ND	ND	108 ± 5	ND
Developed SNF-I	145	35	5	0	188	1.12 (Glycerophosphate)

ND: not detected; SNF-I: fundamental ionic simulated nasal fluid.

Calcium, magnesium, and phosphate were prioritized owing to their susceptibility to compatibility issues with drugs. Given that both calcium phosphate and magnesium phosphate are insoluble compounds, and that the solution must mimic the nasal pH of 6.4, it is essential to prevent the formation of insoluble precipitates. Calcium glycerophosphate was used to introduce an organic phosphate group in place of the inorganic phosphate group, preventing precipitation while supplying calcium ions. To achieve a balance between physiological relevance and composition stability, the calcium concentration was set at 5 mmol/L, the mid-point of the reported range, and the phosphate concentration was determined to be 1.12 mmol/L (*9*). Magnesium was excluded from the fundamental composition due to limited data, but it can be introduced via mucin reagent. The sodium concentration in nasal fluid is reported to range from 75–225 mmol/L, with a maximum plasma reference concentration of 145 mmol/L (*7, 12*). The developed SNFs have the same sodium ion concentration as that of plasma. Similarly, potassium, known for its high concentration in nasal mucus owing to bacteriostatic properties, was standardized at 35 mmol/L based on most reported values (*7, 9, 10*). Chloride ions were used to balance the charge in the final composition. By integrating both robust literature data and practical considerations, the developed SNF composition supports more accurate assessment and development of nasal drug delivery systems.

The in vitro performance testing of nasal products, along with the associated methodological approaches and challenges, has been recently reviewed by a USP Expert Panel, although no official recommendations for performance testing of nasal drug gels or ointments have been established (*13, 14*). Electrolyte solutions have been used in in vitro release experiments for nasal formulations, with one study demonstrating that the concentration of potassium ions in the release medium significantly influences the erosion and release rate of carrageenan-poloxamer 407 hydrogel; however, no studies specifically mention the use of biorelevant nasal fluids (*15–17*). The development of biorelevant methods has been suggested as a clear path forward for future research (*13*). Several drug delivery routes, including the nasal route, currently lack a standardized biorelevant fluid, which is essential for future performance testing and ultimately for achieving an in vitro-in vivo correlation (IVIVC).

It has been demonstrated that porcine nasal mucosa can be used as a model for in vitro assessment of nasal drug delivery, and porcine stomach mucin (type II mucin) has been used to prepare simulated airway or nasal mucus (*18–23*). Higher mucin concentrations significantly decrease particle mobility, with a 44-fold reduction in the particle diffusion rate observed with 0.5% mucin and a 2570-fold reduction with 5% mucin (20). Even a 1% mucin concentration in mucin-hydrogels can lead to a 2500-fold decrease in particle mobility compared to the rate of free diffusion (*24*). A 2% concentration of mucin, which is near physiological concentrations, was used in the developed SNFs to enable more accurate characterization of nasal spray formulation performance.

HNF contains a high concentration of lipids, similar to the lipid composition found in secretions from human respiratory epithelial cells (*25*). Removing some of these lipids decreases the fluid's antimicrobial activity, which can be restored by adding lipids back into the fluid (*25*). In another study, saturated and polyunsaturated fatty acids accounted for 45% and 29% of the nasal total fatty acids, respectively (*26*). Omega-6 fatty acids were predominant in the polyunsaturated fatty acids subgroup, and linoleic acid and arachidonic acid were incorporated in the main nasal phospholipid classes (*26*).

Although nasal mucus contains a variety of lipid categories, integrating lipids into ionic solutions for nasal drug formulations presents challenges. Typically, this integration requires the preparation of lipids either as liposomes or emulsions, which are then capable of dispersing within the aqueous phase. Similar techniques have been employed in the development of simulated lung fluids. For example, liposomes were prepared with phosphatidylcholine to ensure uniform dispersion of lipids and to evaluate in vitro release of itraconazole from nebulized nanoparticle dispersions (*27, 28*). In parenteral nutrition emulsions (e.g., Intralipid), the components contain triglycerides (fatty acid composition containing myristic, palmitic, palmitoleic, stearic, oleic, and linoleic acids) and egg yolk lecithin (mixture of several phospholipids), and the emulsion is a fairly stable liquid system that allows for a ready-to-use composition (*29*). In the developed SNFs, Intralipid was used to simulate the lipid content of nasal mucus at a dosage of 10 mL/L (equivalent to 2 g lipid/L).

