

A Predictive Model To Explore The Effects Of Physicochemical Properties On Mesh-Nebulisation

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Summary

Understanding the effects of three physicochemical properties of formulations: surface tension, conductivity and viscosity, on vibrating-mesh nebulisation has previously employed a 'one variable at a time' (OVAT) approach. These formulations have used a range of components as physicochemical property modifiers and, as such, inter- and intra-literature comparisons are unsuitable for the exploration of physicochemical property interactions and their influences on nebulisation. Using Design of Experiment (DoE) methodology, a predictive model has been developed to parameterise and independently adjust the three physicochemical properties in the form of a generic formulation. The prepared formulations were hypothesised to replicate the output rate (OR) of any formulation with equivalent physicochemical properties in Vectura's FOX[®] vibrating-mesh nebuliser device. The model successfully mapped three active formulations to their parameterised generic formulations. When nebulised, two generic formulations adequately replicated the OR of their active counterparts (a protein-based formulation and a mucopolysaccharide), whereas one generic and active formulation pair (a small molecule formulation) showed a large difference in OR. Therefore, the three physicochemical properties alone were deemed unsuitable as predictors of OR for every formulation. The impact of viscosity on OR was also assessed. When comparing formulations comprising of the same components (the generic formulations), a strong linear relationship was observed. However, this relationship was not applicable to formulations comprising of differing components (the active formulations) and suggest caution should be exercised when comparing the OR of different formulations with respect to their physicochemical properties. Alternative properties, such as interfacial tension, should be explored to fully understand nebulisation and advance formulation development approaches.

Key Message

Three generic formulations were prepared to mimic the physicochemical properties: viscosity, surface tension and conductivity of three active formulations. When nebulised, they did not always show comparable output rates, suggesting alternative physicochemical properties may give a better indicator of a formulation's nebulisation performance, leading to improved formulation development.

Introduction

A fast nebulisation rate (referred to as output rate, OR) is desirable for increasing patient compliance by improving quality of life and is deemed a critical quality attribute of nebuliser performance [1]. Vibrating-mesh nebulisers (VMNs) are considered suitable for the delivery of a range of formulation technologies and molecule classes [2, 3], but achieving a suitable OR is highly dependent on the physicochemical properties of the formulation being nebulised [1, 3]. There have been several significant attempts to understand the effects of formulation properties on the nebulisation mechanism and current research highlights three physicochemical properties: viscosity, surface tension and ion concentration, as the fundamental fluid properties impacting mesh-nebulisation [1, 3, 4]. Understanding the relationship between these formulation properties and nebulisation is essential for optimising drug delivery via inhalation.

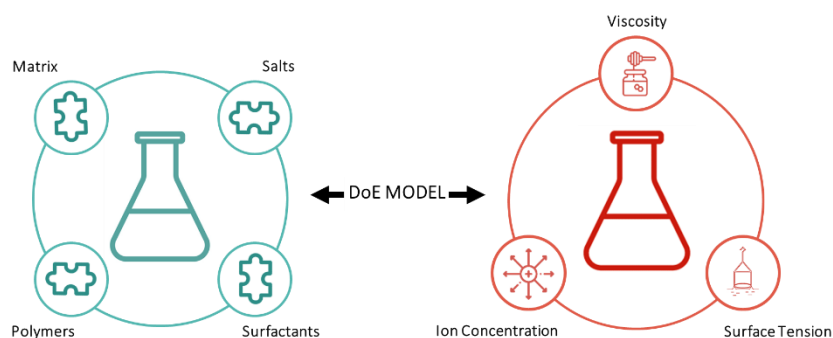


Figure 1 - The statistical model, developed using DoE, allowed for the prediction of four component proportions required to create a solution with pre-defined physicochemical properties, and vice versa.

