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Original Article

Development of a physiologically relevant dripping analytical method using simulated nasal mucus for nasal spray formulation analysis ☆

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ABSTRACT

Current methods for nasal spray formulations have been elementary evaluating the dripping characteristics of a formulation and have not assessed the behavior of the nasal formulation in the presence of varying types of mucus depending on the indication or diseased state. This research investigated the effects of nasal mucus on the dripping behavior of nasal formulations and focused on developing an improved in vitro analytical test method that is more physiologically relevant in characterizing nasal formulation dripping behavior. Method development was performed using simulated nasal mucus preparations for both healthy and diseased states as coatings for the dripping experiment representing a wide range of viscosity. Factors evaluated during development of this in vitro test method included amount of mucus, application of mucus, drying times, and compatibility of the mucus on a C_{18} Thin Layer Chromatography (TLC) substrate. The dripping behavior of nasal formulations containing a range of 1% Avicel to 3.5% Avicel was assessed by actuating the nasal spray on a perpendicular TLC plate coated with either healthy or diseased simulated nasal mucus. After actuation of the nasal spray, the dripping of the formulation on the coated TLC plate was measured after the plate was repositioned vertically. The method that was developed generated reproducible results on the dripping behavior of nasal formulations and provided critical information about the compatibility of the formulation with the nasal mucus for different diseased states, aiding in nasal spray formulation development and physical characterization of the nasal spray.

1. Introduction

Physiologically, the nasal mucosa naturally produces an abundance of nasal mucus when in a healthy state for purposes of protecting the nasal mucosa from drying out and trapping unwanted substances. Excessive nasal mucus may be secreted by patients suffering from allergic rhinitis, sinusitis and the common cold. Nasal mucus roughly consists of 95% water, 2.5% glycoproteins, 1%–2% electrolytes and other still incompletely defined components among which are lysozyme, lactoferrin, complement, and possible liquid fractions similar to surfactant [1–3]. The qualitative and quantitative features of the glycoproteins are primarily responsible for the rheological properties of the nasal secretions [4–6]. Important rheological features of mucus include viscosity, elasticity, adhesiveness, and the ability to be spun ("spinability") and poured ("pourability") [7].

Nasal spray is a unique drug delivery means that has been widely used to deliver medication to the intranasal cavity to treat numerous topical diseases such as allergic rhinitis, sinusitis, and nasal congestion for those suffering from the common cold. Other emerging applications of nasal sprays include vaccination delivery and the treatment for migraine via intranasal delivery. Formulation adherence to mucosa (e.g. residence effectiveness in the nasal cavity) and patient comfort are important factors often considered during nasal spray formulation development. A non-dripping form of a nasal spray formulation has been developed usually containing thixotropic agents such as microcrystalline cellulose to achieve the desired viscosity profile resulting in lower viscosity when shear stress is applied and increasing viscosity in its absence. Analytical methods assessing the dripping behavior of nasal spray formulations have not been commonly used for formulation development. A simple invitro method of employing a paper sheet as the spray substrate has been developed to assess the dripping behavior of nasal spray formulations and is also used to guide formulation development. However, this method hardly predicts or correlates with how the nasal spray formulation behaves inside the nasal cavity. Perhaps besides anatomical structure of the human nose, the most significant factor that should be considered is the interaction of the formulation with nasal mucus. Furthermore, mucus characteristics vary depending on the type and stage of

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diseases, ranging from watery and runny for healthy subjects and those exhibiting allergic rhinitis, to thick and viscous for a patient suffering from chronic sinusitis [8,9]. Current methods in the laboratory evaluating the nasal dripping characteristics of a formulation are elementary and do not assess the behavior of the nasal formulation in the presence of varying types of mucus depending on the indication or diseased state.

Therefore, the goal of this research was to investigate the effects of nasal mucus on the dripping behavior and physical properties of nasal formulations, and to develop an improved invitro analytical test method that is more physiologically relevant in characterizing nasal formulation dripping behavior generating reproducible results. The ultimate objective was to support formulation development by formulating a nasal spray for compatibility with the nasal mucus associated with a particular diseased state or indication for which the nasal spray is intended. To evaluate the interaction of formulation and nasal mucus, healthy simulated nasal mucus and diseased simulated nasal mucus were selected as coatings for the development of an analytical dripping method.

2. Experimental

2.1. Materials

Various types of dyes including allura red AC, methylene blue, alcian blue GX, congo red and crystal violet were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Nano Silica TLC plates, Cellulose Plastic TLC plates, and Cellulose Aluminum TLC plates were all sourced from Sigma-Aldrich (St. Louis, Missouri, USA). The microcrystalline cellulose (Avicel) was sourced internally from Merck MSD (Rahway, New Jersey, USA).

For the preparation of healthy simulated nasal mucus, porcine mucin type II, sodium chloride, and potassium chloride were all sourced from Sigma-Aldrich (St. Louis, Missouri, USA) and the calcium chloride dihydrate was sourced from Fisher Scientific (Fair Lawn, New Jersey, USA).

For diseased simulated nasal mucus, locust bean gum (LBG), saline solution, and sodium dodecyl sulfate (SDS) were sourced from Sigma-Aldrich (St. Louis, Missouri, USA), Bio-world (Dublin, Ohio, USA), and Invitrogen (Carlsbad, California, USA), respectively.

2.2. Instrument and characterization

A stainless steel actuation apparatus for nasal sprays was designed by Merck Engineering for this dripping study (Fig. 1). The apparatus was designed to seat a nasal spray device with the ability for manual actuation, taking into account stroke length for the nasal spray bottle. It also had a metal plate with an affixed TLC plate that can be positioned perpendicular to the nasal spray bottle during actuation and then repositioned 90 degrees vertically after actuation for evaluation of dripping behavior. The distance between the nasal spray tip and this metal plate was set at 3 cm. A digital camera was placed horizontally across from the stainless steel apparatus at a fixed distance and used to record the nasal formulation dripping time-elapse profile.

