Hypertonic saline jet nebulization breathing treatments produce a predictable quantity of aerosolized sodium chloride for inhalation

Martin J. Flores AS¹, MaTais Caldwell BS², Kalyssa D. Passmore AS³, Megan Denney, RRT³, James M. Carr⁷, Kerri Carr, FNP-BC¹³, Jeremy M. Carr, PhD, M.Ed¹²


INTRODUCTION

Jet nebulization is widely regarded as a ubiquitous, cost-effective, and well-tolerated intervention for treating respiratory diseases [1]. Hypertonic saline jet nebulization (HSJN) is a mucolytic, therapeutic subset within this field wherein patients undergoing treatment inhale saltwater droplets. Medical professionals order HSJN breathing treatments to manage symptoms associated with respiratory illnesses [1] including cystic fibrosis [2], tracheostomies [3], and asthma [4]. Despite some skepticism [5], HSJN reduces hospitalization admissions and shortens hospital stay lengths in patients diagnosed with bronchiolitis [6, 7].

During an HSJN treatment, pressurized gas such as room air or oxygen is fed into a jet nebulizer cup containing hypertonic saline—a sodium chloride (NaCl) solution exceeding 0.9% by mass. Within the cup, the saline is drawn into the gaseous stream, shearing the solution into a mist with a variety of droplet sizes [8]. Larger droplets tend to collide with internal baffling within the nebulizer cup, usually returning into the solution reservoir, whereas microscopic droplets escape the cup and are available for patient inhalation.

Numerous questions remain about hypertonic saline breathing treatments. Apart from the intense, ongoing debate over HSJN’s complete mechanism of action [3, 6, 9, 10], relative to albuterol [11] and arformoterol [12], little has been reported about HSJN. By extension, the scientific and medical literature apparently have yet to address the question of drug delivery—quantifying the amount of NaCl escaping the nebulizer cup. Our objective for this study was to determine drug output as a patient undergoes a typical HSJN breathing treatment.

Herein, we report, via laboratory simulation, pre- and post-nebulization NaCl mass differences via conductivity measurement using a portable, medical-grade air compressor. The term “typical” as used in this paper is defined as 5.0 min. This definition is entirely semantic and is used primarily to help clarify language. Moreover, a 5-min breathing treatment reflects recent literature [13] describing patient preference for shorter nebulization times.

¹Central Alabama Community College, Alexander City, AL
²KeBa, LLC, Research Division, Dadeville, AL
³Neurology Consultants of Montgomery, Montgomery, AL
Correspondence: Jeremy M. Carr, Chemistry Department Chair, Central Alabama Community College, 1675 Cherokee Road, Alexander City, AL, 35010, United States of America. Tel: +1 256-215-4342, Email: jcarr@cacc.edu.

Published online at https://www.cjrt.ca on 8 February 2022

This open-access article is distributed under the terms of the Creative Commons Attribution Non-Commercial License (CC BY-NC) (http://creativecommons.org/licenses/by-nc/4.0/), which permits reuse, distribution and reproduction of the article, provided that the original work is properly cited and the reuse is restricted to noncommercial purposes. For commercial reuse, contact editor@csrt.com
Diluted to mark, capped, and inverted 21 times to ensure homogeneity. Analyte solutions were prepared by pouring ~8 mL aliquots into 2 cm × 15 cm glass test tubes.

Solution analysis

All solution conductivities were analyzed via conductivity probe (Vernier, model CON-DIN) and data collected using Logger Lite software (version 1.9.4) under gentle agitation until the measured value remained unchanged for 15 s. Each sample was measured in triplicate, and the average value was utilized in this study. Initial investigations indicated that measured conductivities in excess of 190.3 µS cm⁻¹ and below 2.6 µS cm⁻¹ accurately correlated with solution concentration. Thus, samples in excess of 190.3 µS cm⁻¹ were appropriately diluted with distilled water such that the measured conductivity fell within the probe’s linear dynamic range. The conductivity probe was calibrated daily against standard solutions and did not exhibit drifting throughout this study.

Data analysis

Absolute conductivity measurements were mathematically converted to NaCl masses according to Figure 1. Although gravimetric analysis is not an ideal method for this type of study, actual laboratory mass measurement was performed in solution preparation only; all other measurements were volumetric or conductivity. References to mass throughout this paper were utilized to quantify the amount of drug a patient could potentially receive during HSJN breathing treatments.