METHODS

Chemicals and Reagent Kits

Commercially available SNF products used in the study, referred to as CP1 and CP2, were obtained from Chemazone, Inc (Canada). HNF from a single human donor was purchased from MYBioSource (USA, lot 20-11-598), and pooled HNF (PHNF) was purchased from Innovative Research, Inc (USA, lot 48147).

Sodium chloride (Caledon, Canada, lot 40091), potassium chloride (Merck KGaA, Germany, lot K51693109004), calcium chloride (Aldrich Chemical Company, USA, lot 06924A1), calcium glycerophosphate (TCI America, USA, lot ZQUFB-YM$N), Intralipid 20% (Fresenius Kabi Canada Ltd, lot 10QL3207), mucin from porcine stomach type II (Sigma-Aldrich, USA, lot 0000329256), were used for preparation of the SNF solutions. All other chemicals used were analytical grade.

Melatonin was purchased from Medisca Canada (lot 198251/A). Triamcinolone acetonide was purchased from Thermo Fisher Scientific (lot P251013).

Preparation of SNF Solutions

As shown in Table 2, composition of the developed SNF solutions included sodium, potassium, calcium, and glycerophosphate, with total chloride at 187.76 mmol/L (i.e., sodium chloride 145 mmol/L, potassium chloride 35 mmol/L, calcium chloride 3.88 mmol/L, calcium glycerophosphate 1.12 mmol/L).

Three variations of the developed SNF were prepared according to the quantities specified in Table 3.

SNF-I

The fundamental ionic solution of SNF (SNF-I) was prepared by sequentially adding sodium chloride, potassium chloride, calcium chloride, and calcium glycerophosphate into a 1-L volumetric flask. All reagents were dissolved thoroughly in an appropriate amount of water before adjusting the volume to 1 L. The pH of the solution was adjusted to 6.4 using an appropriately diluted hydrochloride solution.

SNF-IL

For the preparation of the lipid-containing SNF solution (SNF-IL), 100 mL of the SNF-I solution was mixed with 1 mL of Intralipid 20% (an intravenous lipid emulsion). This solution was freshly prepared daily and used within 24 hours; any remaining solution after this period was discarded. The volume of the solution was scaled up or down proportionally as required for experimental needs.

SNF-ILM

To prepare the SNF solution containing both lipid and mucin (SNF-ILM), 100 mL of the SNF-IL solution was mixed with 2 g of mucin. The mixture was thoroughly stirred until the mucin was completely dissolved. The pH was then adjusted to 6.4 using an appropriately diluted sodium hydroxide solution. This solution was freshly prepared and used within 24 hours. The volume of the solution was scaled up or down proportionally as needed.

Table 2. Ion Concentration Conversion for Composition of Developed Simulated Nasal Fluid

Compound	Molecular Weight (g/mol)	Concentration (mmol/L)	Amount (g/L)	Ion Concentrations (mmol/L)
NaCl	58.44	145.00	8.47	Na: 145
KCl	74.55	35.00	2.61	K: 35
$CaCl_2$	110.98	3.88	0.43	Ca: 5
Calcium glycerophosphate	210.14	1.12	0.24	Glycerophosphate: 1.12 Total Cl: 187.76

NaCl: sodium chloride; KCl: potassium chloride; CaCl2: calcium chloride.

Table 3. Compositions of Developed Simulated Nasal Fluid (SNF) Solutions

Category	Compound	SNF-I	SNF-IL	SNF-ILM
Ion composition	NaCl	8.47 g/L	8.47 g/L	8.47 g/L
	KCl	2.61 g/L	2.61 g/L	2.61 g/L
	$CaCl_2$	0.43 g/L	0.43 g/L	0.43 g/L
	Calcium glycerophosphate	0.24 g/L	0.24 g/L	0.24 g/L
Lipid (0.2%, w/v)	Intralipid 20%	-	10 mL (≈ 2 g lipid)	10 mL (≈ 2 g lipid)
Protein (2%, w/v)	Mucin	-	-	20 g/L
		Add water to 1 L		
		Adjust pH to 6.4		

Dash (-) indicates not applicable.
SNF-I: fundamental ionic simulated nasal fluid; SNF-IL: SNF-I with lipids; SNF-ILM: SNF-I with lipids and mucin; NaCl: sodium chloride; KCl: potassium chloride; CaCl2: calcium chloride.