The effects of viscosity [1, 3, 5], conductivity (or ion concentration) [3, 4, 6] and surface tension [3, 4, 7] on OR have been previously explored in literature. However, making comparisons between literature results is exceptionally challenging due to the variation between the formulation components, both within and between studies. Due to the complexity of fluid systems, and the difficulty of independently altering one physicochemical property whilst holding the others constant, the interactions between these properties have not comprehensively been assessed. Hence, the mechanism of nebulisation has not been fully described. As the identified physicochemical properties are founded in fluid intermolecular interactions, work to understand the inter-factor interactions may be hugely beneficial for mesh-nebulisation optimisation, with one author concluding that 'a greater understanding of their role in nebulization performance is a large part of the puzzle towards improved nebulization therapies' [3].

This paper aims to take the first step towards understanding this puzzle, specifically in how the aforementioned properties influence mesh-nebulisation, using Vectura's FOX[®] device. Previous studies have estimated the ranges within which successful nebulisation is expected to occur. An applied statistical approach, in a Design of Experiments (DoE) study has been used to parameterise a generic formulation containing specific components, in terms of surface tension, viscosity and conductivity, as visualised in Figure 1. The proposed parameterisation was carried out using a mixture design DoE study, an effective method in mapping the proportions of a formulation components with a mathematical constraint of summing to unity [8]. Several types of mixture design exist, each capable of dealing with the various restrictions and constraints that can arise when preparing formulations [9] and a custom-made design is often necessary to align with the aims of the experiment. A successful model has provided valuable insight into formulation nebulisation behaviour in the FOX[®] device, and will help to direct formulation development decisions to achieve successful drug/device combination products as well as acting as a tool for use in further research.

Experimental

Materials: NaCl (USP Grade) and poly(ethylene glycol) (PEG, average mol wt 8,000) were purchased from Sigma-Aldrich (Gillingham, UK). Propan-2-ol (IPA, 99.5 %) was obtained from Fisher Scientific (MA, US). Deionised water was produced in-house using a Millipore Milli-Q system (MerckMillipore, MA, US). One FOX[®] device and three vibrating-mesh nebuliser heads (NHs) were manufactured by Vectura, Chippenham, UK. The pore size of the NHs was assessed at manufacture and NHs with a volume median diameter (VMD) of $4.1 \mu\text{m} \pm 0.1 \mu\text{m}$ were selected.

Preparation of Solutions: NaCl (10% w/v) and PEG (10% w/v) Stock Solutions: 10.00 g (± 0.01 g) of solid material was dissolved in 100 mL of deionised water. Generic Formulations: Each fluid component was added volumetrically, according to the proportions calculated by JMP Software, and inverted several times to ensure thorough mixing.

General Physicochemical Characterisation: All measurements were performed at room temperature ($21^\circ\text{C} \pm 1^\circ\text{C}$) and the results were calculated as a mean of three measurements. Where this differs, an additional statement is added. **Viscosity:** Viscosity measurements were carried out using an m-VROC Viscometer (Rheosense, CA, USA) at $25^\circ\text{C} (\pm 0.15^\circ\text{C})$. Samples were measured at a rate to obtain 20-90 % full-scale pressure and the apparent dynamic viscosity was recorded. **Surface Tension:** Surface tension measurements were conducted with a Sigma 700 Force Tensiometer (Biolin Scientific, Gothenburg, Sweden), using a de Noüy ring method. The Sigma 700 Force software package was used to calculate the dynamic surface tension for 10 measurements. The surface tension of each formulation was calculated as the mean of the last five measurements. Prior to each analysis, the de Noüy ring was cleaned with n-hexane and dried under a flame. **Conductivity:** Conductivity measurements were performed using a conductivity probe (Mettler Toledo, Leicester, UK). Each formulation was decanted into three vials. Upon insertion of the probe, the system was stirred for several minutes before a reading was taken.

