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Advancing nasal formulation characterization: Considerations toward a robust and precise method to determine the mass fraction below 10 μm in nasal products

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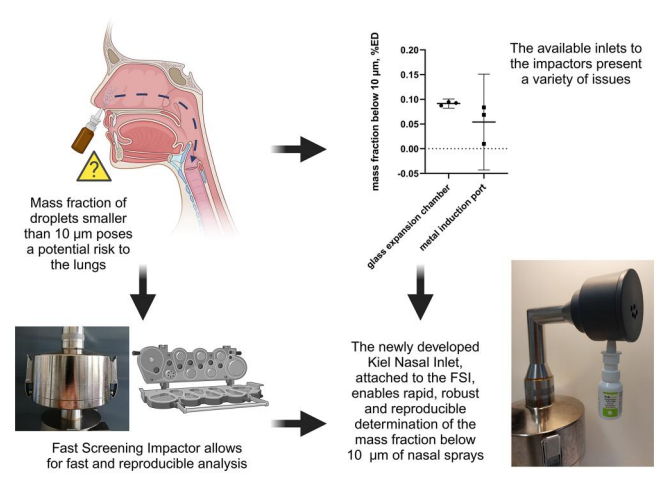
ABSTRACT

The expanding role of the nasal route in drug administration for local and systemic treatments has prompted the need for precise delivery methods to ensure efficacy and patient safety. This study addresses the challenges of evaluating the mass fraction below 10 μm in nasal products, a crucial factor in assessing lung deposition of drugs of nasal formulations. Current regulatory guidelines advocate for this assessment, yet a standardized compendial methodology is lacking. To fill this gap, we comprehensively examined several methods to determine a robust approach for quantifying the mass fraction below 10 μm in nasal products. As model formulation, a commercial nasal product (aqueous solution of sodium cromoglycate) was utilized. The assessment of mass fractions below 10 μm necessitates considerations like general handling, precise assessment of delivered dose, robust recovery, and appropriate impactor size analysis techniques. The choice of impactor and of inlet for size analysis may significantly influence the generated results. In this regard, the study highlights the necessity for careful impactor and inlet selection to ensure accurate measurements. In the course of this, the Kiel Nasal Inlet (KNI) had been designed to optimize nasal product testing, addressing the shortcomings of existing inlets. The KNI performed well across different laboratories and reproducible between impactor types. For the determination of mass fraction below 10 μm, the Fast Screening Impactor is the preferred choice of the authors.

GRAPHICAL ABSTRACT



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Introduction

In recent years, the nasal route of drug administration has gained substantial attraction not only for local treatments but also systemic delivery of drugs. This shift has been particularly evident in the utilization of acute or emergency systemic treatments like migraine relief (e.g., sumatriptan in liquid spray and powder forms), hypoglycemia management (glucagon in powder form), and countering opioid overdose (naloxone in liquid spray), being approved for use. This trend is attributed to the easy access of the nasal mucosa and its high vascularization, facilitating rapid drug absorption. The nasal route of administration also holds great promise for the treatment of central nervous system disorders and the delivery of biopharmaceutical products (Fortuna et al. 2014). The increasing importance of the intranasal route is demonstrated by the rise in clinical trials examining this approach (Keller, Merkel, and Popp 2022). However, the intensified interest in intranasal formulations for both new and repurposed drugs raises questions about optimal formulation deposition within the nasal cavity, emphasizing the pivotal role of precise dose delivery to the nasal cavity in mitigating undesired side effects beyond the nose.

In response to this challenge, both the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) have advocated for assessing the mass fraction of particles and droplets less than 10 μm in size, as part of their guidelines on nasal products (EMA 2006; FDA 2002). This measure intends to ensure that the vast majority of the dose is deposited in the nose and cannot get inhaled to the lungs, thus increasing patient safety. Despite these regulatory requirements, a universally applicable methodology to evaluate the mass fraction below 10 μm in various nasal formulations is yet to be established in the pharmacopeial compendia.

The joint recommendation of the EMA and the FDA to assess the mass fraction below 10 μm is in line with the overall objective to increase patient safety, especially in view of the increasing nasal administration for new treatments and diseases. This study aims to thoroughly examining different impactor methods for quantifying the mass fraction below 10 μm in nasal products to determine a robust approach. The first experimental part of this study utilized a widely-used nasal spray employed for allergy treatment, with the overall objective of developing a reproducible method setup for identifying the mass fraction of droplets or particles below 10 μm within nasal formulations. Notably, an obvious difficulty in this attempt concerns the absence of an appropriate inlet for guiding the nasal aerosol to the impactor during aerodynamic assessment.

Based on these considerations, this study included the design and development of an optimal inlet for nasal product testing as second experimental part. In this context, the Kiel Nasal Inlet (KNI), a newly designed inlet, has been created to facilitate assessment of nasal product performance.

General considerations for aerodynamic testing of nasal products

Nasal products exhibit various application characteristics across products and patients. For example, product orientation or inspiration during application may be specified by the product's patient instruction and should reflect how the patient uses his medicine. Factors such as formulation viscosity, device design, application angle, patient behavior, and individual nasal anatomy contribute to variability in the targeted area within the nasal cavity (Kundoor and Dalby 2011; Warnken et al. 2018). For detailed insight into these factors, we refer to Gao, Shen, and Mao 2020. Various nasal cast models have been proposed to assess the regional deposition of nasal products. Since the first nasal casts made from cadavers were available (Häußermann et al. 2002) and had been used for testing of nasal deposition (with all their drawbacks basically being large cavities not representing any physiological characteristics of the nasal cavity), much progress has been made. This progress substantiates in the use of *in vivo* CT scans to develop the models (Hughes et al. 2008; Le Guellec et al. 2014), but also relates to better cast materials, better sectioning (Schönbrodt et al. 2010) and more individual segmentation to allow for the assessment of regional distribution. Recent research resulted in comparative computer simulation studies of various individual CT scans (Kiaee et al. 2019) and the development of the Alberta Idealized Nasal Inlet (AINI) (Chen et al. 2020). This single side nasal cast model can be fitted onto an impactor and is believed to mirror an average nasal deposition. The journey is well summarized in the several reviews (Deruyver et al. 2021; Williams and Suman 2022).