Analysis of SNF Components

Composition of the SNF-I, SNF-IL, and SNF-ILM solutions was analyzed and compared with two commercially available SNF products (CP1 and CP2) and two human nasal fluid samples (HNF, PHNF). Additionally, a 0.2% lipid solution and 2% mucin solution were analyzed as controls to study the additional components introduced by lipid emulsion Intralipid and mucin.

Chemical composition analysis of all nasal fluid solutions was performed using the Easy RA reagent kit and Easy RA clinical chemistry analyzer (Medica, USA). This instrument was used to detect the concentrations of sodium, potassium, calcium, magnesium, chloride, phosphorus, as well as total protein, albumin, and triglycerides. Following the standard operating procedures of the Easy RA analyzer, the probes and ion-selective electrodes were thoroughly cleaned. The instrument was then calibrated and system-checked using standard solutions. Appropriate reagents kit and blanks (purified water) were placed in the reagent rack, and 500 µL of each test sample solution was placed in the sample rack. For solutions exceeding the upper limit of the linear range, appropriate dilutions were made, and the samples were reanalyzed. The obtained results were recorded for further calculation and comparison.

The mucin content in the SNF solutions and HNFs was determined using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kit to detect and quantify Human MUC2 levels in biological fluids (Thermo Fisher Scientific, Canada, lot WU11J8HT0707). Following the protocol provided in the kit, the absorbance readings of the samples were obtained and compared to a standard curve to determine the concentration of MUC2 in each sample.

Analysis of SNF Properties

pH

The pH and conductivity of the fluids was measured using an Accumet AB200 meter purchased from Fisher Scientific (USA).

Density and Viscosity

The relative density of various solutions was measured using a 5-mL graduated bicapillary pycnometer (model 13377-92, Shangqiu Ruiboer Chemical Glass Instrument Co., China). The measurements were conducted according to the methods outlined in ISO 3838:2004. Calibration was performed at a constant room temperature of 22 °C to determine the density of purified water. The procedure included weighing the empty pycnometer, filling the dry pycnometer with the test solutions, allowing it to stabilize at room temperature for 30 min, then recording the liquid levels and corresponding weights to obtain at least 4 measurements. A linear regression between the height of the liquid level and the mass was established ($r^2 > 0.999$). The relative density was then determined by comparing the mass of the pycnometer filled with the test liquid to the mass of the pycnometer filled with water at the room temperature.

Viscosity of the fluids was measured using a portable field viscometer (PDVdi-120, Stony Brook Scientific, Ltd., USA). Following the setup instructions provided in the manual, 25 mL of each sample was added to the viscometer body. An extension bar was screwed into the needle, which was then inserted into the viscometer body until the needle top aligned with the viscometer body. The needle was then released to fall freely, and the time taken for the needle to pass between two pre-marked points on the extension bar was measured using a video recording. Each sample was measured three times, and the average fall time was calculated. The fall time was then substituted into the viscosity conversion formula provided with the instrument, viscosity = $9.1463 \times (\rho s - \rho f) \times t$, to obtain the viscosity results of the solutions, where ρs is needle density (2.9263 g/cm^3) and ρf is fluid density (g/cm^3), and t is time.

Surface Tension

Surface tension (dyn/cm) was measured with flexible video system (FTÅ200, First Ten Angstroms, USA) to measure the contact angle in conjunction with a 1 mL luer-lock syringe and a blunt needle. The test parameters were set as follows: pump rate 2.0009 µL/s, camera frame rate 40.0 fps, and all other parameters used the system's default settings. From the series of captured images, the photo with the largest droplet volume just before detachment was selected for analysis using the accompanying software. The measurements were taken in quadruplicate, and the average value was reported.

Zeta Potential

Zeta potential of the SNF solutions was measured using the Zetasizer Nano ZS (Malvern Instruments, UK). The zeta potential of the fluids was determined by disposable plain folded capillary zeta cells. All measurements were carried out at room temperature in triplicate.