In addition, characterization of the simulated nasal mucus was performed by testing viscosity and surface tension. The surface tension was evaluated on a Kruss DSA-3 Surface Tension Analyzer in pendant drop mode (single drop measurement). The viscosity of each of the coatings and formulations were determined on a Brookfield Viscometer DV-II Pro. A 5 mL sample was transferred into the sample cup and equilibrated for 30 min in an undisturbed water bath at 37 °C. Rotations at 100 rpm were started after the 30 min and measurements were recorded at 0, 5 and 10 min. A

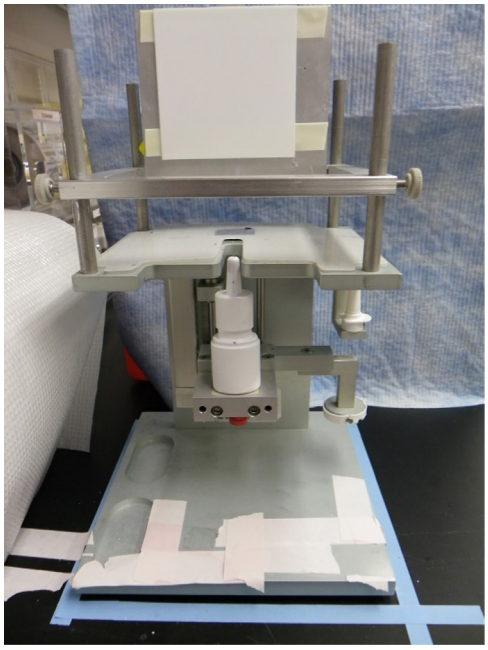

Fig. 1. Nasal spray apparatus.

Spraytec® laser diffraction system (Malvern Instrument Ltd., Westborough, MA) measured the droplet size distribution of the nasal sprays. The SprayView NSP system equipped with a high-speed camera (Proveris Scientific Corp., Westborough, MA) measured spray pattern of the nasal sprays.

2.3. Nasal formulation preparation

Six placebo formulations were prepared for this study containing varying amounts of Avicel (1%, 1.5%, 2%, 2.5%, 3% and 3.5% (m/m)). A stock solution was made by gradually charging 40 g of Avicel to 800 g of water in a steel compounding vessel (temperature 25 ± 5 °C) and continuously mixed at 350 rpm while recirculating through an in-line homogenizer (IKA Ultra Turrax T25) at a flow rate of 1 L/min for 30 min. After 30 min, 200 g of water was added to the compounding vessel and continued mixing for another 10 min. Variable amounts of stock solution and water were combined to achieve each of the desired concentrations of Avicel. Dye was added to each formulation at a concentration of 0.05% (m/m) followed by agitation once more at 350 rpm for 10 min. The final formulation was filled into a nasal spray bottle with a spray pump to deliver 100 μL per actuation.

2.4. Simulated mucus preparation and coating

Healthy nasal mucus was simulated by preparing a solution of porcine mucin in buffer exhibiting properties such as watery and non-viscous [10]. Conversely, a type of mucus that may exemplify a diseased state such as chronic sinusitis displaying highly viscous properties was simulated by a preparation of LBG and SDS in saline solution [11]. The healthy simulated nasal mucus was prepared by adding porcine mucin type II to a buffer solution consisting of 7.5 mg/mL of sodium chloride, 1.3 mg/mL of potassium chloride, and 0.3 mg/mL of calcium chloride dihydrate to attain an overall concentration of porcine mucin of 8% (m/m) in buffer. The diseased simulated nasal mucus was prepared by heating a beaker containing 100 mL of saline solution and SDS at a concentration of 6 mM to 80 °C. Once the solution reached a temperature higher than 70 °C, the LBG was slowly added while mixing until a final temperature of 80 °C was attained and the LBG was dissolved.

After cooling for about 2 h, additional saline solution was added and mixed in to achieve mass balance from losses during heating, mixing and evaporation. This preparation of mucus has similar physical properties to real human diseased mucus exhibiting high viscosity due to the high concentration of LBG.

2.5. Method development for coating application

Multiple experiments were performed to develop a method to assess the dripping behavior of the nasal spray formulations with simulated nasal mucus coated on a TLC plate as a substrate. TLC plates (10 cm × 10 cm) were selected because various types of coatings (i.e. different chemistry) were available on the plates with porous and hydrophilic characteristics compatible with the simulated human mucus. Different types of TLC plates were evaluated during the coating experiments to determine which was most compatible with the simulated human mucus displaying a uniform coating without beading on the plate. In addition, the plates were the ideal size to fit in the nasal spray apparatus and large enough to capture the dripping experiment over 30 s. Further experiments were performed to optimize the volume and application technique of the nasal mucus for development of a repeatable and reproducible method. In order to achieve this, the key parameter of non-dripping was assessed to assure that the simulated mucus when applied to the TLC plate did not drip when positioned horizontally (upside down) during nasal spray actuation and vertically at a 90° angle during the dripping analysis part of the experiment. A visual check of the simulated nasal mucus after application to the TLC plate was also evaluated to ensure that it did not dry. Therefore, application experiments for the simulated mucus focused on determining a final method which was bracketed by a lower and upper range in terms of volume and drying time (if necessary) that would still produce no dripping of the applied simulated mucus during the test. The application method, such as brushing technique during distribution of the simulated mucus, was also evaluated to ensure control and consistency. Once the parameters were optimized, they were assessed by testing replicates ($n=2$) for the 1% Avicel and 3.5% Avicel formulations, bracketing the complete range of Avicel in the formulations. The replicates for each of the formulations were very comparable when assessed for dripping behavior at 5, 10, 15, 20, 25 and 30 s. Both of the methods assessing the healthy simulated mucus with porcine mucin in buffer and the diseased simulated mucus with LBG were developed with this approach, achieving the goal of analyzing the dripping behavior of nasal spray formulations in a robust and reproducible way.

For the healthy simulated nasal mucus with buffer method, 1.0 mL of solution was applied to the cellulose side of a cellulose plastic TLC plate (10 cm × 10 cm). Droplets of the mucus were applied evenly on the periphery and the interior of the TLC plate to allow for even dispersion. The mucus was then distributed with a paintbrush for even application covering the entire TLC plate and air dried for 1 min prior to the dripping analysis.