Notably, when a nasal product is actuated at high speed at the correct angle and reinforced by a harsh inhalation, a substantial portion of the formulation may reach the lungs (Suman, Laube, and Dalby 1999). This dual targeting of nasal and pulmonary sites, although sometimes desirable for diseases of the upper respiratory tract like bacterial infections (Seow et al. 2022), is not envisaged in the Pharmacopoeias of both

the US and Europe. In contrast, from the early 2000s, EMA and FDA guidelines (EMA 2006; FDA 2002) have advocated for assessing (and limiting) the inhalable fraction below 10 μm in nasal products in drug product development and routine quality control and there are several methods available (e.g., laser diffraction and Morphology-Directed Raman spectroscopy) yet a harmonized method for such assessment remains absent in the literature (Doub et al. 2023).

Two main techniques for particle or droplet size analysis, laser diffraction (LD) and impactor analysis, are available. However, LD has limitations in distinguishing solid from liquid particles in suspension formulations (Williams, Blatchford, and Mitchell 2018) and is not drug-sensitive. To address this, aerodynamic assessment offers a comprehensive option to evaluate various formulations, spanning (pressurized) solutions, suspensions, and powders. In the view of the authors, the evaluation of mass fraction below 10 μm in nasal products as a quality measure necessitates an aerodynamic assessment method.

For both size analysis methods, reproducible nasal spray actuation is essential, as atomization dynamics change with actuation force variations (Kundoor and Dalby 2011; Trows et al. 2014). Ideally, automated actuation with predefined parameters is used to maximize reproducibility. The parameters should reflect actuation by human hand. Elevated actuation forces can support the determination of the maximum possible mass fraction below 10 μm by atomizing solution and suspension formulations into smaller droplets (Trows et al. 2014). Yet, overly elevated forced may overestimate the inhalable fraction.

The mass fraction below 10 μm is expected to be low for nasal products. Doub et al. (2012) determined the fraction to be 0.60% ± 8.68% (RSD) for an aqueous nasal beclomethasone dipropionate suspension (42 μg per actuation) using an Andersen impactor with glass expansion chamber as experimental setup. Williams, Blatchford, and Mitchell (2018) investigated the droplet fraction below 14 μm facilitating an NGI-glass expansion chamber setup with both an azelastine solution (137 μg per actuation) and fluticasone propionate suspension (50 μg per actuation). The fraction was 0.07 ± 0.11% of total recovered dose for the solution and 0.5 ± 0.4% for the suspension.

Hence, the experimental setup and analytical methodology must allow quantification of low amounts of active pharmaceutical ingredients.

As mass fraction below 10 μm shall be assessed, precise knowledge of the emitted dose (ED) is crucial. Accurate analysis and precise determination of the emitted dose of the nasal product require effective recovery of the entire nasal product dose from the experimental setup. The *Nasalia* monograph in Ph. Eur. 11.0 describes the Dosage Unit Sampling Apparatus (DUSA) from *Inhalanda* as feasible option for determining delivered dose, and similarly, USP refers to the Delivered Dose Uniformity sampling apparatus A.

The DUSA, however, was found unsuitable for high volume aqueous nasal sprays (data not shown) like Pollicrom (sodium cromoglycate [SCG]) used in this study. It was observed that the nasal spray wetted the filter and liquid was spilling through the filter material, which could not be collected and quantified in a reproducible manner. Therefore, the 1 l glass expansion chamber was used for dose collection in the current study, while a more widely applicable system is still needed.

Available impactors are the Next Generation Pharmaceutical Impactor (NGI, Figures 1a and b), the reduced NGI (reduced Next Generation Pharmaceutical Impactor [rNGI], Figures 1b and c) featuring a filter in one selected stage nozzle, and the Fast Screening Impactor (FSI, Figures 1d–f). Impactors that efficiently collect the mass fraction below 10 μm without dispersing it into multiple samples are expected to yield more accurate results. Precision, reproducibility and very good recovery are key, while considerations like ease of use, automation feasibility, and impactor compatibility must also guide the selection process.

In conjunction with impactor choice, the inlet selection may influence the mass fraction below 10 μm. The USP throat aims for horizontal administration, which is impossible for nasal spray pumps. Thus, an adapter/inlet is required which allows to adjust the angle of the inlet to accommodate orientations mentioned in the patient information leaflet (typically either upright with vertically directed spray or slightly tilted upwards). Available inlets comprise the metal induction port (MP) (Figure 1e), described by Williams et al. (2013), and the glass expansion chamber (Figure 1a).

The extent of spray cloud expansion may affect the inhalable fraction, with overly restricted or broad inlets potentially leading to underestimation or overestimation, respectively. The metal induction port is crafted from a metal tube with an angle of 60° from inlet to outlet. Nasal sprays might lead to coating of the inlet with the product. Hence, this might lead to building up a layer where further aerosol droplets may deposit more easily, despite being small, leading to an underestimation of mass fraction below 10 μm.

Figure 1. Commercially available impactors and inlets for aerodynamic assessment. The top half shows the Next Generation Pharmaceutical Impactor closed (a) and opened (b). (c) Illustrates the abbreviation of the NGI to the reduced NGI by inserting a filter holder and a filter into a selected nozzle of the NGI. The lower half shows the Fast Screening Impactor with a USP throat attached (d) and the metal induction port (e). Into the corpus of the FSI a 10 µm cut off plate at 30 L/min is inserted (f). A commercially available nasal inlet is the glass expansion chamber (a, 1 l variant shown).