Drug Solubility

Solubility of model drugs melatonin and triamcinolone acetonide, was determined using a 12-well plate. Each well was filled with 3 mL of various media, with three replicates for each medium. An appropriate amount of each model drug was added to the different media, and

the wells were covered with a parafilm membrane. The 12-well plate was placed on the incubated microplate shaker (model 130000, Boekel Scientific Jitterbug, USA) and shaken at 37 °C, mix level 7, for 36 hours. After the incubation period, the media were subjected to centrifugation at 10,000 g for 20 minutes or filtration using 0.45-µm Basix nylon syringe filters. For samples containing mucin, acetonitrile was used to precipitate the mucin before injection. The filtered solutions were analyzed using a high-performance liquid chromatography (HPLC) system (Shimadzu Corp., Japan) equipped with an Kinetex-C18 column (150 × 4.6 mm, 5 µm), SPD-M10A VP UV–VIS detector, LC-10AS pumps, and SIL-10A auto injector. The chromatographic conditions for melatonin and triamcinolone acetonide were employed as specified in the United States Pharmacopeia (USP) monograph for melatonin and triamcinolone acetonide (*30*).

RESULTS AND DISCUSSION

The components and properties of the developed SNF solutions were systematically compared with those of commercially available SNF solutions (CP1 and CP2) as well as human nasal fluid samples (HNF and PHNF). The comparative data are comprehensively presented in Tables 4 and 5.

Ion Composition

Comparative analysis of ion concentrations in human nasal fluid samples (HNF and PHNF) with previously reported values revealed some notable differences. Sodium, chloride, and phosphorus were within the expected range, whereas potassium and calcium levels were significantly lower. Magnesium levels were also below the expected range, but to a lesser extent. Additionally, the concentrations of albumin in HNF and PHNF were below the detection limits. These findings highlight the inherent variability in nasal fluid composition, influenced by sampling techniques, individual biological differences, health status, physiological changes, and external environmental factors (*6*, *31*).

In terms of ionic interactions, sodium, potassium, and chloride primarily contribute to the osmolarity of solutions. Due to their compatibility with drug ingredients, there is minimal evidence of these ions causing degradation or precipitation in drug formulations. Calcium, magnesium, and phosphorus, however, often lead to precipitation and degradation in sensitive drugs. Tetracycline antibiotics (including tetracycline, minocycline, and doxycycline), known chelating agents, form insoluble complexes with divalent and trivalent metal cations such as calcium and magnesium, adversely affecting drug

Table 4. Comparison of Newly Developed Simulated Nasal Fluid (SNF), Commercially Available Simulated Nasal Fluids, and Human Nasal Fluids

Components	0.2% Lipid	2% Mucin	SNF-I	SNF-IL	SNF-ILM	CP1	CP2	HNF	PHNF	Ref. Value
Na^+ (mM)	< LLQ	< LLQ	138.2 ± 0.3	137.4 ± 0.4	147.1 ± 0.6	133.8 ± 0.1	147.3 ± 0.8	106.0 ± 0.3	106.6 ± 0.1	75–225
K^+ (mM)	< LLQ	3.64 ± 0.02	35.41 ± 0.02	35.97 ± 0.20	37.45 ± 0.12	5.43 ± 0.00	6.58 ± 0.06	18.81 ± 0.02	14.32 ± 0.08	17–69
Ca^{2+} (mM)	< LLQ	0.36 ± 0.03	3.80 ± 0.11	4.05 ± 0.13	4.29 ± 0.10	1.22 ± 0.03	1.78 ± 0.03	0.44 ± 0.02	0.97 ± 0.02	1–14
Mg^{2+} (mM)	0.04 ± 0.01	2.03 ± 0.11	< LLQ	0.04 ± 0.00	2.05 ± 0.04	1.73 ± 0.01	1.77 ± 0.03	0.45 ± 0.01	0.92 ± 0.01	5
Cl^- (mM)	< LLQ	< LLQ	171.7 ± 1.8	171.6 ± 1.9	186.5 ± 0.4	147.0 ± 4.0	146.7 ± 0.7	109.0 ± 0.6	127.3 ± 0.4	103–217
P^{2-} (mM)	< LLQ	1.71 ±0.01	< LLQ	< LLQ	1.79 ± 0.01	0.80 ± 0.01	1.15 ± 0.03	0.78 ± 0.02	2.04 ± 0.01	0.72–7
Total protein (g/L)	5.3 ± 0.6	6.0 ± 0.0	< LLQ	3.0 ± 0.0	12.3 ± 0.6	< LLQ	4.0 ± 0.0	< LLQ	1.3 ± 0.6	4.14–8.95 (*7*)
Albumin (g/L)[a]	< LLQ	< LLQ	< LLQ	< LLQ	< LLQ	< LLQ	< LLQ	< LLQ	< LLQ	0.31–1.05 (*7*)
Triglycerides (mM)	4.38 ± 0.09	0.25 ± 0.01	0.43 ± 0.01	4.26 ± 0.06	4.66 ± 0.05	4.24 ± 0.19	2.63 ± 0.01	0.04 ± 0.00	0.39 ± 0.02	-
Mucin (ng/mL)[b]	-	-	-	-	< LLQ	< LLQ	< LLQ	8.94	0.89 ± 0.06	2% (*51*)