The method for dripping analysis with the diseased simulated mucus with LBG as a coating, entailed adding 2 g of mucus to the cellulose side of a cellulose aluminum TLC plate (10 cm × 10 cm). A 1 inch brush was utilized to evenly distribute the diseased nasal mucus. A net weight of 1.5 g was targeted after brushing in a uniform pattern (up/down) covering the entire TLC plate. The dripping analysis occurred immediately after the final step of application of the mucus with no drying time.

2.6. Dripping assessment method

The dripping assessment method was performed on each of the formulations listed in Section 2.3 containing a range of 1%–3.5% Avicel. In addition, two commercial formulations designated "commercial A no drip" and "commercial B" were included in the study. Once simulated nasal mucus was applied to a TLC plate (10 cm × 10 cm) and dried, if necessary, the TLC plate was affixed to the nasal dripping study apparatus. A primed nasal spray bottle containing formulation was then shaken in a horizontal plane for 10 s prior to placement in the nasal bottle seating and was immediately actuated vertically. Once actuated, the metal plate holding the horizontal TLC plate was moved in a 90° vertical direction and the dripping distance was measured at 0, 5, 10, 15, 20, 25, and 30 s while the camera simultaneously captured images of the formulation dripping at these times. If the formulation dripped off the TLC plate, no further data was collected for that particular replicate. Duplicate replicates ($n=2$) were performed for each formulation and the dripping distances (mm) were averaged for each time point. In addition to dripping distance, the dripping speed (mm/s) was also calculated for further analysis about the dripping behavior of the formulations.

3. Results and discussion

3.1. Selection of dye and characterization experiments

Dye was added to the nasal formulation during its preparation to aid in visualization of the dripping process. Several dyes were evaluated including allura red AC, methylene blue, alcian blue GX, congo red and crystal violet. Dyes were assessed and selected based on having a high absorptivity and good solubility in water. Preliminary experiments showed the crystal violet dye exhibited intense color when added to the formulations even at a relatively low concentration of 0.05% (m/m). Further testing conducted confirmed that the addition of crystal violet dye did not cause any significant changes in physical properties to the formulations. Viscosity, spray pattern and droplet size testing were all performed to compare the formulation with dye and without. A direct comparison of a formulation with dye and without showed no practical significant difference in viscosity ($\leq 5\%$). In addition, droplet size distribution data (Dv10, Dv50, Dv90, and span) and spray pattern data such as minimum spray diameter (Dmin), maximum spray diameter (Dmax), ovality, and % area (Fig. 2) further confirmed that there was no significant impact of adding crystal violet dye with respect to affecting the physical characteristics of the formulation which would ultimately affect the dripping behavior of the nasal spray. Therefore, for purposes of visualizing dripping behavior, crystal violet dye was selected to add into all nasal spray formulations. Six placebo formulations with 0.05% (m/m) crystal violet dye were prepared for this study containing increasing amounts of Avicel including 1%, 1.5%, 2%, 2.5%, 3% and 3.5% (m/m). It should be noted that there was an error during the preparation of the 2.5% Avicel formulation which was not included in the subsequent nasal dripping experiments.

The droplet size data (Dv10, Dv50, and Dv90) appear to demonstrate a general trend of an increase in larger droplets during actuation as the % Avicel in the formulation is increased irrespective of the addition of dye. There is also an apparent shift to higher span values confirming the effect that higher concentrations of Avicel have on droplet size distribution. A comparison of the placebo formulation and the placebo with dye formulation shows no significant differences in droplet size distribution, suggesting that the dye has no impact on the physical properties of the formulation. Therefore, the droplet size data support that adding the dye for purposes of assessing the dripping behavior for this nasal spray formulation is acceptable.

An evaluation of the spray pattern data suggests that the addition of dye to the placebo formulation does not affect the spray pattern attributes since the data is comparable between the