The glass expansion chamber allows for full aerosol cloud expansion and may foster droplet evaporation potentially leading to an overestimation of the mass fraction below 10 μm.

Considering the limit of quantification, minimizing sample solvent volume to be used for sample recovery is crucial. While reducing the solvent volume enhances the likelihood of successful drug quantification, it also enhances the risk upon solvent evaporation, namely potentially overestimating the mass fraction below 10 μm. The use of internal standards can mitigate this risk. Quantification should be undertaken

with a 2D method like high-performance liquid chromatography (HPLC) to reduce variability and increase specify as well as selectivity of the method.

Methods and materials

Experimental setup

The overall aim of the study was the comparative assessment of different impactor setups for the determination of the fraction below 10 μm in nasal spray products. For this method, being intended as quality control measure, which needs to be robust and reproducible, no anatomically correct or *in vivo* mimicking setups (such as the AINI) were used intentionally. In the course of impactor testing it became clear that the available options to guide a nasal spray into an impactor (the metal inlet and the expansion chamber) were unsuitable for this purpose. For this reason, the Kiel Nasal Inlet (KNI) had been developed and tested. Thus, the experimental setup served two aspects: identifying the best impactor setup and assessing suitability of the KNI. The KNI in combination with NGI, rNGI and FSI, respectively, were examined in three different laboratories (Kiel University, AstraZeneca Gothenburg and Emmace Lund).

Nasal product

Pollicrom (15 ml, Ursapharm, Saarbrücken, Germany), containing 2.8 mg of sodium cromoglycate per spray shot (140 μl) in solution form, was used as model formulation in the study because of its high drug load per actuation which eases drug quantification, and its low viscosity, which is in a typical range for a nasal spray solution. Prior to use, the containers were prepared according to patient information. The droplet size distribution was characterized by laser diffraction ($n = 3$): $x_{10} = 21.5 \pm 0.3$ μm, $x_{50} = 57.6 \pm 1.8$ μm, $x_{90} = 173.1 \pm 18.7$ μm. Automated actuation was utilized (parameters below).

Determination of active ingredient content

Kiel University

For drug quantification, isocratic, reversed-phase high-performance liquid chromatography with UV detection and Empower 3 analysis software (HPLC, Waters Corporation, Milford, MA, USA) were employed. For this, a C-18 stationary phase (LichroCHART 250-4, LiChrospher 100 RP-18, 5 μm with pre-column, Merck, Darmstadt, Germany) and a mobile phase consisting of 40% methanol and 60% 10 mM phosphate buffer adjusted to pH 2.4 were used. The column oven temperature was set to 50 °C. Detection was performed at 240 nm and the quantification limit (LoQ) was determined to be 0.1 μg/ml according to the corresponding ICH guideline (CPMP/ICH/381/95). The quantitative calculations were based on an external standard calibration curve (0.1–150 μg/ml, $R^2 > 0.99$). Salicylic acid (5 μg/ml) was additionally used as an internal standard to reduce the influence of evaporation. The retention times were 3.2 min for sodium cromoglycate and 8.6 min for salicylic acid. The samples were centrifuged at 10,000 g for 10 min at 22 °C before analysis to eliminate any possible particles. All solvents used were of chromatographic grade. Two injections of 30 μl were made, and the mean peak area was considered for the calculation of drug content of each sample.

Emmace, Lund

Gradient, reversed-phase high-performance liquid chromatography with UV detection (240 nm) and Chromeleon analysis software were employed. An Acquity UPLC BEH C18 1.7 μm column was used at a column temperature of 25 °C. The mobile phase and gradient are described in Table 1. A flow rate of 0.4 mL/min was used and 5 μl were injected per run. Detection was performed at 240 nm and the LoQ was determined to be 0.1 μg/ml. The quantitative calculations were based on an external standard calibration curve (0.1–137 μg/ml, $R^2 > 0.99$). Salicylic acid was additionally used as an internal standard to reduce the influence of evaporation. The retention times were 3.5 min for sodium cromoglycate and 3.9 min for salicylic acid. Samples containing fibers from glass filters were centrifuged.

AstraZeneca, Gothenburg used the same method as Emmace with slight modifications.

Aerodynamic assessment

Impactors

Measurements were performed with 30 L/min airflow and in triplicate. Air flow was measured with an air flow meter (Kiel University: Flow Meter DFM 2000,

Table 1. Composition of the mobile phase during gradient elution.

Time	A (%), 0.1% TFA in water	B (%), 0.1% TFA in ACN
0	95	5
3	10	90
3.2	95	5
4	95	5

Table 2. Sample solvent volumes used for drug recovery.

Component/analytical site	Kiel University	Emmace, Lund	AstraZeneca, Gothenburg
Glass expansion chamber	30 ml	Not assessed	
Metal induction port	20 ml	Not assessed	
KNI and USP throat combined	30 ml	25 ml KNI 5 ml throat	25 ml
FSI corpus	15 ml	5 ml	20 ml
FSI filter	10 ml	5 ml	10 ml
NGI stages	5 ml (stage 1, MOC: 10 ml)	5 ml	5 ml
rNFI filter	5 ml	5 ml	5 ml

Copley Scientific, Nottingham, UK; AstraZeneca and Emmace: TSI4040 Flowmeter, Driesen + Kern GmbH, Bad Bramstedt, Germany) prior to each analysis.

The assessment of the mass fraction below 10 μm was carried out using a Next Generation Pharmaceutical Impactor (NGI, Figures 1a and b), a reduced variant of the NGI (rNGI, Figures 1b and c) and a Fast Screening Impactor (FSI, Figures 1d and e) with a 10 μm cut off plate (Figure 1f; all Copley Scientific, Nottingham, UK). For the reduction of the NGI, a filter support was inserted into the nozzle well above stage 3 (6.39 μm cut off at 30 L/min) followed by a glass fiber filter and a split ring for fixation of filter plate and filter (Figure 1c).

