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Research Paper

Assessment of Nasal Spray Deposition Pattern in a Silicone Human Nose Model Using a Color-Based Method

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Purpose. To develop a simple and inexpensive method to visualize and quantify droplet deposition patterns.
Methods. Deposition pattern was determined by uniformly coating the nose model with Sar-Gel® (a paste that changes from white to purple on contact with water) and subsequently discharging sprays into the nose model. The color change was captured using a digital camera and analyzed using Adobe® Photoshop. Several tests were conducted to validate the method. Deposition patterns of different nasal sprays (Ayr, Afrin, and Zicam) and different nasal drug delivery devices (Afrin nasal spray and PARI Sinustar nasal nebulizer) were compared. We also used the method to evaluate the effect of inhaled flow rate on nasal spray deposition.
Results. There was a significant difference in the deposition area for Ayr, Afrin, and Zicam. The deposition areas of Afrin nasal spray and PARI Sinustar nasal nebulizer (2 min and 5 min) were significantly different. Inhaled flow rate did not have a significant effect on the deposition pattern.
Conclusions. Lower viscosity formulations (Ayr, Afrin) provided greater coverage than the higher viscosity formulation (Zicam). The nebulizer covered a greater surface area than the spray pump we evaluated. Aerosol deposition in the nose model was not affected by air flow conditions.

KEY WORDS: air flow; bioequivalence; deposition pattern; formulation; nasal spray; spray pattern.

INTRODUCTION

Nasal drug administration is an established way to deliver drugs for both local and systemic action; examples include anti-inflammatory corticosteroids, such as fluticasone propionate for treatment of allergic and non-allergic rhinitis, and 5-hydroxytryptamine $(5\text{-HT})_{1B/1D}$ receptor agonists, such as zolmitriptan for migraine treatment. Nasal delivery is considered promising for numerous other conventional drugs and biologics as a result of the nose's large, microvilli-covered mucosal surface area and excellent blood profusion resulting in fast absorption and onset of action. Venous blood from the nose avoids first-pass hepatic metabolism, and access to the nose via spray systems is arguably easier than achieving this via the lungs (1,2). Over the years, various nasal drug delivery devices, such as drops, propellant-pressurized sprays, aqueous spray pumps, catheters and dry powders have been used, but aqueous spray pumps are now dominant (3). Such pumps are differentiated into various subtypes, including those which discharge their formulation in response to most applied forces, those that require a minimum force to actuate, and those that only discharge formulation when their metering chamber is completely liquid-filled. Top and side-actuated variants exist, as well as preservative-free units designed to mechanically prevent microbial contamination of the bulk formulation following first use. A plethora of metered volumes and nozzle designs, in conjunction with the formulation and mode of patient use, are known or assumed to impact spray volume (and thereby dose to target site), droplet size distribution, plume shape and spray duration. Interest in assessing the impact of many of these factors on nasal deposition pattern is considerable for new drug, formulation and hardware development; life-cycle management activities for existing products and recent patent expirations on high-value, nasally administered corticosteroid products offer the prospect of generic availability. GlaxoSmithKline's Flonase (fluticasone propionate) lost patent protection in February 2006, and Sanofi-Aventis's Nasacort AQ (triamcinolone acetonide) lost patent coverage in January 2007 (4). Understanding the interactions between these complex variables would facilitate many of these activities and teach us much about what really influences nasal deposition, but progress has been slow for several reasons. No existing bench-top techniques, such as particle size analysis and plume shape evaluation, are generally accepted as predictive of droplet deposition site in the nose, and computational fluid dynamic (CFD) models that seek to do this are not well-validated and remain uncorrelated with clinical outcomes or pharmacodynamic endpoints (5). Subsequently, the US Food and Drug Administration (FDA) and pharmaceutical companies lack a validated methodology to establish nasal deposition patterns. An inexpensive tool to assess deposition pattern would potentially serve as a bioequivalence metric or to bridge spray product formulation/hardware changes during early development. The