Values are expressed as mean ± SD (n = 3) or range unless otherwise noted.
[a]Albumin lower limit of quantification is 4 g/L.
[b]Due to limited sample volume, a single measurement is reported for HNF and duplicate measurements are reported for PHNF.
Dash (-) indicates not applicable; SNF-I: fundamental ionic simulated nasal fluid; SNF-IL: SNF-I with lipids; SNF-ILM: SNF-I with lipids and mucin; CP1: commercial product 1; CP2: commercial product 2; HNF: human nasal fluid; PHNF: pooled human nasal fluid; LLQ: lower limit of quantification; Na: sodium; K: potassium; Ca: calcium; Mg: magnesium; Cl: chloride; P: phosphorus.

Table 5. Properties of Simulated Nasal Fluid (SNF) Solutions

Property	Water	SNF-I	SNF-IL	SNF-ILM	CP1	CP2
pH	-	6.38 ± 0.03	6.35 ± 0.02[a]→6.4	3.54 ± 0.04[a]→6.4	7.37 ± 0.03	6.89 ± 0.05
Conductivity (ms/cm)	-	19.86 ± 0.06	19.61 ± 0.02	20.10 ± 0.02	15.14 ± 0.12	13.81 ± 0.06
Relative density[b]	-	1.0081	1.0083	1.0155	1.0101	1.0181
Viscosity, μ(cP)	-	3.33 ± 0.17	3.33 ± 0.22	4.19 ± 0.14	3.33 ± 0.29	6.46 ± 0.22
Surface tension (dyn/cm) (n = 4)	-	72.25 ± 0.62	56.65 ± 0.49	101.53 ± 2.82	70.81 ± 0.67	64.84 ± 0.44
Zeta potential (mV)	-	–11.18 ± 1.09	–2.17 ± 0.46	–3.80 ± 1.40	–19.96 ± 2.37	–34.53 ± 1.20
Melatonin solubility (mg/mL)	2.05 ± 0.08	3.00 ± 0.10	2.98 ± 0.15	1.62 ± 0.26	1.51 ± 0.11	1.39 ± 0.17
Triamcinolone acetonide solubility (μg/mL)	24.9 ± 0.6	21.7 ± 0.7	22.9 ± 0.4	25.4 ± 1.7	23.6 ± 7.1	23.5 ± 3.5

Values are expressed as mean ± SD (n = 3).
[a]pH measured after adding lipid and mucin before adjusting pH.
[b]The relative density was without replicates, thus no standard deviation was calculated. The high precision of the measurement is indicated by the linear regression coefficient ($r^2 > 0.999$).
Dash (-) indicates not applicable; SNF-I: fundamental ionic simulated nasal fluid; SNF-IL: SNF-I with lipids; SNF-ILM: SNF-I with lipids and mucin; CP1: commercial product 1; CP2: commercial product 2.

absorption and reducing bioavailability (*32, 33*). Similarly, quinolone antibiotics like ciprofloxacin and levofloxacin are incompatible with multivalent cations, forming insoluble complexes that lead to incompatibility (*34, 35*). Furthermore, reports have indicated that dicalcium phosphate dihydrate is incompatible with various drugs including ceronapril, oxprenolol, quinapril, metronidazole, parthenolide, famotidine, and temazepam (*36*). Therefore, the variability of calcium, magnesium, and phosphorus in SNF can significantly impact the evaluation of nasally administered drugs. Given the known physicochemical properties of drugs sensitive to multivalent ions, it is advisable to prepare SNF solutions using higher values of calcium, magnesium, and phosphorus than what has been reported in the literature.

Comparison of commercially available products (CP1 and CP2) with HNFs revealed that magnesium was significantly higher than both the reported values and the actual measured values, whereas calcium and phosphorus were close to the lower limits of physiological ranges. Comparison with the developed SNF-ILM solution revealed that the magnesium, calcium, and phosphorus levels more closely approximated the mid-values of physiologic ranges than CP1 and CP2. Therefore, the developed SNF-ILM more accurately reflects the physiological concentrations of ions in HNF.