Inlets

Three inlets for nasal products were assessed. The metal induction port (unrestricted geometry, see Figure 1e), the 1 L glass expansion chamber (see Figure 1a; Copley Scientific, Nottingham, UK) and the newly developed Kiel Nasal Inlet which is introduced in detail below (see Figures 4 and 5). The Kiel Nasal Inlet (KNI) was manufactured of rigid polypropylene (GEHR PP®, GEHR GmbH, Mannheim, Germany) by milling.

Automatic actuation

Nasal sprays were actuated automatically, if not indicated otherwise. A Mighty Runt MR (Innova Systems Inc., Marlborough, MA, USA) with the following parameters was used: dose time = 0.2 s, force-to-actuate 5 kg, hold-time 2 s (Williams, Blatchford, and Mitchell 2018). The nasal spray was weighed before and after actuation to calculate delivered mass.

The spray shot was delivered in vertical orientation, as per the patient information. This was not possible with the metal induction port due to the fixed angle of the inlet.

Drug recovery

Double distilled water was used as the sample solvent. The internal standard (salicylic acid, 5 μg/ml) was dissolved in the sample solvent before washing. Table 2 shows the used volumes for drug recovery.

Deviating from the described method, AstraZeneca and Kiel University assessed the combination of NGI and KNI by washing stages 4–8 consecutively with 5 ml of sample solvent. Emmace washed each stage with a volume of sample solvent as described in Table 2.

Reference dose

Emitted dose (ED) was assessed by automatically actuating one spray shot of nasal product into the 1 L glass expansion chamber without air flow. The outlet of the chamber was covered with a particle free tissue to avoid formulation escaping the chamber. The chamber and the tissue were washed with 30 ml sample solvent. Each spray actuation was weighed and the emitted dose was related to the shot mass (μg drug/mg formulation).

Validity of determination and calculation of mass fraction below 10 μm

The performed analysis was considered valid when the recovery of each individual analysis was 98–102% of the expected dose. This narrow range was chosen as a low fraction below 10 μm was expected and thus, all further uncertainties had to be reduced to a minimum to allow a statistically reliable result. The expected dose was calculated from the weighted mass of the given spray shot and the mean emitted dose per spray shot.

$$recovery = \frac{total\ drug\ in\ impactor\ and\ inlet}{spray\ shot\ mass \cdot ED} \cdot 100\%$$

The mass fraction below 10 μm was calculated from the drug found below 10 μm aerodynamic size and recovered dose from the experimental setup. The following equation was used for the FSI:

$$mass\ fraction < 10\,\mu m = \frac{drug\ amount\ below\ 10\,\mu m}{recovered\ dose\ in\ impactor\ and\ inlet} \cdot 100\%$$

For NGI and rNGI, the mass fraction ($m_{<10\,\mu m}$) was determined according to Ph.Eur. The amount of

droplets was interpolated between stages 2 and 3 with an aerodynamic diameter (d_{ae}) cut off of 11.66 and 6.39 μm at 30 L/min, respectively. The following equation was used for the NGI and rNGI:

$$m_{<10\,\mu m} = m_{stage\,3} + \frac{m_{stage\,2} - m_{stage\,3}}{d_{ae\ \ stage\,2} - d_{ae\ \ stage\,3}} \cdot \left(10\,\mu m\ \ d_{ae\ \ stage\,3}\right)$$

Statistical analysis

Statistical evaluation was performed using Microsoft Excel (version 2108, Microsoft Corporation). Confidence intervals (CIs) (95%) are reported unless otherwise stated.

Results

Experiments were conducted in three phases: A screening was performed to evaluate available impactors for their advantages and disadvantages. After selecting a suitable impactor, inlets were assessed. As none appeared to be the optimal one, a new inlet was developed and validated in the last step.

Impactor screening

The mass fraction below 10 μm is expected to be low for nasal products as discussed in the general considerations (above). The 1 l glass expansion chamber was chosen as the nasal inlet for the first step because it allows for almost complete spray cloud expansion. An underestimation of the inhalable fraction by impaction to inlet walls was assumed to be lowest here for all available inlets.

The FSI was operated with a 10 μm cut off plate at 30 L/min. Figure 2 shows how the choice of impactor can influence the captured mass fraction less than 10 μm. Due to the distribution of the mass fraction over a large number of stages in the NGI, the fraction could not be quantified since the amount of drug being diluted to many samples is below LoQ and overall recovery was low (92.97 ± 1.65%). The drug was thus dispersed and diluted to the extent that quantification by UV detection was no longer possible. The rNGI and FSI were more sensitive and comparable in terms of recovery (99.39 ± 2.14% and 101.83 ± 2.32%, respectively), but despite being in the same overall range (lower than 0.1%) the determined mass fraction less than 10 μm differed significantly ($p = 7.05$ E-05) between the two setups.

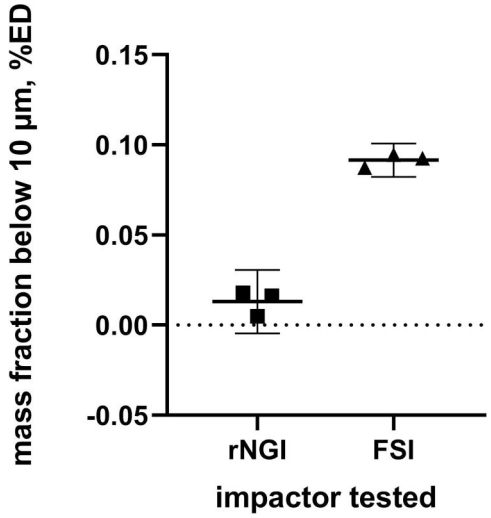

Figure 2. Comparison of impactors. Mass fraction below 10 µm reported as percentage of determined emitted dose. Inlet: 1 l glass expansion chamber, one spray shot per assessment, 30 L/min. The mean is displayed and error bars indicate confidence intervals (95%), $n = 3$.