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lack of correlation between spray shape metrics and clinical outcome means that most generic approvals have been based on a finding of statistical similarity in range of *in vitro* tests that favor discriminating power over therapeutic relevance. Companies developing new nasal products face expensive clinical trials when proposing even minor product changes. Scintigraphic and dye-based methods of assessing nasal spray deposition are not FDA-favored techniques because they require adulteration of the product, and we expect that expensive, laser-based systems used to visualize unobstructed plumes sprayed into the air will remain difficult to correlate with deposition pattern within the confines of the morphologically complex and volume-restricted nasal cavity. We have therefore begun to look at an inexpensive spray-visualization technique that allows simulation of gross nasal anatomy and airflow and is able to visualize (without need of a specific drug assay or addition of dye), quantify and discriminate droplet deposition patterns.

The purpose of this study was to design and validate an inexpensive method to visualize and quantify droplet deposition patterns in a nose model and demonstrate the utility of this approach by comparing the deposition patterns of different commercially available nasal sprays and different nasal drug delivery devices and assessing the effect of air flow conditions on nasal spray deposition. It is not our intention to suggest that the model we used is superior to other nasal casts or representative of all nasal geometries.

MATERIALS AND METHODS

Sar-Gel® (Sartomer Company Inc.), a commercially available water level indicating paste, which changes from white to purple on contact with water (which is the main ingredient by weight in all nasal sprays), was used to visualize deposition of aqueous droplets within an anatomically correct, transparent, silicone human nose model (Koken Co., Ltd.). We evaluated Ayr Saline Nasal Gel No-Drip Sinus Spray (B.F. Ascher & Co., Inc.), Afrin No Drip Original 12 Hour Pump Mist (Schering-Plough Healthcare Products, Inc.), and Zicam No-Drip Liquid Nasal Gel Non-Drowsy Seasonal Allergy Relief (Zicam LLC) because they represent a wide range of formulation viscosities (6). For the nasal nebulizer, we used PARI Sinustar reusable nebulizer with nasal adapter (PARI Respiratory Equipment, Inc.). All the images were captured using a digital camera (Canon Power Shot SD100 6.1MP Digital ELPH Camera w/3x Optical Zoom) and were quantified using Adobe® Photoshop (CS3 Version).

Design and Validation of a Color-Based Method

Design

The interior of the nose model was uniformly covered with Sar-Gel using a brush before being clamped onto a customized stand which incorporated a transparent Plexiglas model septum and airtight seal from the nasopharynx to a hose connector. The septum of the nose model was positioned approximately 8 cm in front of the digital camera (the distance and magnification are not critical due to use of a calibration square described later). Afrin nasal spray was manually actuated at a 45° angle to the horizontal and at a nostril insertion depth of 5 mm. Nasal spray was used as obtained from a pharmacy (without adulteration). The experiment was repeated five times. Before and after lateral spray images were captured using a digital camera under standardized photographic conditions with respect to lighting, camera position and magnification. Sequential photographs were taken approximately 10 seconds apart, and deposition area was measured from the first photograph in each series. The region of color change was quantified using Adobe Photoshop.

For quantification using Photoshop, the image size was first adjusted to 20×20 cm with a resolution of 100 pixels per cm. The 2000 × 2000 pixels image contrast was then adjusted so that only the purple area was selected using *Hue Saturat*ion. The *Magic Wand* tool was used to identify the purple color indicating nasal spray deposition after the *Tolerance* level was adjusted. The *Similar* command was then used to automatically select the entire purple region whose projected area in pixels could be automatically obtained using the *Histogram* tool. Dividing the pixel area by resolution gives the projected spray area in cm^2 (7).

Validation

Sensitivity of Sar-Gel. Increasing volumes of water from 0.5μL to 10μL were pipetted onto a horizontal glass plate coated with Sar-Gel to establish the sensitivity of Sar-Gel to water.