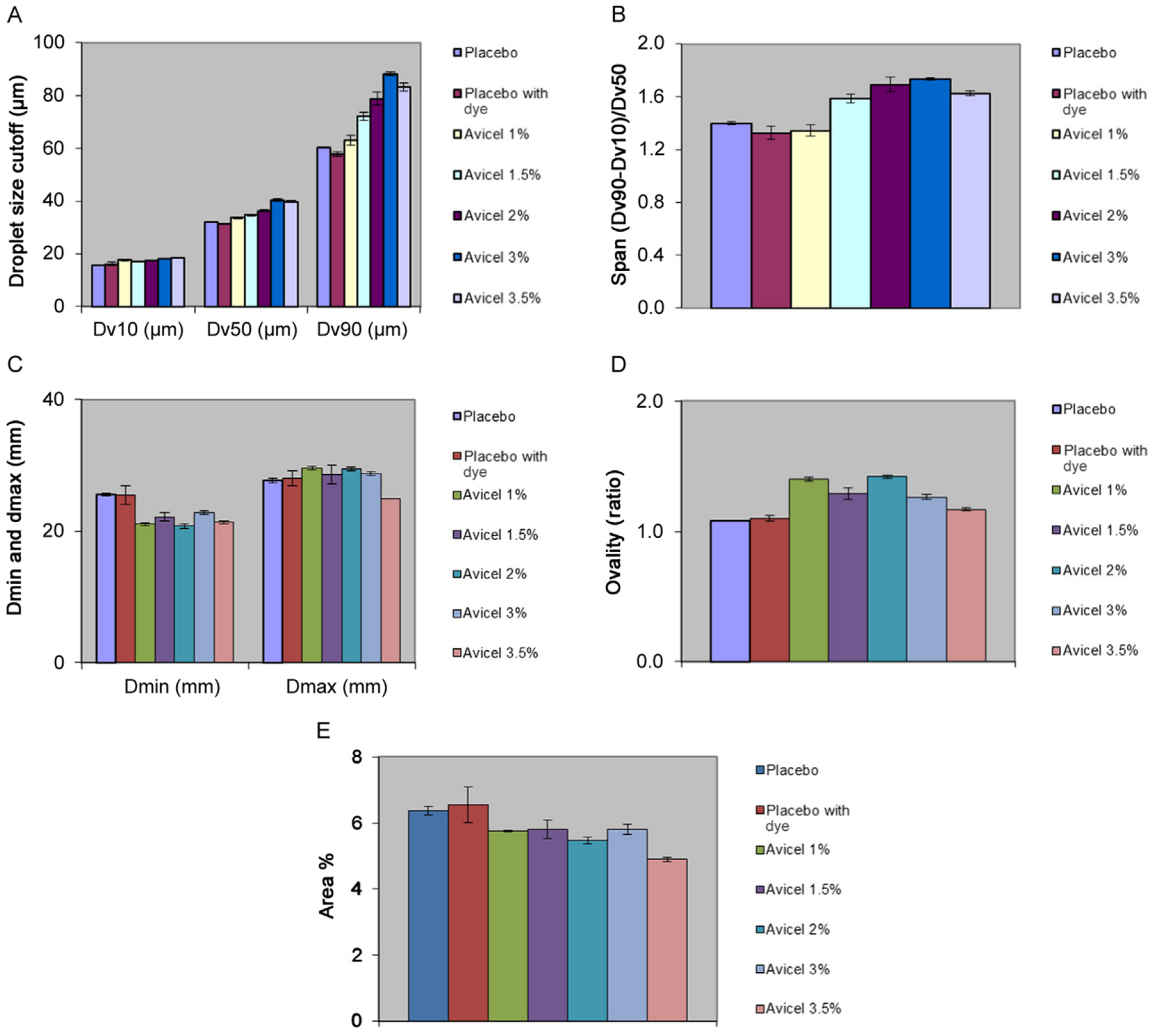

Fig. 2. Nasal spray formulation characterization data: (A) droplet size distribution, (B) span, (C) Dmin and Dmax, (D) Ovality, and (E) Area %.

placebo and placebo with dye formulations for Dmin, Dmax, ovality and area %. However, the spray pattern data with respect to increasing the % Avicel in the nasal spray formulations appears to be somewhat inconclusive with no obvious trend for ovality, Dmin and Dmax while exhibiting a trend of decreasing spray pattern area %. This may be attributed to the increased viscosity of the formulation as the % Avicel is increased.

3.2. Selection of coatings and finalized coating technique methods

Different coatings and coating techniques were assessed for the nasal dripping experiment. Simulated mucus representing healthy mucus with a watery, thin consistency and diseased mucus similar to very viscous, thick mucus commonly associated with chronic sinusitis were selected to investigate the interaction and dripping properties of mucus with formulation containing various amounts of Avicel ranging from 1% to 3.5%. As stated earlier, viscosity is an important characteristic of nasal mucus and therefore, the two simulated mucus types were a good option to bracket the full range of all types of nasal mucus. Low viscosity was associated with healthy mucus and a very high viscosity was typical for the diseased mucus with values of 13 cP and 1400 cP, respectively. Both types of simulated mucus had similar values in surface tension (approximately 30 mN/m). The property of surface tension is also important for nasal mucus because it is related to adhesiveness, another characteristic of nasal mucus, and may provide some critical information about the interaction of the simulated nasal mucus and formulation. The coating substances selected for this experiment were a healthy simulated nasal mucus made with porcine mucin in buffer and a diseased simulated nasal mucus comprised of LBG and SDS in saline solution. For the experiment assessing nasal dripping on paper, no coating was applied. The final method evaluating healthy simulated nasal mucus involved applying 1.0 mL of mucus to the cellulose side of a cellulose plastic TLC plate, evenly distributing droplets on the periphery and interior of the TLC plate to allow for even dispersion. The healthy mucus was dried for 1 min prior to analysis. The final method assessing diseased simulated nasal mucus involved adding 2 g of mucus to the cellulose side of a cellulose aluminum TLC plate and evenly distributing a target net weight of 1.5 g after brushing in a uniform pattern. Both of the coating methods described for simulated nasal mucus resulted in robust methods generating reproducible results.

Further characterization was performed on the coatings by testing viscosity, surface tension and density. The viscosity data generated for the diseased simulated mucus with LBG confirms that it is very viscous (~1400 cP) and is representative of a diseased state such as chronic sinusitis in which the mucus is described as thick and viscous as determined by Majima, et al. [9]. Conversely, the viscosity of the healthy mucus is low (~13 cP)