Further experiments were carried out with the Fast Screening Impactor, as it seemed most sensitive and reproducible in the analysis of the mass fraction below 10 μm. As the method shall serve as safety assessment, it appears reasonable to use the setup giving a higher fraction below 10 μm (as worst case scenario).

Finding a suitable inlet

Two already available inlets for nasal product testing were evaluated in combination with the FSI (Figure 3), the glass expansion chamber and the metal induction port (depicted in Figures 1a and e, respectively). The assessed mass fraction below 10 μm was higher with the glass expansion chamber (not significant) which may be due to excessive droplet evaporation following almost complete expansion of the nasal spray cloud as discussed earlier. The metal induction port revealed handling difficulties as the liquid being sprayed into the inlet at the fixed angle of 60° deposited at the inner walls and dripped back toward the entry resulting in spill, irreproducible collection of dose and overall much larger measurement variability for the Pollicrom nasal spray. Further experiments could be done to assess whether this result is due to the actuation angle being inappropriate for the particular spray or whether this is a general issue. Nevertheless, a fixed angle is useless for a universal setup.

Using the glass expansion chamber with the FSI and a 10 μm cut off plate at 30 L/min gave a good and reproducible estimate of the maximum mass fraction

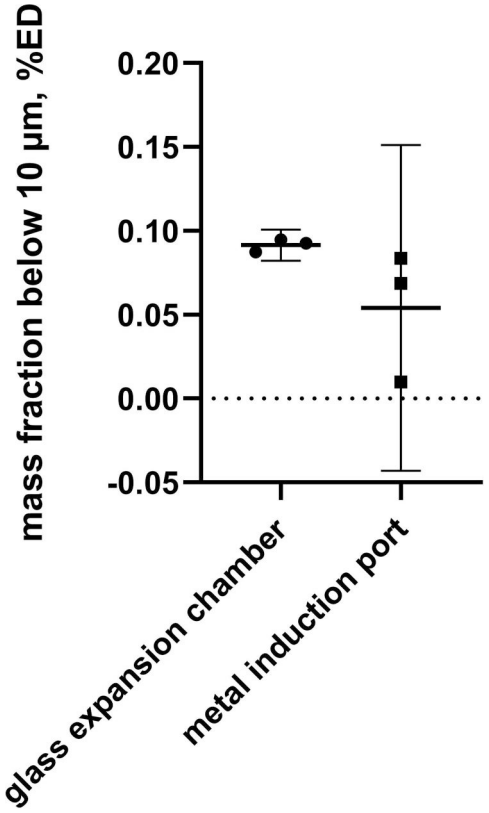

Figure 3. Comparison of nasal inlets. Mass fraction below 10 µm is reported as a percentage of the determined emitted dose. One spray shot per assessment, Fast Screening Impactor equipped with a 10 µm cut off plate at 30 L/min. The mean is displayed and error bars indicate confidence intervals (95%), $n = 3$.

less than 10 μm. Yet, both inlets do not display a convenient inlet for the above-mentioned reasons. A good nasal inlet shall serve as an adapter between the nasal spray product and the USP throat being mounted on an FSI, NGI or rNGI. It shall introduce the spray's droplets into the airflow toward the impactor without compromising the particle size distribution. It shall not be an anatomical or physiological relevant nasal cast model in terms of geometry, volume or alike, but provide an appropriate adapter which allows the delivery of a nasal spray product into an impactor to facilitate aerodynamic particle sizing. Following this line of thought, a nasal product inlet was designed to overcome the shortcomings of the existing inlets.

Development of the Kiel Nasal Inlet (KNI)

The newly developed Kiel Nasal Inlet (Figure 4) has been molded from a rigid block of polypropylene and meets many of the above-mentioned general considerations. It consists of a corpus that is attached to the USP throat and has a plate with aeration holes at the opposite side (see Figure 4 right). This prevents the operation of the nasal product under negative pressure as the vast majority of nasal product do not allow airflow through the container. At the bottom is an inlet for the insertion of the nasal product. The insertion port for the nasal spray tip in the KNI is equipped with a tight sealing to prevent spilling of the nasal product. The product Pollicrom can be administered between an upright up to and slightly tilted position (approx. 60° from the vertical plane) depending on the cutout of the rubber seal. The airflow passes through the KNI in orthogonal orientation to the administration of the nasal spray to minimize interference and allow aerodynamic assessment. A detailed technical drawing is shown below (Figure 5).

The material is chemically resistant and already established as material of the tube of the DUSA known from inhalation product characterization. The connections from the cover to the body of the inlet and those of the inlet to the USP throat are tight to prevent any leakage of formulation. The geometry and internal volume of the KNI (approx. 65 ml) do not allow a full expansion of the spray cloud to reduce the probability for overestimation of small droplets by evaporation. This geometry was chosen because it allows for easy attachment of the KNI to the commonly used USP induction port for the assessment of inhalation products like dry powder inhalers or pressurized metered dose inhalers utilizing an impactor. Hence, it requires standard equipment to operate, which is available in many laboratories. Actuation can be performed manually or automated.

Characterization of the Kiel Nasal Inlet

The KNI was tested with FSI, NGI and rNGI. A special focus was put on recovery of emitted dose since this is crucial for the assessment of the mass fraction below 10 μm.

A typical image of formulation deposition in the KNI is shown in Figure 4, right. Droplets, which were too large to be entrained by the airflow toward the FSI remained in the upper part of the inlet and no drainage into the lower half was visible. There was no formulation spill into the sealing between inlet and lid.