No Color Change of Sar-Gel in Response to Moisture in Ambient Air. To detect if the Sar-Gel-coated nose model spontaneously showed a color change in response to ambient air, the flat section of the nose model was coated with Sar-Gel using a brush and was exposed to ambient air.

Proof of No Beading of Sar-Gel on Nose Model Surface. The human nose model is made of silicone, which is hydrophobic, whereas Sar-Gel is hydrophilic. In order to determine whether Sar-Gel uniformly and completely coated the nose model (a prerequisite for detecting water droplets), a flat section of silicone was coated with Sar-Gel, and the uniformity was visually assessed after 0, 1, 10 and 20 min.

Proof of No Post-Deposition Migration or Dripping of Formulation. Increasing volumes (0.5μL to 200μL) of formulations (Afrin, Ayr and Zicam) were pipetted as a single spot onto a vertical surface coated with Sar-Gel to determine the conditions under which dripping of the nasal formulation on a Sar-Gel surface might occur.

Stability of Deposition Patterns Following Spraying. Pictures were taken every 10 sec for 1 min after initial nasal spray deposition (Afrin) in the nose model. Deposition area of the initial image was compared to the deposition areas of subsequent images ($n=5$).

Selected Area Depends on Size, Not on Intensity of Color. Since we manually brushed Sar-Gel onto surfaces, it is possible that some regions would be more thickly coated than others. In order to see if this had any effect on the calculated deposition area, 1 cm^2 squares were thickly or lightly coated with Sar-Gel and subsequently exposed to water spray prior to evaluation using Photoshop ($n=5$).

Accuracy of Adobe Photoshop Deposition Area Estimates. In order to determine the accuracy of image analysis, purple regions of known area (1 cm^2 and 4 cm^2) were generated by spraying water through a square stencil. Before and after pictures were taken and quantified for comparison with the starting area standards (n=5).

In order to correct for variations in the starting image size and the deposition area that result from use of different camera-to-nose model distances, we incorporated a 1 cm^2 purple square, which served as an area standard and was imaged simultaneously with deposition pattern in all nose model photographs. The variations in starting image size could then be corrected using the known area of the reference square.

Viscosity Studies

The bulk viscosity of each nasal spray formulation was measured using a cone and plate rheometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) at 2 RPM after 5 min. Five measurements per formulation were made at 25°C.

Comparison of Deposition Patterns of Commercially Available Nasal Sprays

We evaluated three commercially available nasal sprays, Ayr, Afrin, and Zicam, because they represent a wide range of formulation viscosities. The nose model was uniformly coated with Sar-Gel, and nasal sprays were discharged at an angle of 45° to the horizontal at a nostril insertion depth of 5 mm. Before and after spray images were captured under standardized photographic conditions, and the region of color change was quantified using Photoshop. The projected deposition area was calculated with reference to a 1 cm^2 purple square (n=5).

Comparison of Deposition Patterns of Nasal Spray and Nasal Nebulizer

Normal saline was added to a container fitted with an Afrin nasal spray pump and discharged into the nose model uniformly coated with Sar-Gel. Normal saline was added to a Pari Sinustar nasal nebulizer with nasal adaptor specifically designed to simultaneously administer aerosol into both nostrils. The nasal nebulizer was connected to a Pulmo Aide® compressor and run for 2 and 5 min with the output from one limb of the adaptor discharging into the coated nose model prior to image capture (n=5).