Prior to experimentation, a calibration curve comparing measured conductivity against NaCl concentration was developed and utilized to convert between microsiemens per centimeter (µS cm⁻¹) and molarity (M) dimensions. Then, based on analyte volumes, solution concentrations were converted into moles, and subsequently into mass using the NaCl average atomic weight molar mass (58.44 g mol⁻¹). All mass values in this study were reported in milligrams (mg).

In this study, “nebulized” saline was equated with solute mass difference (ΔMass) between mean pre-nebulization (Mass₁) and post-nebulization (Mass₂) experiments as ΔMass = Mass₂ - Mass₁. Note that masses were calculated directly from conductivity measurements according to Figure 1. We assumed that the term “nebulized” referred to sodium chloride that had escaped the nebulizer cup into the laboratory. Although “escaped the nebulizer cup” does not mean “inhaled”, the generated mist is, in essence, available for patient inhalation. The quantity of NaCl a patient receives could be the subject of future, follow-up studies.

Each solution was analyzed at least 3 times to ensure that an average NaCl mass and corresponding standard deviation could be calculated. All data collected in this experiment were evaluated using Microsoft Excel 2018 (version 16.16.18). Experimental data are available for review in the Supplementary Material. In instances where relative error exceeded 5%, the data set was examined via Grubbs test to identify potential outliers with 95% confidence. These data points were removed, and averages/standard deviations were recalculated.

RESULTS

Calibration curve

The conductivity/NaCl concentration correlation for this study (Figure 2) was assumed to be linear. The resulting trendline

1 Supplementary Material is available on the journal website at: https://www.cjrta/wp-content/uploads/Supplement-cjrta-2021-055.pdf.
Predictable quantities of sodium chloride during hypertonic saline jet nebulization

The comparison between actual and predicted values (Table 3) shows that these absolute percent differences range from 2% to 16%, where the largest deviations tended to occur for the most dilute solutions, variations that are consistent with existing literature [16]. The average percent difference between the actual and predicted values in Table 3 was 9%. Collectively, these results suggest that our data plot provides a reasonable model for predicting saline output for a typical jet nebulization breathing treatment.

**DISCUSSION**

Clinical relevance

Because HSJN breathing treatments can disrupt ionic interactions within mucus gel [17], higher concentration saline solutions likely cause enhanced mucolytic properties and even improved patient outcomes. It is also well known that nebulized saline triggers coughing reflexes [17], which are beneficial to sputum clearance but could be potentially anomalous low error for our 5.0% saline solutions, standard deviations tended to increase with initial saline concentration. Based on existing literature [12], these results are comparable to similar, portable HSJN systems available for home, clinic, and hospital use.

Figure 3 also indicates that for a 5-min saline breathing treatment, the amount of NaCl escaping the nebulizer cup is predictable. For instance, the trendline in Figure 3 predicts that over a 5-min breathing treatment, a 4.0 mL sample of 5.0% saline produces 53 mg of sodium chloride. This result is 1 mg, or 2%, different from the laboratory result. The comparison between actual and predicted values (Table 3) shows that these absolute percent differences range from 2% to 16%, where the largest deviations tended to occur for the most dilute solutions, variations that are consistent with existing literature [16]. The average percent difference between the actual and predicted values in Table 3 was 9%. Collectively, these results suggest that our data plot provides a reasonable model for predicting saline output for a typical jet nebulization breathing treatment.

**TABLE 3**

Compiled experimental data showing average NaCl masses prior to and following 5.0 min of jet nebulization

<table>
<thead>
<tr>
<th>Saline concentration (wt%)</th>
<th>Pre-nebulization</th>
<th>Post-nebulization</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean NaCl mass (mg)</td>
<td>Standard deviation (mg)</td>
<td>Upper range (mg)</td>
</tr>
<tr>
<td>0.9</td>
<td>37.1</td>
<td>0.2</td>
<td>37.3</td>
</tr>
<tr>
<td>3.0</td>
<td>136</td>
<td>3</td>
<td>140</td>
</tr>
<tr>
<td>5.0</td>
<td>230</td>
<td>9</td>
<td>242</td>
</tr>
<tr>
<td>7.0</td>
<td>295</td>
<td>5</td>
<td>303</td>
</tr>
<tr>
<td>9.0</td>
<td>418</td>
<td>4</td>
<td>421</td>
</tr>
<tr>
<td>12.0</td>
<td>569</td>
<td>6</td>
<td>574</td>
</tr>
</tbody>
</table>

Note: The final shaded column shows the average difference between post-nebulization and mean pre-nebulization masses. Each experiment was performed at least three times to report an average, range, and standard deviation.