Protein Content

C SNF-ILM is an artificial composition solution designed to mimic the properties of natural nasal secretions. The total protein content in SNF-ILM is three times greater than that of commercial products, which also exceeds the protein levels found in HNFs. This elevated protein concentration is especially significant due to the presence of mucins, as glycoproteins play a crucial role in maintaining the viscosity and elasticity of nasal mucus (*1, 37, 38*). Mucins, by virtue of their structure and high molecular weight, contribute extensively to the gel-like consistency of mucus, making it an effective barrier against pathogens and particulate matter (*1, 39, 40*). They bind to water molecules, thereby ensuring that the nasal passages remain moist, which is essential for the proper functioning of cilia in the respiratory epithelium (*40, 41*). This moisture retention also facilitates the trapping of airborne particles and microbes, preventing them from reaching the lungs and causing infection (*38*).

In addition to its role in maintaining nasal mucus properties, mucin interacts with drug carriers through hydrogen bonding, electrostatic interactions, and hydrophobic forces, which significantly influence drug release, retention time, and absorption within the nasal cavity. Research indicates that mucins can act as selective diffusion barriers, potentially limiting drug delivery and affecting the bioavailability and absorption of drugs (*42, 43*). In the presence of mucin, especially at higher concentrations (≥ 0.6%), the diffusion of compounds is significantly hindered (i.e., atenolol, caffeine, naproxen, and hydrocortisone) (*42*). Positively charged chitosan exhibits strong adhesion to negatively charged mucin, whereas negatively charged or neutral polymers display weaker or negligible interactions (*44*). Moreover, mucin can adhere to different polymers through various mechanisms: anionic polymers like carboxymethyl cellulose bind via electrostatic attraction; pectin and carboxymethyl chitosan form stable structures through hydrogen bonding; and hydroxypropyl methylcellulose

and hydroxypropyl cellulose enhance adhesion through molecular entanglement and van der Waals forces (*45*). Additionally, many excipients in nasal powders have demonstrated effectiveness in prolonging nasal residence time by interacting with mucin and altering mucus rheology (46). Mucin content influences the hydration state of the mucus layer, with proper hydration being crucial for maintaining the optimal viscoelasticity needed for effective ciliary clearance (*47*). Therefore, negatively charged mucin has been identified as a key target for developing in situ nasal gels. The high mucin content in SNF-ILM enhances its utility as a representative medium for evaluating the release of nasal formulations, assessing their potential for prolonged contact time, and their ability to penetrate the mucus barrier.

The ELISA results revealed that mucin levels in HNF and PHNF differed by a factor of 10. The commercial products and developed SNF solutions did not yield detectable results due to the absence of human-derived mucin in these compositions (porcine-derived mucin was used in the developed SNFs).

Albumin Content

The absence of detectable albumin in both commercial products and SNF-ILM, which deviates from the expected values of 0.31–1.05 g/L, can be attributed to the albumin levels being below the lower limit of detection (4 g/L) (*7*). Designed primarily for blood albumin level detection, the Easy RA and its reagents lack the analytical sensitivity necessary to detect minute quantities of albumin present in nasal fluids. This limitation can result in undetectable albumin levels in samples that fall below the threshold.

The presence of albumin in nasal fluids has been reported based on samples of natural secretions, which are influenced by various physiological and environmental factors, resulting in a complex and dynamic protein composition (*48*). Albumin, primarily found in plasma, is a crucial carrier for both hydrophilic and hydrophobic drugs, enhancing their solubility and systemic distribution. By forming complexes with drugs, albumin can protect them from rapid degradation or elimination, thereby extending their half-life within the circulatory system. However, the presence of albumin in nasal fluids is notably low, typically constituting only 1–2% of its concentration in blood plasma (*7*). This minimal concentration suggests that in nasal drug delivery systems, due to the limited volume of mucus within the nasal cavity (≈ 1 mL), small amounts of albumin are unlikely to significantly affect drug solubility and permeability. SNF solutions are typically designed for specific therapeutic or evaluative purposes and are not intended to replicate the complete protein spectrum of natural nasal secretions. Consequently, the composition process often focuses more on other components deemed more critical for the intended applications, excluding albumin.