Effect of Inhaled Flow Rate on Regional Deposition Pattern

The nasopharynx of the model was either connected to a Harvard pump, which simulated a 15 breaths/min breathing pattern with a ratio of inspiration to expiration of 40/60 and cycle volumes of 700 ml/stroke (which we considered a moderate inhalation) or 50 ml/stroke (which we considered a shallow inhalation), or the nasopharynx of the model was connected to a regulated vacuum pump operated at 20 L/min or without any air flow. When breathing patterns were evaluated, discharge of the spray was coordinated with the onset of a simulated nasal inhalation. The nose model retained all the main structures of the human nasal cavity, such as the three projecting turbinates and the nasopharynx. We coated interior surfaces of the model using the nasal vestibule, turbinates and olfactory region as landmarks, but while the areas are reproducible, they remain somewhat arbitrary. Care was taken to cover the same area of each region with Sar-Gel during separate experiments before manually actuating each product at a 45° angle to the horizontal at a nostril insertion depth of 5 mm (n=5). We evaluated Ayr, Afrin and Zicam. Nasal spray products were used as obtained, but were not discharged as the patient instructions indicated. This was done so that different formulations could be compared without altered nozzle orientation and insertion depth confounding the deposition area results.

Statistical Analysis

All data are presented as mean±standard deviation (SD). A Kruskal-Wallis one way analysis of variance test was used to identify significant differences in deposition area

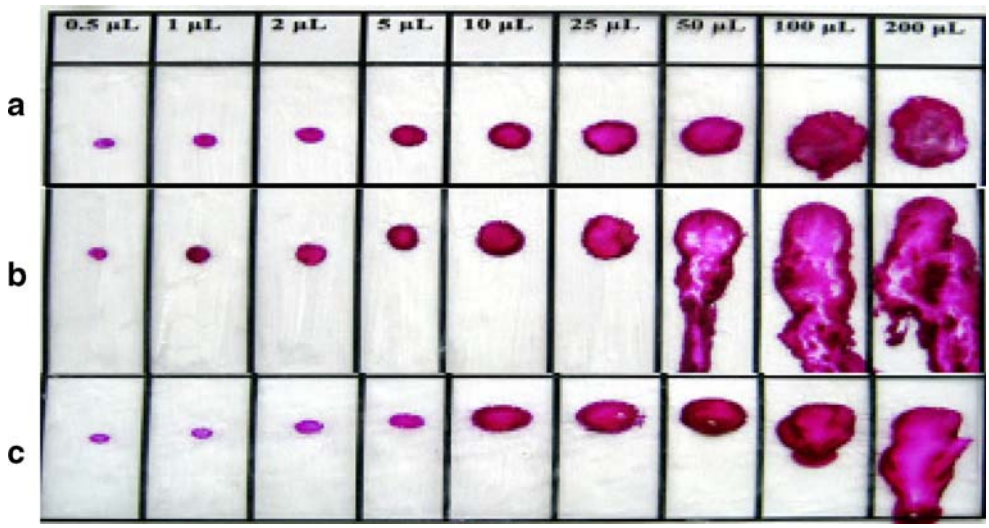


Fig. 1. Visualization and evaluation of dripping with Afrin (a), Ayr (b) and Zicam (c) one minute after the indicated formulation volume was pipetted onto a vertical surface coated with Sar-Gel.

Fig. 2. Deposition patterns of Afrin at 0 sec (a), 30 sec (b) and 1 min (c).

in the nasal vestibule, turbinates and olfactory regions. This nonparametric test was used because it does not require any assumption of normal distribution, and was most appropriate for our small sample size. *P* values less than 0.05 were judged to represent statistical differences.

RESULTS

Assessment of Deposition Pattern by the Color-Based Method. There was a significant and quantifiable change from white to purple when droplets or liquid streams from nasal spray products came into contact with Sar-Gel. The mean nasal spray deposition area for Afrin nasal spray was 7.82 ± 1.02 cm^2.

Sensitivity of Sar-Gel. Water volumes as low as 0.5μL became clearly visible after contact with Sar-Gel.

No Color Change of Sar-Gel in Response to Ambient Air. No color change was observed when the nose model coated with Sar-Gel was exposed to ambient air over a period of 24 hours.