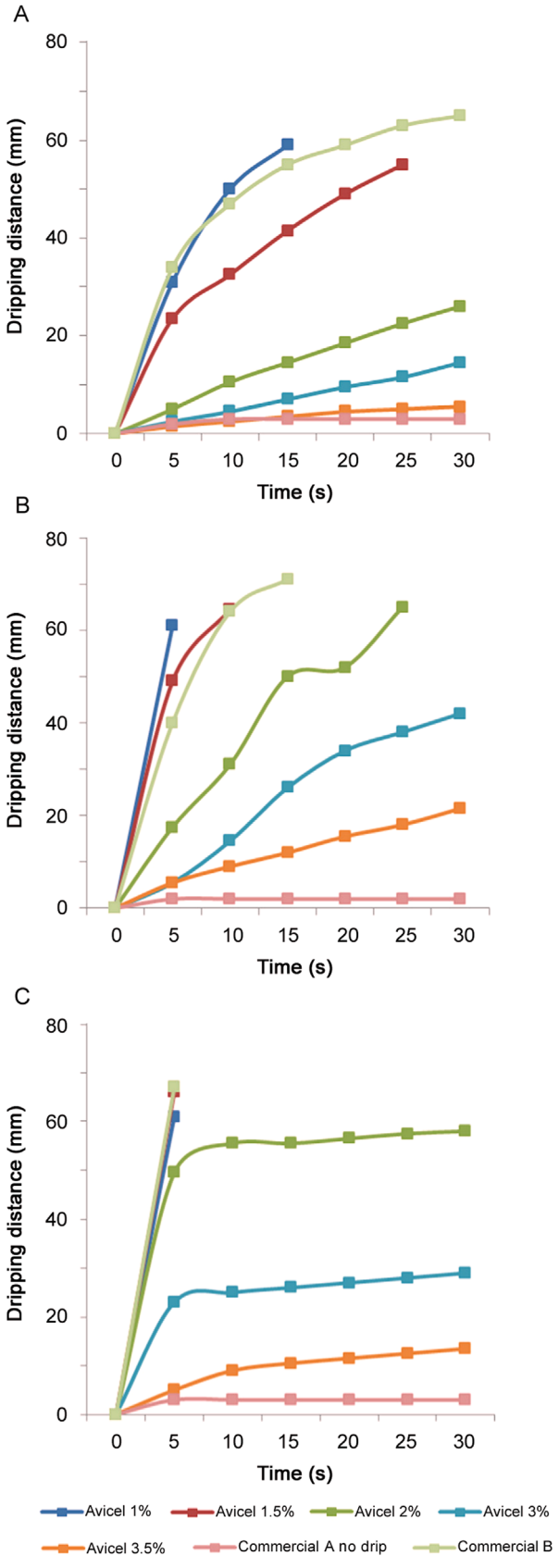

Fig. 3. Formulation dripping distance vs. time with (A) no coating on paper, (B) healthy simulated nasal mucus and (C) diseased simulated nasal mucus.

Table 1.
Average dripping speed of formulation with simulated mucus at different time points.

Mucus	Formulation	Average dripping speed (mm/s)						
		0	5	10	15	20	25	30
None (Paper)	Commercial A no drip	0	0.4	–	–	–	–	–
	Commercial B	0	8.0	6.4	4.7	–	–	–
	Placebo	0	2.6	5.0	4.3	3.4	–	–
	Avicel 1%	0	12.2	–	–	–	–	–
	Avicel 1.5%	0	9.8	6.5	–	–	–	–
	Avicel 2%	0	3.5	3.1	3.3	2.6	2.6	–
	Avicel 3%	0	1.1	1.5	1.7	1.7	1.5	1.4
	Avicel 3.5%	0	1.1	0.9	0.8	0.8	0.7	1.7
Healthy simulated mucus	Commercial A no drip	0	0.4	0.3	–	–	–	–
	Commercial B	0	6.8	4.7	3.7	3.0	2.5	2.2
	Placebo	0	3.8	2.4	1.8	1.5	1.2	1.1
	Avicel 1%	0	6.2	5.0	3.9	–	–	–
	Avicel 1.5%	0	4.7	3.3	2.8	2.5	2.2	–
	Avicel 2%	0	1.0	1.1	1.0	0.9	0.9	0.9
	Avicel 3%	0	0.5	0.5	0.5	0.5	0.5	0.5
	Avicel 3.5%	0	0.3	0.3	0.2	0.2	0.2	0.2
Diseased simulated mucus	Commercial A no drip	0	0.6	–	–	–	–	–
	Commercial B	0	13.4	–	–	–	–	–
	Placebo	0	12.6	–	–	–	–	–
	Avicel 1%	0	12.2	–	–	–	–	–
	Avicel 1.5%	0	13.2	–	–	–	–	–
	Avicel 2%	0	9.9	5.6	3.7	2.8	2.3	1.9
	Avicel 3%	0	4.6	2.5	1.7	1.4	1.1	1.0
	Avicel 3.5%	0	1.0	0.9	0.7	0.6	0.5	0.5

"–" indicates no measurement calculated since formulation dripped off the substrate.

which is reflective of the normal thin, watery mucus in the nasal cavity of healthy individuals [12]. The two types of mucus exhibit two extremes in terms of nasal mucus with respect to viscosity and encompass a wide range of viscosity values for different types of mucus.

The surface tension data of approximately 30 mN/m for both the healthy simulated nasal mucus and the diseased nasal mucus demonstrate that both have a relatively low surface tension. Further, the surface tension values are critical because it provides information on how well the simulated mucus will disperse when applied to the TLC substrate. During the experiment, both the healthy and diseased simulated nasal mucus dispersed with ease across the TLC plate with no beading. The density of the healthy and diseased simulated nasal mucus was also tested for informational purposes and yielded a density of approximately 1.00 g/mL and 0.94 g/mL, respectively.

3.3. *Dripping study*

The dripping behavior of five formulations of 1%, 1.5%, 2%, 3%, 3.5% Avicel and two commercial formulations (commercial A no drip and commercial B) were evaluated when applied to TLC plates with coatings representing simulated nasal mucus for a healthy and diseased individual. In addition, the dripping behavior of the formulations was also assessed on paper with no coating to serve as a control for the experiment.

The results comparing the formulation dripping behavior on paper with no coating demonstrated a trend in which increasing