Lipid Content

HNF contains a rich array of lipids. Intralipid was utilized to simulate the lipids in nasal fluid, while also accommodating the solubility of lipid particles. The triglycerides in a lipid emulsion form are capable of dispersing relatively uniformly in an aqueous solution. Both commercially available products and the newly developed SNF solutions contained triglyceride levels significantly higher than those found in HNF. This might be because lipids in nasal fluid are not exclusively in the form of triglycerides but also include diglycerides, free fatty acids, cholesterol, and phospholipids.

Based on the analysis of nasal fluid components, the high inherent variability in nasal secretion compositions suggests that using a fixed composition of SNF cannot truly represent the physiological range across a diverse population. It is necessary to determine a composition range that can cover the physiological concentration ranges under various conditions for most of the population. Therefore, a fundamental composition that allows for flexible adjustments in component composition would be more appropriate to mimic this nasal secretion inherent variability.

Introducing lipids and mucin into the SNF-I solution altered its ionic concentrations. Specifically, the addition of 0.2% lipid introduced trace amounts of magnesium, and its main contribution was triglycerides.

Compared to the minimal impact of the lipid solution on ionic concentrations, the mucin solution had a more pronounced effect. In addition to the expected contribution to detectable protein levels, the addition of mucin introduced additional trace amounts of potassium and calcium, significant quantities of magnesium and phosphorus, and trace amounts of triglycerides.

The addition of lipids and mucin together substantially enhanced the physiological relevance of the SNFs. The most notable changes were increases in sodium, calcium, magnesium, total protein, albumin, triglycerides, and mucin concentrations. These alterations bring the composition of the SNFs closer to that of human nasal fluid, making SNF-ILM particularly well-suited for studies that aim to mimic the biological environment of the nasal cavity for drug delivery evaluation.

Properties of SNF solutions

pH

The natural pH of nasal secretions generally ranges from 5.5–6.5 in adults and from 5.0–7.0 in infants (*2*). In the developed SNFs, the addition of mucin significantly lowered the pH, requiring adjustments during preparation. In contrast, the pH values for CP1 and CP2 were 7.37 and 6.89, respectively. These deviations from the pH range suggest that commercially available products may not accurately reflect the physiological pH environment.

Electrical conductivity, indicative of a solution's ionic strength, plays a crucial role in nasal fluid by ensuring effective mucociliary clearance, maintaining drug stability and solubility, and enhancing mucosal absorption. In developed SNFs, the addition of lipids and mucin did not significantly alter the conductivity. Conductivity of CP1 and CP2 was lower than that of the developed SNF solutions, which is a normal manifestation of composition differences.

Density and Viscosity

Density and viscosity characteristics significantly impact the retention time of solutions within the nasal cavity. Higher viscosity can enhance the retention of nasal drugs, but it may also affect the rate of drug dissolution and absorption. CP2 exhibited relatively higher density and viscosity compared to other SNF solutions, whereas the other SNF solutions showed no significant differences in these properties.

Surface Tension

Surface tension determines how a solution spreads over a surface. Lower surface tension enhances the spreadability of nasal formulations on mucosal surfaces. This ensures greater surface area coverage, facilitating more extensive contact between the drug and the mucosal tissue, thereby potentially improving drug absorption. In developed SNF solutions, the addition of lipids alone led to a noticeable increase in surface tension, whereas the addition of lipids and mucin decreased this parameter.

Zeta Potential

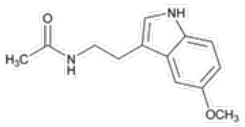
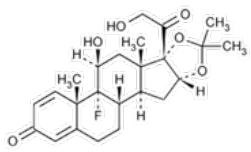
Zeta potential influences the interactions between drug delivery systems and the mucosal layer. Current developments in nasal delivery are focusing on nano and liposome carriers, where the zeta potential of nasal mucus can indicate compatibility with the ions of nasal dosage forms and potential impacts on mucosal permeability. A low zeta potential for the SNF-IL and SNF-ILM solutions suggests that both were unstable dispersion systems.

Drug Solubility

Based on reported physicochemical properties of melatonin and triamcinolone acetonide, both compounds exhibit relatively high pKa values (Table 6) (*49*). At physiological pH values (≈ 5–7.4), both drugs predominantly exist in their nonionized forms. Consequently, their solubility is not expected to be pH-dependent, and the solubility in the commercial and developed SNF solutions should remain consistent. Due to the sample preparation steps involved in the solubility determination process (i.e., filtration, acetonitrile precipitation, and centrifugation) the relative standard deviation (RSD) for the solubility measurements of melatonin and triamcinolone acetonide was as high as 30%.