No Beading of Sar-Gel on Nose Model. 20 min after application, no beading of Sar-Gel was detectable on the nose model's silicone surface. Subsequent experiments were conducted within 10 min of coating the model surface with Sar-Gel.

Maximum Spray Volume in One Area That Did Not Result In Dripping of Formulation. Dripping was observed with Ayr when the formulation volume was 50μL and above. For Zicam, dripping was observed at a volume of 200μL, and no dripping was observed with Afrin at all formulation volumes (Fig. 1).

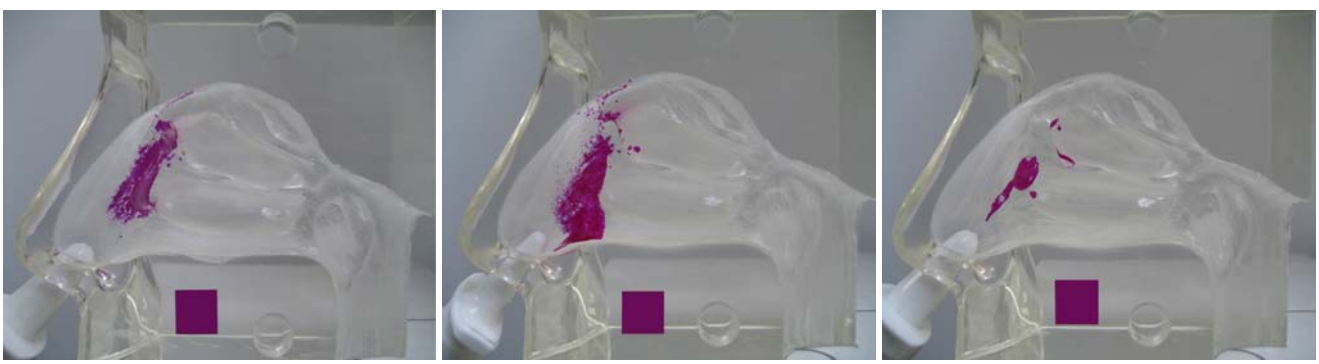
Stability of Deposition Patterns Following Spraying. The deposition area of the initial image was 6.95 ± 0.61 cm^2. There was an approximately 1% increase in the deposition area of the images taken at 30 sec and 1 min when compared to the deposition area of the original image (Fig. 2).

Selected Area Depends on Size, Not on Intensity of Color. Lightly and heavily coated Sar-Gel 1 cm^2 squares resulted in the same apparent mean area of 4.76 ± 0.3 cm^2 irrespective of the color intensity changes associated with coating thickness. Magnification by the digital camera lens and the camera-to-model distance resulted in an apparent area of 4.76 cm^2 for the 1 cm^2 reference squares.

Accuracy of Adobe Photoshop. The image processing method yielded areas directly proportional to the purple area on the original photograph. The area calculated by Photoshop for the 1 cm^2 purple reference square was 4.06 ± 0.04 cm^2, and that of the 4 cm^2 was 16.05 ± 0.07 cm^2.

The total mean nasal spray deposition area for Afrin nasal spray calculated using the reference square was found to be 1.45 ± 0.06 cm^2.

Viscosity Studies. Of the three nasal sprays tested, Zicam had the highest viscosity of $1{,}288.4 \pm 47.2$ cP, whereas the



Afrin Ayr Zicam

Fig. 3. Deposition patterns of Afrin, Ayr and Zicam.