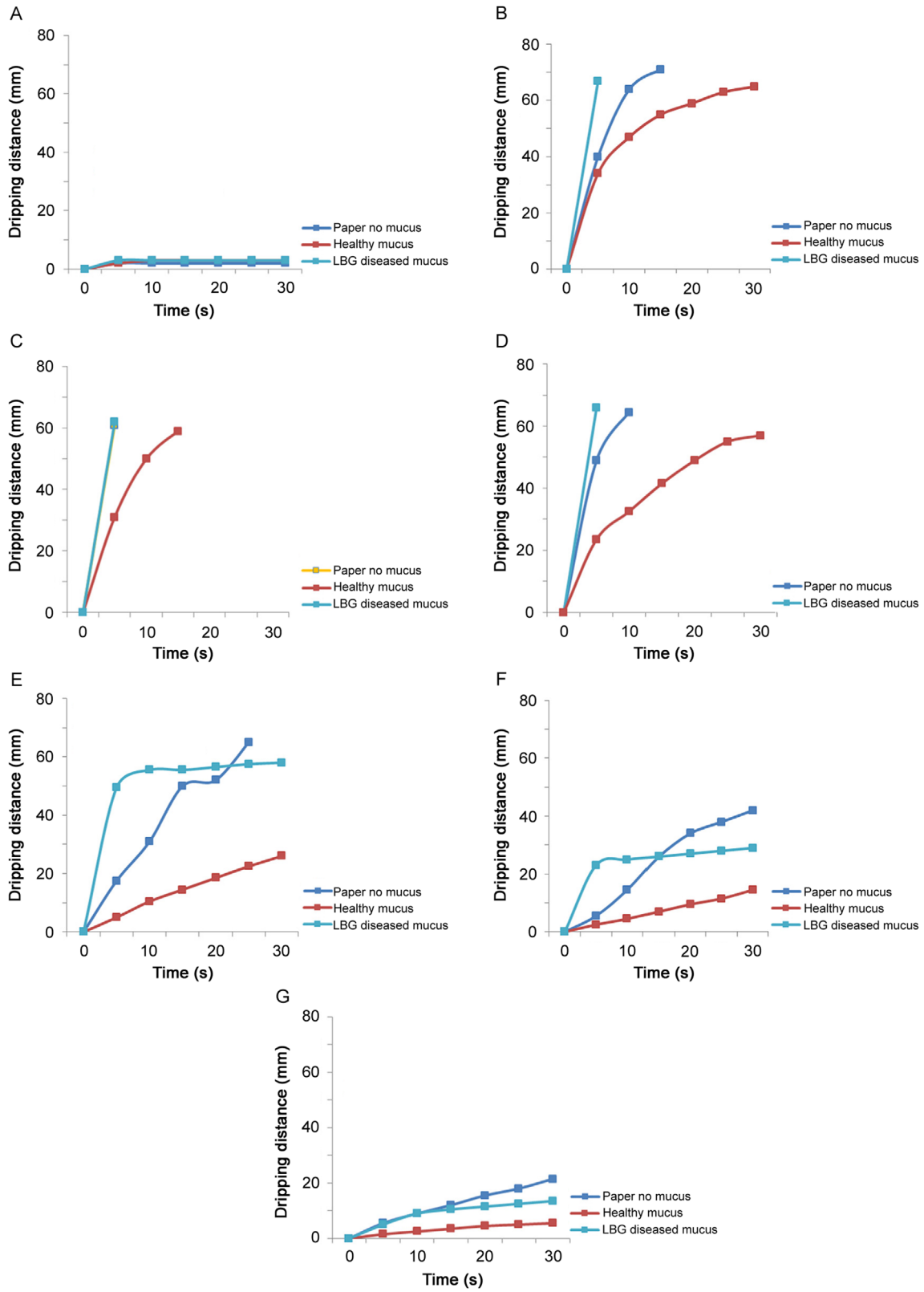

Fig. 4. Dripping distance vs. time for (A) commercial A no drip, (B) commercial B, (C) 1%, (D) 1.5%, (E) 2%, (F) 3%, and (G) 3.5% Avicel formulations with different coatings.

the % Avicel in the formulation results in a slower dripping rate (Fig. 3A). Formulations with 1.5% Avicel or less will drip off the paper in about 10 s. The commercial A no drip formulation exhibited a true no drip profile by traveling less than 5 mm during the 30 s test. The paper method, however, does not provide any information on how the formulation will interact with nasal mucus and may only be useful in comparing different formulations during formulation development to assess dripping behavior. In addition, paper is porous and may absorb some of the formulation when actuated. The presence of mucus may result in more