Nebulization experiments

Results from nebulization experiments appear in Table 2. Prenebulization solution conductivities gave average NaCl masses ranging from 37 mg to 569 mg with standard deviations of 0.2 mg and 9 mg, respectively. After 5.0 min of nebulization, conductivity measurements indicated that solution masses ranged from 19 mg to 457 mg with standard deviations ranging from 2 mg to 11 mg, respectively. The differences in NaCl masses between pre- and post-nebulization ranged from 19 mg to 114 mg, with corresponding standard deviations ranging from 2 mg to 12 mg, respectively.

Pre- and post-nebulization mass differences strongly correlated with initial saline concentration (Figure 3). The resulting linear trend (y = 784.43 µS cm⁻¹ M⁻¹ x + 13.155 µS cm⁻¹) exhibited a coefficient of determination of 0.993. Conductivities ranging from 172.3 to 10.3 µS cm⁻¹ correlated with saline concentrations ranging from 2.10 × 10⁻¹ to 8.16 × 10⁻³ M, respectively. Concentrations exceeding 2.10 × 10⁻¹ M were found to deviate from the established linear trend. Similarly, saline concentrations below 8.16 × 10⁻³ M gave inaccurate conductivity values. For instance, when 1.03 × 10⁻³ M saline and distilled water were both analyzed, both exhibited conductivities of 0.5 µS cm⁻¹.

Because HSJN breathing treatments can disrupt ionic interactions within mucus gel [17], higher concentration saline solutions likely cause enhanced mucolytic properties and even improved patient outcomes. It is also well known that nebulized saline triggers coughing reflexes [17], which are beneficial to sputum clearance but could be potentially anomalous low error for our 5.0% saline solutions, standard deviations tended to increase with initial saline concentration. Based on existing literature [12], these results are comparable to similar, portable HSJN systems available for home, clinic, and hospital use.

Figure 3 also indicates that for a 5-min saline breathing treatment, the amount of NaCl escaping the nebulizer cup is predictable. For instance, the trendline in Figure 3 predicts that over a 5-min breathing treatment, a 4.0 mL sample of 5.0% saline produces 53 mg of sodium chloride. This result is 1 mg, or 2%, different from the laboratory result. The comparison between actual and predicted values (Table 3) shows that these absolute percent differences range from 2% to 16%, where the largest deviations tended to occur for the most dilute solutions, variations that are consistent with existing literature [16]. The average percent difference between the actual and predicted values in Table 3 was 9%. Collectively, these results suggest that our data plot provides a reasonable model for predicting saline output for a typical jet nebulization breathing treatment.

**DISCUSSION**

Clinical relevance

Because HSJN breathing treatments can disrupt ionic interactions within mucus gel [17], higher concentration saline solutions likely cause enhanced mucolytic properties and even improved patient outcomes. It is also well known that nebulized saline triggers coughing reflexes [17], which are beneficial to sputum clearance but could be potentially anomalous low error for our 5.0% saline solutions, standard deviations tended to increase with initial saline concentration. Based on existing literature [12], these results are comparable to similar, portable HSJN systems available for home, clinic, and hospital use.

Figure 3 also indicates that for a 5-min saline breathing treatment, the amount of NaCl escaping the nebulizer cup is predictable. For instance, the trendline in Figure 3 predicts that over a 5-min breathing treatment, a 4.0 mL sample of 5.0% saline produces 53 mg of sodium chloride. This result is 1 mg, or 2%, different from the laboratory result. The comparison between actual and predicted values (Table 3) shows that these absolute percent differences range from 2% to 16%, where the largest deviations tended to occur for the most dilute solutions, variations that are consistent with existing literature [16]. The average percent difference between the actual and predicted values in Table 3 was 9%. Collectively, these results suggest that our data plot provides a reasonable model for predicting saline output for a typical jet nebulization breathing treatment.
detrimental to patients. For example, a patient with pseudotumor cerebri who has comorbid conditions requiring the use of mucolytics proposes a unique challenge. With pseudotumor cerebri, not only is the patient on a strict salt-intake limit, but symptoms are also exacerbated by triggers such as prolonged coughing [18], which will likely result in irreversible blindness if left uncontrolled. Consequently, providers must evaluate the gain of utilizing HSJN versus the risk of triggering a life-altering cough. Our findings can help clinicians precisely determine the quantity of NaCl a patient could potentially receive during a 5-min breathing treatment based on the initial saline concentration.