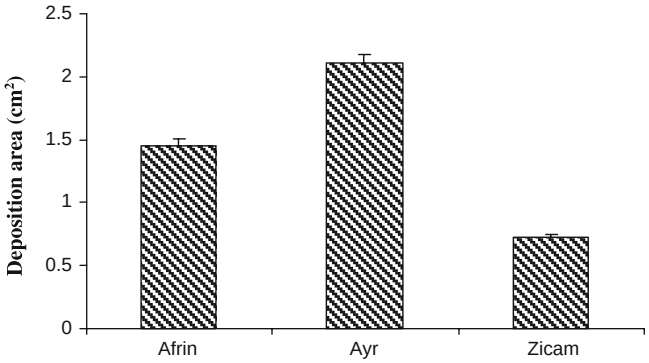


Fig. 4. Projected deposition areas of Afrin, Ayr, and Zicam. All values are expressed as mean±standard deviation of the mean (n=5).

viscosities of Afrin and Ayr were 923.5±30.1 cP and 667.6± 21.5 cP, respectively.

Comparison of Deposition Patterns of Commercially Available Nasal Sprays. Images for the deposition pattern of Ayr, Afrin and Zicam nasal sprays without any air flow are shown in Fig. 3. The nasal spray deposition areas determined from these photographs are shown in Fig. 4.

Comparison of Deposition Patterns of Nasal Spray and a Nasal Nebulizer. The deposition patterns of Afrin nasal spray bottle filled with normal saline and PARI Sinustar nasal nebulizer at 2 and 5 min are shown in Fig. 5. The deposition area of the nasal nebulizer both at 2 min and 5 min was significantly (p<0.001) higher compared to the deposition area of the nasal spray (Fig. 6).

Effect of Inhaled Flow Rate On Regional Deposition Pattern. Projected deposition areas of Ayr, Afrin and Zicam in the nasal vestibule, turbinates and olfactory regions under different air flow conditions are shown in Fig. 7.

DISCUSSION

Method Validation

The smallest droplet of water we could pipette, 0.5 μL, was easily visible when in contact with Sar-Gel, which also allowed visualization of individual droplets at the periphery of sprays, suggesting even greater sensitivity to liquid water. When Sar-Gel was painted on a silicone surface, no beading was observed at 20 min suggesting that all spray impacting on the model would be detected. Formulation dripping or color spreading (by diffusion) was not observed with any test formulation placed on a Sar-Gel surface as a single drop until the drop volume exceeded 25μL. Most nasal sprays deliver a volume no larger than 140μL per spray. Since the spray typically fans out as a cone from the tip of the nasal actuator, it is unlikely that 25μL of formulation will deposit at a single spot, so dripping or post-deposition spreading was expected to be minimal. This assessment was later confirmed by the stability of deposition patterns taken at different times following spraying. Deliberately thinly or thickly coated Sar-Gel 1 cm^2 squares changed to different purple color intensities by a water mist showed the same mean area. This indicates that the area calculated using Photoshop was dependent on the size of purple area but not on its color intensity. Areas calculated using Photoshop for 1 and 4 cm^2 purple reference squares were directly proportional to their actual areas. Inclusion of a 1 cm^2 purple reference square in all nose model photographs allowed for easy corrections of apparent deposition area to actual area, rendering the technique accurate even when the zoom magnification or camera to model distance varied.

Analysis of the Nasal Spray Deposition Patterns

These data suggest that the lower viscosity formulations (Ayr and Afrin) generated significantly (p<0.001) enhanced coverage of the nasal cavity compared to the higher viscosity Zicam formulation. The enhanced surface coverage of Ayr and Afrin may be attributed to the production of smaller droplets more able to navigate the nasal cast airspaces. In the absence of air flow, their trajectories are more likely to be flattened under the influence of gravity, resulting in a wider plume. In contrast, the higher viscosity Zicam formulation displayed a narrower plume and focused deposition directly in front of the spray orifice. This finding agrees with the results of a previous study (8).