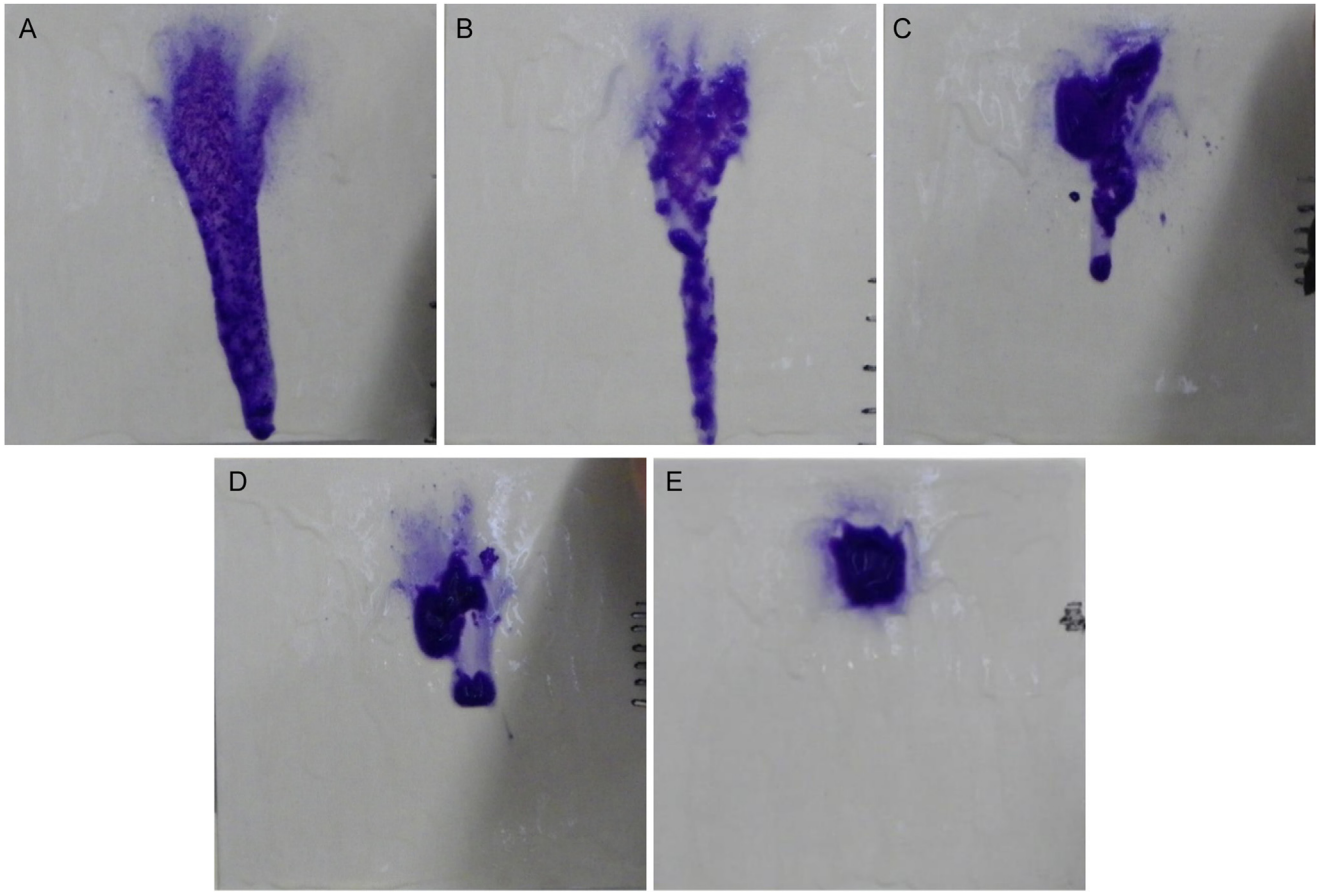

Fig. 5. Images of (A) 1%, (B) 1.5%, (C) 2%, (D) 3%, and (E) 3.5% Avicel formulations at 30 s after actuation on a TLC plate coated with healthy simulated mucus.

dripping of the formulation or less depending on the physical properties of each when interacting. This test may not always correlate with how the nasal spray will perform in vivo. For example, nasal anatomy, nasal temperature and nasal humidity can also have significant effects. It is possible that some nasal formulations may exhibit a completely different dripping profile when actuated into a human nose. However, with the addition of simulated nasal mucus to a TLC substrate, depending on the indication that is targeted, it may provide relevant information for formulation development by comparing varying amounts of Avicel in the formulation by qualitative analysis. It is also a critical analytical test that performs physical characterization of a nasal formulation.

For the experiment assessing dripping distance when applied to a healthy mucus coating, there appears to be a trend in which increasing the % Avicel in the formulation correlates to the total dripping distance decreasing through 30 s indicating a slower dripping rate (Fig. 3B). It also indicates that formulations with an increased Avicel content also demonstrate dripping behavior that is more linear and then levels off to a non-dripping state once the Avicel level is greater than or equal to 3%. The healthy mucus made with porcine mucin and buffer salts diluted in a buffer solution reflects that of a healthy individual and also is similar to mucus produced by those with allergic rhinitis and is watery and has low viscosity in nature. Therefore, this experiment may prove to be useful in assessing formulations for allergic rhinitis and also newer indications in which diseased viscous mucus is not a factor. Delivery of drug by a nasal spray with the potential for the drug to cross the blood brain barrier may be assessed for much newer indications in which nasal sprays historically have not been a dosage form. Therefore, there is potential in using this method for evaluating the dripping behavior of these types of formulations which may contain different excipients contributing to different dripping profiles.

The LBG coating representing diseased mucus appears to show a trend that as the % Avicel is increased in the formulation, the less probable it is to drip as measured by a shorter dripping distance after actuation (Fig. 3C). In addition, the majority of dripping occurs within the first 5–10 s after actuation as indicated by a steeper slope and then tends to stabilize afterwards through the 30 s of measurement. The formulations containing less than 2% Avicel display immediate dripping off the TLC substrate coated with mucus whereas the formulations containing greater percentages of Avicel demonstrate a slower, almost constant dripping rate after the initial 5 s. This data supports that the formulation has an affinity for the LBG mucus from a diseased state when increased levels of Avicel are present, indicating that it would not repel the formulation. Furthermore, it suggests that the residence time of the formulation may be increased in the nose since there is much less dripping especially with formulations containing higher percentages of Avicel.

The average dripping speeds of all formulations were also calculated when analyzed with both types of mucus and without. In all three experiments, there is an obvious trend that as the % Avicel increases in the formulation, the dripping speed decreases. The formulations also tend to show a relatively constant or decrease in speed when evaluated over time for all 3 experiments. Table 1 displays the results for the calculated average dripping speeds for all formulations in the presence of mucus and without. The dripping speed is much slower for the Avicel formulations when analyzed on a TLC plate coated with healthy simulated mucus when compared to the results for diseased simulated mucus and