Table 3 (first data set from Kiel) shows the results of the assessment of mass fraction below 10 μm utilizing the three different impactors in combination with the KNI as inlet. The mass recovery was excellent, being close to 100% regardless of the impactor used. As shown in Figure 6, the recovered fraction is dominated by KNI + USP throat deposition accounting for

Table 3. Mass fraction below 10 μm as determined at different laboratory sites and different impactors with the KNI at 30 L/min.

Site	Impactor	Mass fraction below 10 μm in % emitted dose	Recovery	Shot mass
University Kiel*	FSI	0.08 ± 0.01%	101.11 ± 5.33%	148.17 ± 8.02 mg
	rNGI	0.05 ± 0.01%	101.24 ± 0.54%	156.60 ± 1.30 mg
	NGI	0.06 ± 0.01%	99.35 ± 3.26%	156.87 ± 0.91 mg
AstraZeneca Gothenburg[m]	FSI	0.07 ± 0.01%	99.51 ± 1.34%	162.05 ± 2.22 mg
	rNGI	0.03 ± 0.02%	99.67 ± 2.53%	163.33 ± 4.82 mg
	NGI	0.03 ± 0.01%	98.66 ± 2.15%	159.62 ± 1.04 mg
Emmace Lund[m]	FSI	0.09 ± 0.03%	99.42 ± 1.87%	155.97 ± 4.46 mg
	rNGI	0.02 ± 0.01%	100.88 ± 2.64%	158.19 ± 3.20 mg
	NGI	0.04 ± 0.02%	99.37 ± 3.54%	155.86 ± 4.28 mg

Target shot volume 140 μl (approx. 140 mg). Data reports mean of three values with 95% confidence intervals. *Automated actuation; [m]manual actuation.

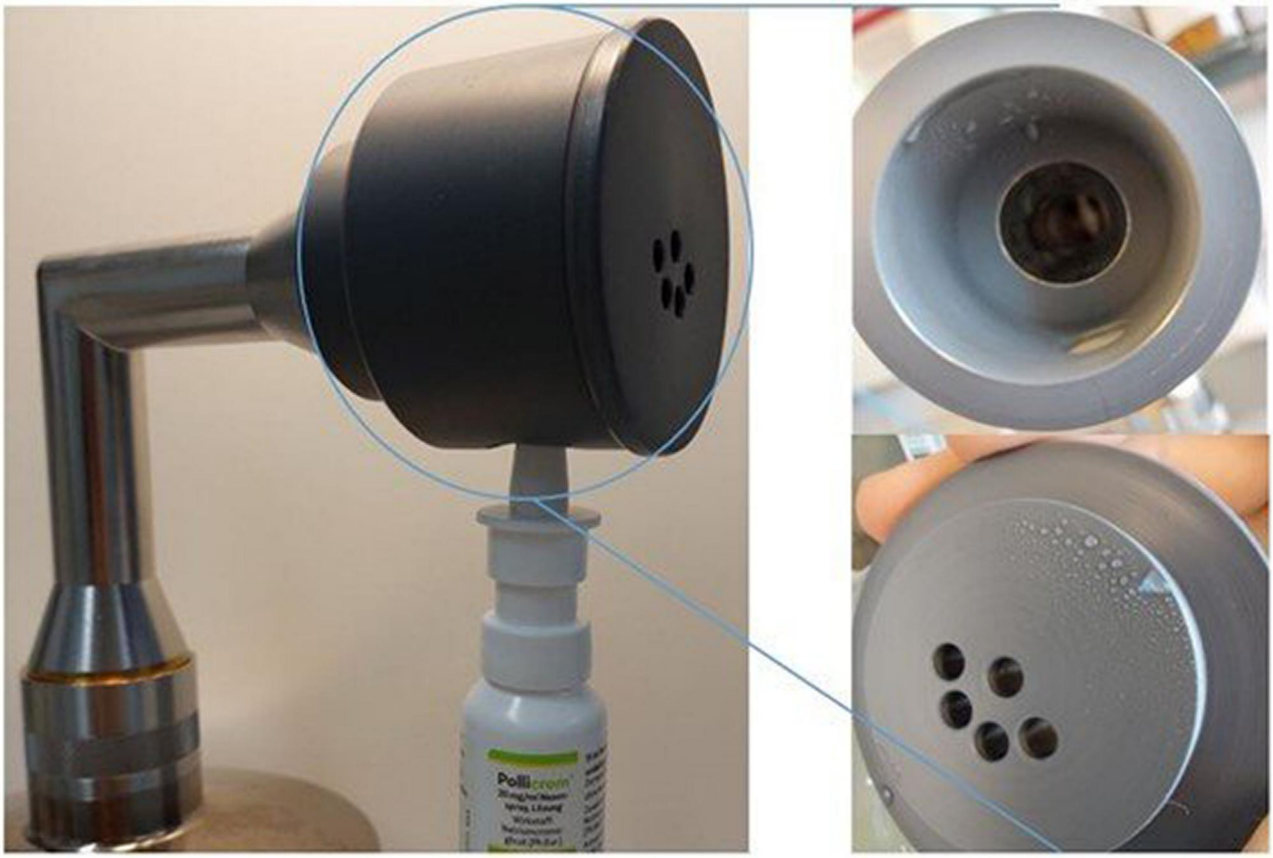

Figure 4. Left: KNI attached to the USP throat with a nasal spray inserted at the entry. Right: KNI after distribution of one spray shot (140 μl) of Pollicom in the chamber. The aqueous solution deposits to the KNI ceiling.

more than 99% of the overall captured amount of API.

The mass fraction below 10 μm between the different impactors was in the same range (below 0.1%), but the FSI resulted in the highest determined fraction.

Contrary to first observations with the glass expansion chamber (see Figure 2) the mass fraction below 10 μm could be assessed in NGI with the KNI. This was obtained by washing stages 3–8 consecutively with 5 ml of sample solvent which were otherwise used per stage.