Comparison of Deposition Patterns of Nasal Spray and a Nasal Nebulizer

Results from the nasal nebulizer image analysis showed significantly (p<0.001) increased deposition beyond the anterior nasal cavity compared to the spray pump under

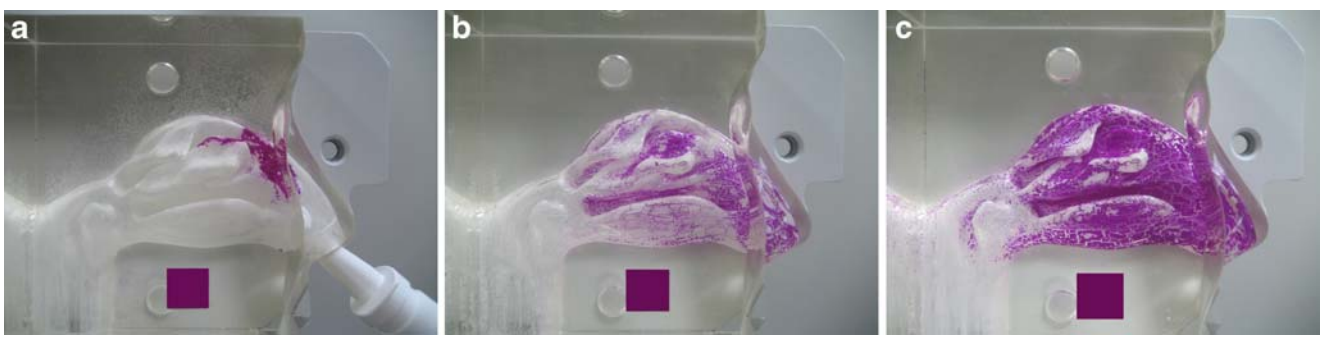


Fig. 5. Deposition patterns of Normal saline in Afrin spray bottle (a), Pari Sinustar (2 min) (b) and Pari Sinustar (5 min) (c).

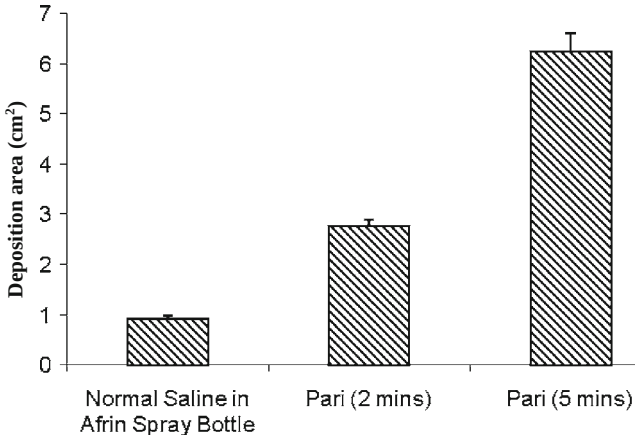


Fig. 6. Projected deposition areas of Normal saline in Afrin spray bottle, Pari Sinustar (2 min) and Pari Sinustar (5 min). Values are expressed as mean±standard deviation of the mean (n=5). **Note:* To have space to locate the nasal adaptor used in conjunction with the nebulizer in the model nostril, images were photographed through the silicone model rather than through the Plexiglas model septum as we did in previous experiments.

conditions of no airflow. These findings were consistent with the scintigraphic study in volunteers by Suman *et al*. where they demonstrated that the nasal nebulizer covered greater surface area and deposited droplets beyond the anterior nasal cavity, whereas the spray pump deposited larger, fast-moving droplets primarily in the anterior portion of the nasal cavity in human volunteers as a result of the inferior turbinate serving as a baffle/impaction surface (9). Smaller droplets generated by the nebulizer deposited in more posterior regions as a result of their smaller size, lower exit velocity and some airflow through the nose resulting from use of compressed air to generate the nebulized aerosol.