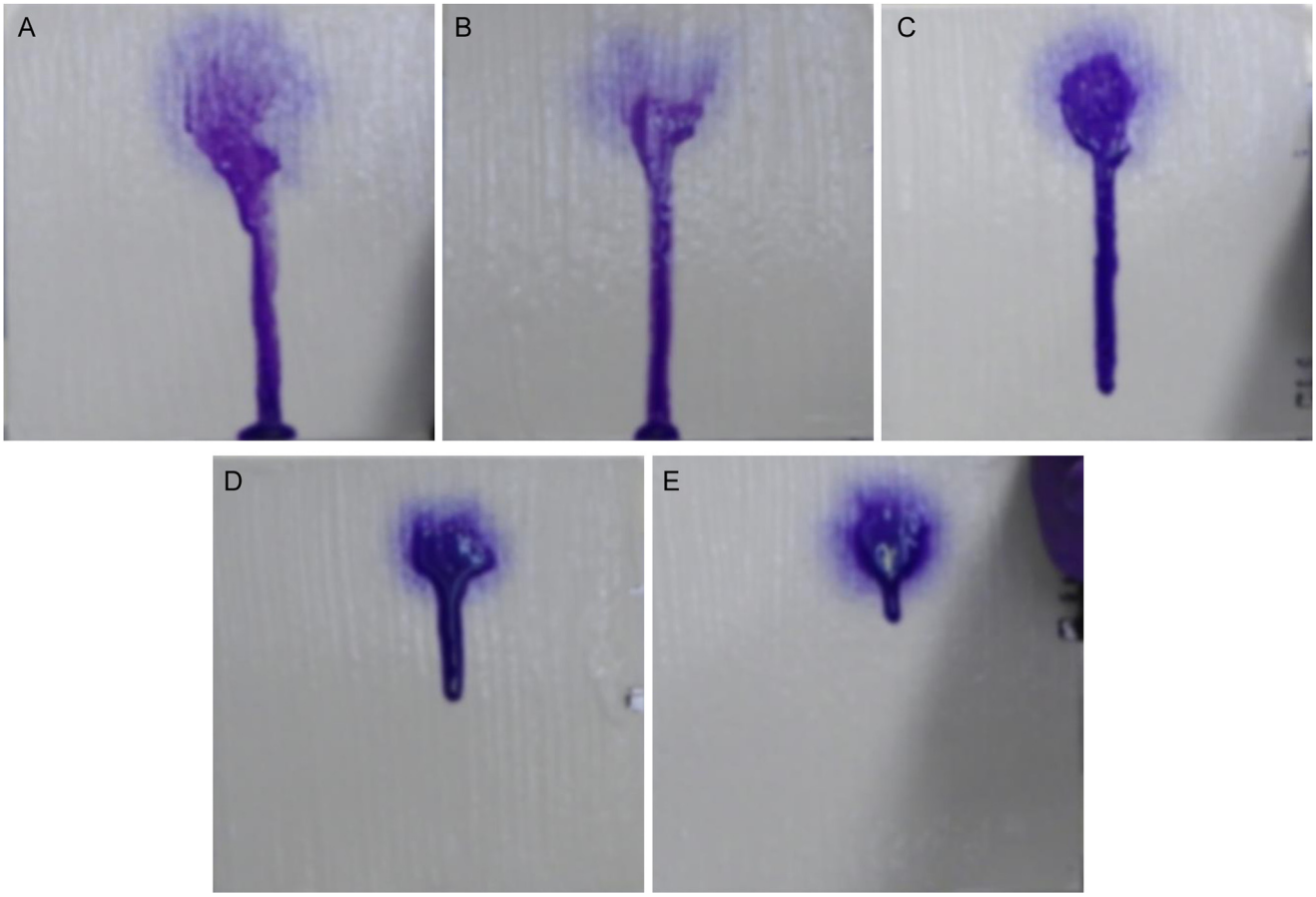

Fig. 6. Images of (A) 1%, (B) 1.5%, (C) 2%, (D) 3%, and (E) 3.5% Avicel formulations at 30 s after actuation on a TLC plate coated with diseased simulated mucus.

paper with no coating. This may suggest that these formulations have more similar properties to the healthy simulated mucus and therefore, have less of a tendency to drip. Conversely, the formulations, ranging in viscosity from 80 to 500 cP may be less similar to the highly viscous diseased simulated mucus (about 1400 cP), possibly resulting in a faster dripping rate.

The dripping behavior of each formulation (distance vs. time) was also individually analyzed and compared to the healthy and diseased simulated mucus and paper (no mucus) methods (Fig. 4). Images were captured for each of the formulations at 30 s after actuation on a TLC plate coated with the healthy simulated nasal mucus (Fig. 5) and diseased simulated nasal mucus (Fig. 6), respectively.

All figures for the 1%–3.5% Avicel formulations demonstrate a trend of decreased dripping as the % Avicel increases in the formulation when analyzed on a TLC plate with healthy and diseased simulated mucus and paper with no coating. The commercial A no drip formulation appears to exhibit a true no drip profile (Fig. 4A). The healthy simulated mucus appears to have the least dripping distance for all of the formulations.

The diseased simulated mucus and paper no coating methods appear to generate more similar results with increased dripping distances. Interestingly, the nasal spray formulations containing lower % Avicel (1%–2%) show increased dripping for the diseased simulated nasal mucus when compared to the paper method with no coating. However, this relationship changes when the % Avicel is increased in the formulation to 3%–3.5% with less dripping occurring for the diseased simulated mucus method. The increase in viscosity of the formulations becoming more similar to the highly viscous diseased simulated mucus may be the reason for this change.

4. Conclusion

The tendency to drip when a formulation is actuated onto a coated surface may provide useful information about how effective or efficacious a formulation might be when used for a certain diseased state. Even though the complex anatomy of the nasal cavity is not investigated in this study, the compatibility of a formulation with the nasal mucus on a TLC plate provides critical information about the interaction between the two. This is important because of the abundance of nasal mucus that exists in the nasal cavity regardless of an individual in a healthy state or afflicted with chronic sinusitis, allergic rhinitis or the common cold. Furthermore, a formulation that has very different characteristics from a particular nasal mucus may have more of a tendency to drip or, in human subjects, drip out of the nose with less residence time, so no therapeutic benefit is achieved.

From a formulation and patient use perspective, the % Avicel added to a nasal formulation is critical because it may affect patient comfort when administering the nasal spray to the nasal cavity. Higher percentages of Avicel added to a formulation can decrease the overall size of the spray pattern (with a narrower plume), resulting in a much more bullet-like effect actuating into the posterior nasal cavity correlating to higher patient discomfort. This is most likely related to the increased viscosity of the formulation [13]. Conversely, lower % Avicel formulations have a larger spray pattern (with wider plume) from the lower viscosity of the formulation and may deposit in the more anterior region of the nose with the undesirable effect of dripping out of the nose. Therefore, there is a balance that must be achieved when formulating nasal sprays and how the formulation will interact in the presence of various types of nasal mucus associated with different

indications may need to be investigated during formulation development. By developing an in vitro test method using various coatings to simulate nasal mucus, there is more biorelevance in characterizing the dripping behavior of nasal spray formulations which further supports formulation development and may provide more information about nasal spray deposition.

The development of a method evaluating the dripping behavior of a nasal spray formulation is important for characterization during formulation development. By assessing the dripping behavior in the presence of simulated nasal mucus, there is increased knowledge and understanding of the developed formulation by the differentiating factor with the addition of the nasal mucus. Furthermore, with nasal sprays being developed for new indications, different excipients may be employed requiring greater understanding of the formulation interaction with nasal mucus. Therefore, it is useful in developing a nasal spray dripping method which aids in formulation development and provides critical information about the compatibility of the formulation with nasal mucus, resulting in a method more physiologically relevant than a paper-based dripping method.

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