Validation of the method setup for the assessment of mass fraction <10 μm

Inter-laboratory variability

In order to validate the results and to substantiate the decision for the impactor on a broader database, the experiments described above were repeated at the laboratories of AstraZeneca, Gothenburg, and Emmace, Lund. Within the same impactor type, the outcomes were found to be very comparable in terms of recovery and mass fraction while differences between impactor types followed the same trend across labs (Table 3).

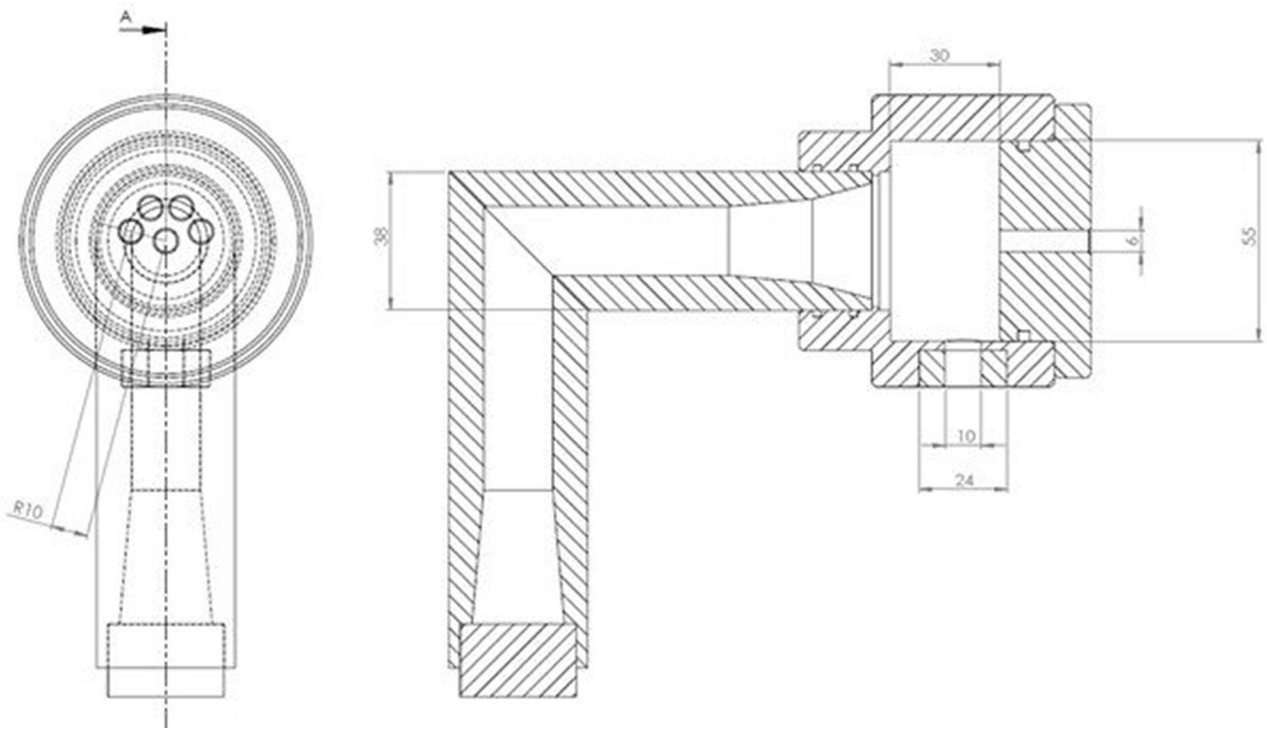

Figure 5. Technical drawing of the Kiel Nasal Inlet attached to the USP throat. Frontal (left) and lateral (right) view. Dimensions given in millimeter. Reproduced with kind permission of Herbert Wachtel.

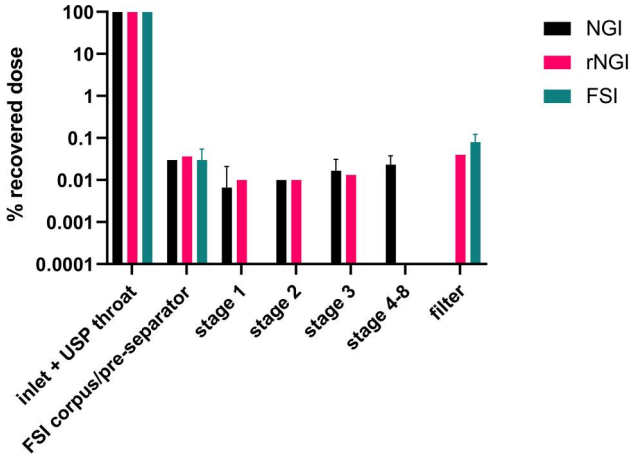

Figure 6. Deposition profile of the formulation in the impactors using the KNI as inlet. Logarithmic representation. One spray shot per assessment, 30 L/min. Error bars indicate confidence intervals (95%), $n = 3$. Some error bars are too small to be visible.

Robustness

To assess the robustness of the preferred setup (KNI + FSI), the flow rate of the FSI was varied within the compendial limits of ±5% and the number of spray shots was increased from one to seven spray shots (corresponding to 140–980 μl liquid).

Figure 7 shows that the method was independent of the applied air flow in the compendial limits. No significant differences were found.

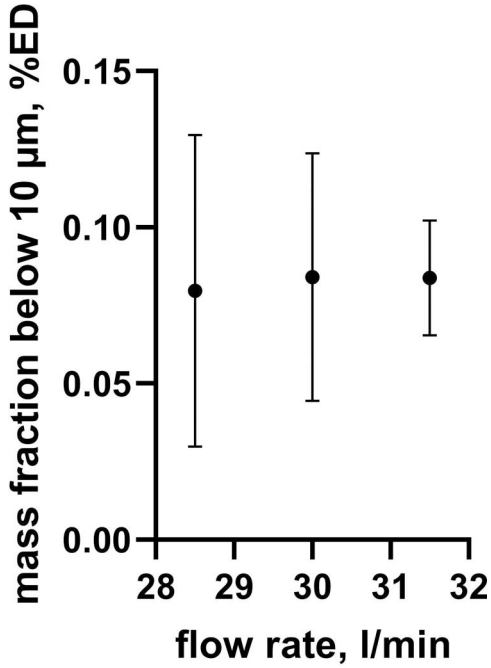

Figure 7. Comparison of determined mass fraction below 10 µm depending on the flow rate of the air flow. No significant differences were found ($p = 0.930$, one-way ANOVA). One spray shot per assessment, 30 L/min. Error bars indicate confidence intervals (95%), $n = 3$.