Effect of Inhaled Flow Rate on Regional Deposition Pattern

Deposition area was higher in the nasal vestibule and turbinate region and smaller in the olfactory region for both Ayr and Afrin. No Zicam deposition in the olfactory region was seen under any airflow conditions we evaluated. This data suggest that the low viscosity formulations (Ayr and Afrin) might achieve more coverage than a high viscosity formulation (Zicam), which may be ascribed to the production of smaller droplets sprayed at a wider plume angle. It is also possible that the smaller droplets had their paths more closely aligned with the air stream and were subsequently carried deeper into the nose. At different flow rates the regional distributions were not visibly different with the three nasal sprays. This might be due to the initial high velocity of the droplets exiting the spray nozzle impacting the surface of the nose model irrespective of the air flow rate in which they were introduced (8). The finding that the inhaled flow rate had little effect on the deposition pattern is supported by the work of Guo *et al*. (8). They showed that the regional distribution (assessed by scintigraphy) of nasal spray formulations differing in viscosity was not significantly influenced when tested using fast- and slow-breathing profiles in a nasal model.

CONCLUSION

A simple and inexpensive spray-evaluation technique which is able to visualize and quantify droplet deposition pattern has been developed. It also offers distinct advantages over plate- or laser-based spray pattern imaging and scintigraphy in terms of experimental simplicity, not requiring a specific drug assay or addition of dye or radiolabel. While this is a very small step towards demonstrating a correlation with meaningful biometric and clinical results, the methodology has the advantage of not exaggerating differences between spray products and could be the basis for a low-cost alternative to spray pattern and plume geometry testing as they are typically practiced. Moreover, the ambiguities of spray pattern edge detection that result from operator-defined boundaries can be avoided by the use of predefined and validated Photoshop settings. The method is also amenable to use with automated actuation stations and allows testing under a range of air flow conditions, which is not possible using laser sheet evaluation method. It could potentially be used with any nose model constructed using transparent materials. This approach might be developed into an alternative tool to justifiably establish *in vitro* bioequivalence of nasally administered, locally acting drug solutions, could provide a scientific rationale for justifying patient instructions for use, and may be a way to efficiently validate computational models of aqueous droplet deposition. We are also evaluating the use of Sar-Gel on cascade impactor stages

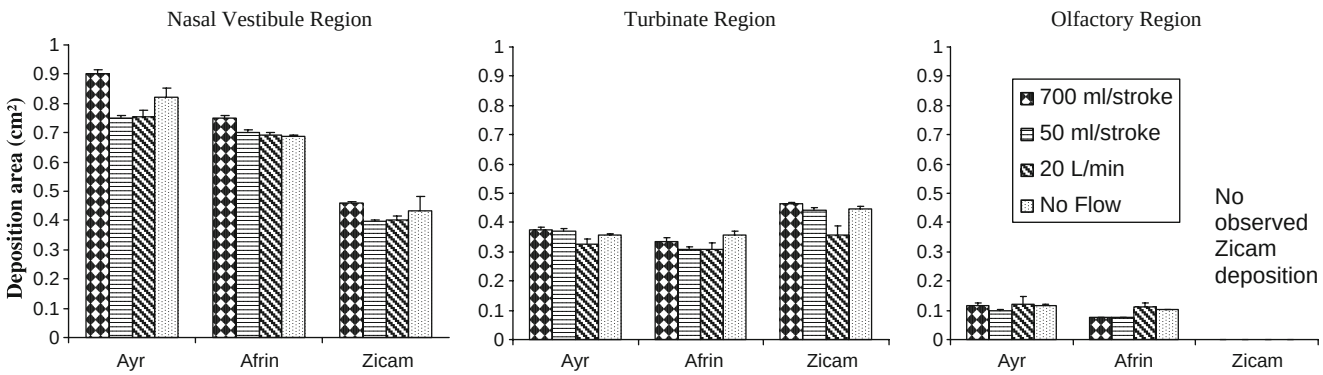


Fig. 7. Projected area associated with droplets deposition in each region of the nasal cavity under different airflow conditions. Values are expressed as mean±standard deviation of the mean (n=5).

(to detect droplet deposition) and on model faces fitted with facemasks (to quantify unintended facial and ocular droplet deposition associated with nebulizer use).

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