Colligative effects
The linear data trends offer input as to whether colligative effects influence HSJN output. Because colligative properties such as surface tension [19, 20] and viscosity [14, 21], general factors of drug-related performance [16], are directly related to solute concentration, we anticipated that these effects might influence the amount of NaCl being produced for patient inhalation [22]. However, the linear trend in Figure 2 suggests that HSJN is, at operating flow rate of 5 L min⁻¹, minimally influenced by colligative properties. If colligative properties play a role in aerosol generation, the force of compressed air acting upon the saline solution likely negates any observable effect.

Comparison/contrast with existing literature
The results from this study bear some similarities with existing literature. For example, similar studies tended to utilize homecare nebulizer setups [12], dilute samples to an appropriate concentration prior to nebulization [11] or during post-nebulization sample collection, prior to analysis [16]. However, there are novel differences between our study and existing literature. Key prior studies relied entirely upon gravimetric analysis [16, 23] to evaluate drug delivery efficiency or output droplet size. Other studies evaluated the nebulization of organic-based, spectrophotometrically active molecules [11, 12, 16]. Our use of conductivity and volumetric measurements to evaluate drug output offers the medical community an inexpensive strategy for studying HSJN reliably.

Study limitations and future work
Although our study measured NaCl outputs at 5-min intervals, the HSJN kinetic aspects—how solution conductivity (and, by extension, mass) changes over time—have yet to be addressed in the scientific and/or medical literature. Currently, our group is in the process of devising an experimental plan for evaluating this aspect of HSJN with the goal of establishing a process (i.e., reaction) order, an apparent void in the scientific and medical literature.

Another limitation of our study was the sole use of jet nebulizers with portable air compressors. One reoccurring theme throughout the literature [23, 24] was an output comparison between traditional jet nebulization versus vibrating mesh technologies. Thus, future work could involve a conductivity probe analysis that evaluates the performance of jet nebulizers versus vibrating mesh nebulized saline solutions or even metered doses.

Li et al. [25] recently opined that the physical and chemical properties of aerosols, and their generation in medical application, remain largely unevaluated and warrant further investigation. The fact that colligative properties play an apparent role in the nebulization of a solution [14, 16, 19–21] warrants how these effects influence HSJN. Although

**Table 3**

<table>
<thead>
<tr>
<th>Saline concentration (wt%)</th>
<th>Actual mean NaCl mass difference (mg)</th>
<th>Predicted mean NaCl mass difference (mg)</th>
<th>Absolute difference (mg)</th>
<th>Predicted mass percent difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>19</td>
<td>16</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>3.0</td>
<td>31</td>
<td>35</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>5.0</td>
<td>54</td>
<td>53</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>7.0</td>
<td>64</td>
<td>71</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>9.0</td>
<td>100</td>
<td>90</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>12.0</td>
<td>114</td>
<td>117</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
one obvious extension of studying these effects is to vary output flow rate, contributions from the carrier gas (i.e., air vs. pure oxygen) could also prove useful in the development of improved technologies.

Finally, although many nebulization studies are performed in vitro, our solutions were nebulized to room air rather than into an adult lung bench model [12, 23, 24]. Because mist that escapes the nebulizer cup is only available for patient inhalation, another follow-up study could precisely examine how much NaCl a patient receives during a typical breathing treatment.

CONCLUSION
We sought to determine exactly how much NaCl escaped the nebulizer cup during a 5-min HSJN breathing treatment. Pre- and post-nebulization NaCl mass differences indicated that between 19 mg and 114 mg of NaCl escaped the nebulizer cup, depending upon the initial saline concentration. The resulting regression analysis reliably predicts how much NaCl is likely to escape the nebulizer cup during a typical breathing treatment. Linearity in the data suggests that colligative properties (e.g., osmolality) minimally affect the quantity of NaCl that escaped the nebulizer cup. To our knowledge, these findings are the first quantitative report on pre- and post-nebulization NaCl mass differences during HSJN breathing treatments. The potential applications of these findings are boundless, this seminal work represents a key step in definitively determining how much NaCl a patient could potentially receive during a typical breathing treatment.

ACKNOWLEDGMENTS
The authors wish to thank Kaylen Thomas (Encompass Health Corporation) for her donation of jet nebulizer equipment.

DISCLOSURES

Contributors
MC, MJF, KDP were primarily responsible for collecting laboratory data. MC, MJF, KDP, JMC, and JMC were responsible for study design and data analysis. KEHC and MD were responsible for providing clinical applications for collected data. JMC and JMC were responsible for literature searching, manuscript preparation/review/editing, and project supervision.

Competing Interests
The authors declare no conflict of interest.

Funding
This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical Approval
Not required.

REFERENCES