The presence of mucin results in decreased solubility of melatonin. This reduction may be due to the possible formation of a complex between mucin and melatonin, which is subsequently removed by filtration, thereby reducing the detectable concentration of melatonin in the solution. Other studies have reported that mucin can delay precipitation and stabilize the supersaturation of poorly water-soluble drugs, such as carvedilol and piroxicam, without significantly affecting their solubility (*50*).

Table 6. Properties of Model Drugs (49)

	Melatonin	Triamcinolone Acetonide
Chemical structure		
Molecular formula and weight	$C_{13}H_{16}N_2O_2$ 232.28 g/mol	$C_{24}H_{31}FO_6$ 434.5 g/mol
pKa values	0.6, 16.5	13.3
Log P Log D (pH 4–8)	1.148	1.944

Cost Advantages of the Developed SNFs

The developed SNFs provide significant advantages over commercial products and HNFs, particularly in terms of economic feasibility and practical application in vitro and ex vivo research settings. A cost analysis highlights that producing 1 L of the developed SNF solution costs approximately $50 CAD (Table 7). This is substantially lower than that of CP1 and CP2, which are priced at $975 CAD for an equivalent volume. Even more pronounced is the contrast with HNFs, which cost $710–790 CAD per milliliter and are typically available only in extremely limited volumes of 0.5 mL or 1 mL. These limitations render HNFs impractical and prohibitively expensive for routine laboratory studies.

Table 7. Cost of Newly Developed Simulated Nasal Fluid (SNF)

Component	Required amount per 1 L of SNF	Price per Unit	Cost per Liter (CAD)
NaCl	8.47 g	$155.23 / 1 kg	$1.30
KCl	2.61 g	$115.00 / 1 kg	$0.30
$CaCl_2$	0.43 g	$130.12 / 1 kg	$0.10
Calcium glycerophosphate	0.24 g	$370.05 / 1 kg	$0.10
Intralipid (20%)	10 mL	$339.91 / (10 × 100 mL)	$3.40
Mucin (2%)	20 g	$1110.00 / 500 g	$44.40
			Total: $49.60

Price sourced from Fisher Scientific (accessed May 24, 2024): https://www.fishersci.ca/ca/en/home.html
CD: Canadian Dollars; NaCl: sodium chloride; KCl: potassium chloride; $CaCl_2$: calcium chloride.

The developed SNF, costing only $50 CAD per liter, which translates to 5 cents per milliliter, emerges as an economically viable alternative (Table 8). The developed SNF offers a more accessible option for extensive experimental and pharmaceutical research, often constrained by the high costs and scarcity of human nasal fluid. The affordability and greater volume availability of SNF allow for broader and more diverse applications, facilitating comprehensive research that natural nasal fluids cannot support due to their high cost and limited supply.

Table 8. Cost Comparisons of Simulated Nasal Fluid (SNF)

	Developed SNF	CP1	CP2	HNF	PHNF
Volume	1 mL	1 mL	1 mL	1 mL	1 mL
Cost (CAD)	$0.05	$0.975	$0.975	$790.00	$710.00

CP1: commercial product 1; CP2: commercial product 2; HNF: human nasal fluid; PHNF: pooled human nasal fluid; CAD: Canadian Dollars.

Moreover, adjustability of the SNF composition allows researchers to tailor the fluid's properties to meet specific research objectives. This flexibility is crucial for studying the solubility characteristics of nasal fluids, as it enables the simulation of various physiological conditions that may affect drug solubility and release. By understanding how different components of nasal mucus influence these properties, researchers can significantly optimize the design of potential nasal drug performance tests.

CONCLUSION

Developing a standardized SNF is crucial for accurately assessing the solubility and uptake of drugs intended for nasal delivery and for the development of future standardized performance tests. Current alternatives, including limited human donor nasal fluids and commercial products, fail to consistently replicate the complex environment of the nasal mucosa and may be cost-prohibitive. The novel composition of SNF described herein addresses these shortcomings by incorporating mucin and lipid components to mimic the natural conditions of the nasal cavity more closely. The developed SNF provides a more reliable medium for evaluating nasal drug formulations, thereby enhancing the accuracy of performance assessments. Such advancements are vital for optimizing nasal drug delivery systems, ultimately improving therapeutic outcomes and expanding the potential for nasal administration of various pharmaceuticals.

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DISCLOSURES

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