Table 4 shows that the setup (consisting of FSI and KNI) can be used with a higher number of spray shots without affecting the mass fraction below 10 μm.

Table 4. Robustness testing of the KNI in regards to the number of spray shots.

Number of spray shots	Mass fraction below 10 μm in % emitted dose	Recovery	Shot mass
1	0.08 ± 0.02%	101.11 ± 4.71%	148.17 ± 07.09 mg
3	0.07 ± 0.01%	99.59 ± 9.26%	464.07 ± 11.52 mg
5	0.06 ± 0.00%	98.76 ± 4.33%	793.33 ± 10.00 mg
7	0.06 ± 0.00%	Not assessed (dripping)	1118.57 ± 15.80 mg

$n = 3 \pm 95\%$ confidence interval.

This is crucial for testing low dose nasal products where an increase in spray shots might be needed to exceed LoQ. The recovery was also independent of the number of nasal spray shots administered (Table 4). Only at seven spray shots did the formulation drip out of the inlet indicating overload. For seven spray shots, the fraction below 10 μm was therefore calculated from the expected drug amount. As Pollicrom administers approximately 140 μl per spray shot, the KNI was able to handle at least 700 μl before the formulation drips out of the inlet port. The confidence interval was the biggest for the assessment of one spray shot due to the low amount of API and quantification close to LoQ.

Discussion

The presented work aims at the identification of a setup being suitable for the assessment of mass fraction below 10 μm in nasal sprays as a quality control measure. The data presented in this study were obtained with the marketed low viscosity aqueous nasal spray solution Pollicrom. Aqueous nasal sprays represent the majority of nasal products on the market. Three different impactors had been selected, which all generally allowed the determination of a mass fraction below 10 μm upon aerodynamic classifying, namely NGI, rNGI and FSI. Further, different nasal inlets were assessed as a nasal spray requires some adapter to be usable with an impactor being designed for horizontal administration. This turned out to be a challenge, as discussed above.

The determined mass fraction below 10 μm was impacted by both the use of the impactor and the nasal inlet and must be interpreted alongside the method employed. Precise knowledge of the emitted dose per mass of formulation is necessary to relate the obtained results to the administered dose. With advancement beyond locally acting drugs toward systematically acting ones, the calculation of the mass fraction below 10 μm based on the label claim may be too imprecise. Intra-container testing of nasal spray is permitted by Ph. Eur. Nasalia monograph (11.0) with an emitted dose within 85–115% of the label claim. In order to proceed with further analysis, the emitted dose needs to be assessed accurately. The emitted dose gains even more relevance with suspension formulations. Nasal spray suspensions present greater complexity, as they tend to sediment and lose homogeneity over time after redispersion.

The dose collection device specified for measuring the emitted dose in the Nasalia monograph of the Ph. Eur., the DUSA tube, proved unsuitable for high volume nasal spray, Pollicrom, due to filter saturation causing loss of dose (data not shown). Thus, a custom glass expansion chamber approach was employed. However, there is a need for a more versatile method that can accommodate all nasal formulations, and the KNI may also be suitable for this function.

In this study, the mass and drug recovery were excellent, being close to 100% for all combinations of KNI or glass expansion chamber with the tested impactors. Shot mass variation in between shots given from one container and furthermore between different containers of the nasal spray product were observed. The shot mass should be reported in conjunction with the recovery to narrow down reasons for variability in recovery.

The recovery of the nasal formulation from the set up should be as high as possible to assure accurate determination of the mass fraction below 10 μm. The authors suggest setting the acceptable range at 95–105% mean recovery with low variability unless otherwise justified.

Conclusion

A suitable method for determining the mass fraction less than 10 μm in an aqueous solution nasal spray was developed. Impactors, nasal inlets and their combinations were tested, in which the inlet serves the purpose of applying the nasal spray to the air flow toward the impactor. The Fast Screening Impactor was found to be the most suitable impactor for this purpose of the ones tested in this study.

None of the commercially available nasal inlets appeared to be suitable and/or practicable for directing the nasal product toward the impactor.

Therefore, the Kiel Nasal Inlet was developed to overcome these challenges. The KNI is attached to the

USP throat which is included in the compendial set-up in respiratory product characterization laboratories. A nasal product can be inserted between vertical orientation and up to 60° from the vertical plane. The inlet retains at least 700 μL of a low viscosity formulation, allowing the evaluation of nasal sprays with low drug concentration utilizing several spray shots to exceed the limit of quantification. The material is durable and the production cost by molding is low.

The combination of Kiel Nasal Inlet and the Fast Screening Impactor equipped with a 30 L/min cut off plate allows the precise and reproducible aerodynamic assessment of the mass fraction below 10 μm. The method is independent of the applied spray shots and insensitive to variability in the air flow within the specification of the FSI. Repetition of the experiments in other laboratories gave comparable results. The Fast Screening Impactor allows fast analysis of nasal products due to easy operation and a comparably low number of analytical samples.

The applicability of the developed method to nasal spray suspensions, nasal powders and pressurized nasal sprays will be the objective of further studies. Preliminary tests with powder and suspension formulations look promising. The usefulness of the Kiel Nasal Inlet will also be investigated for other nasal product characterizations such as collection of emitted dose.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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