General Aerosol Characterisation: All aerosol testing was carried out in triplicate, with a 1 mL fill volume and a flow rate of $15.00 \pm 0.5 \text{ L min}^{-1}$. All experiments were conducted at room temperature ($21^\circ\text{C} \pm 1^\circ\text{C}$), with a relative humidity between 40-60 %. **Output Rate Determination:** As the FOX[®] device is breath-actuated, a simulated breath was required and set for a four second duration. The delivered dose was determined gravimetrically by taking the difference between the mass of the assembled device prior and post-nebulisation. The number of breaths were recorded as the number of simulated breaths before the device empty detected and automatically turned off. The output rate was calculated using Equation 1.

$$\text{OR (g min}^{-1}\text{)} = \frac{\text{Dose delivered (g)} * 60 \text{ s min}^{-1}}{(\text{Nebulisation duration per breath (s)} * \text{Number of breaths})}$$

(1)

Experimental Design (DoE): The response variables were defined as the three physicochemical properties known to influence successful nebulisation: surface tension, viscosity and conductivity. The range of the design space was determined by previous internal research. A constrained mixture design was established with the components and response variables. The DoE platform provided with the statistical software, JMP (SAS Institute) and all experiments were randomised. The proposed design comprised of 12 experimental generic formulations, including one centre point to identify two-way interactions and three replicate points to allow analysis of lack of fit. For each response variable, all experiments were conducted on the same day and as such, further design modifications to estimate day-to-day variance was considered unnecessary. **Model Analysis:** A regression model was fit to the experimental data for each response variable, using stepwise multiple linear regression with an Akaike Information Criterion corrected for a small sample size (AICc) to select the best model. Verification of each model was carried out with an additional 15 generic formulations using physicochemical properties across the known range of successful nebulisation. The proportions required for each verification experiment were generated using the model. The actual and predicted physicochemical properties were compared to the calculated model prediction intervals to assess model accuracy. **Model Use:** Three formulations containing active drug substances were identified and their physicochemical properties were mimicked using the developed model. The generic formulations were prepared, and the physicochemical properties and nebulisation characteristics of each were compared to internal data on the active counterparts. **Statistical Analysis:** Data is presented as mean ($\pm$ S.D.). Statistics were prepared using JMP software and significance was determined to the $p < 0.05$ confidence interval.

Results and Discussion

A model was designed and verified to predict the proportions of four components (10 %w/v NaCl, 10 %w/v PEG, IPA and deionised water) required to produce a 'generic formulation' of pre-specified values of the identified physicochemical properties (surface tension, viscosity and conductivity). The accepted predictive model was used to mimic the physicochemical properties of three inhalation formulations containing an active substance. The active formulations were selected so as to cover some classes of drug molecules that are commonly nebulised and are described as a mucopolysaccharide (MPS), a small molecule (SM) and protein-based formulation (PBF).

It was established that the three generic formulations were acceptable matches to the physicochemical properties of their active counterparts, the results of which are summarised in Table 1.

Table 1: Physicochemical properties of formulation pairs (a – data obtained from internal Vectura reports).

	Viscosity /mPa s ($\pm$ S.D)	Surface Tension /mN m ⁻¹ ($\pm$ S.D)	Conductivity /μS cm ⁻¹ ($\pm$ S.D)
Active MPS	2.2 ^a	49 ^a	23200 ^a
Generic MPS	2.1 (0.0)	47 (0)	27600 (0)
Active SM	1.2 ^a	~60 ^a	3570 ^a
Generic SM	1.2 (0.0)	61 (0)	4600 (0)
Active PBF	1.6 ^a	48 ^a	44640 ^a
Generic PBF	1.5 (0.0)	45 (0)	45700 (0)

The literature suggests that the three selected physicochemical properties are the most important in nebulisation and it was expected that generic formulations would perform comparably to the actives in the FOX[®] device. OR assessment of each generic and active formulation pair was carried out and the comparisons are shown in Figure 2. There is no discernible difference between the average output rates of the MPS pair, however variability in performance observed between them was noted as being potentially different, and the small disparity (0.03 g min⁻¹) between the PBF pair is not acknowledged as unusual for the vibrating-mesh nebuliser device. A larger sample size is required to assess the significance of these results.

Conversely, the output rates of the active and generic SM formulations show a considerable unexplained difference (0.41 g min⁻¹) which cannot be reasonably attributed to physicochemical property differences, nor the approximation of the active formulation surface tension. The observed difference in performances suggests that, contrary to the literature, the three selected physicochemical properties are not always suitable for assessing and predicting nebulisation characteristics, despite the positive MPS and PBF results.

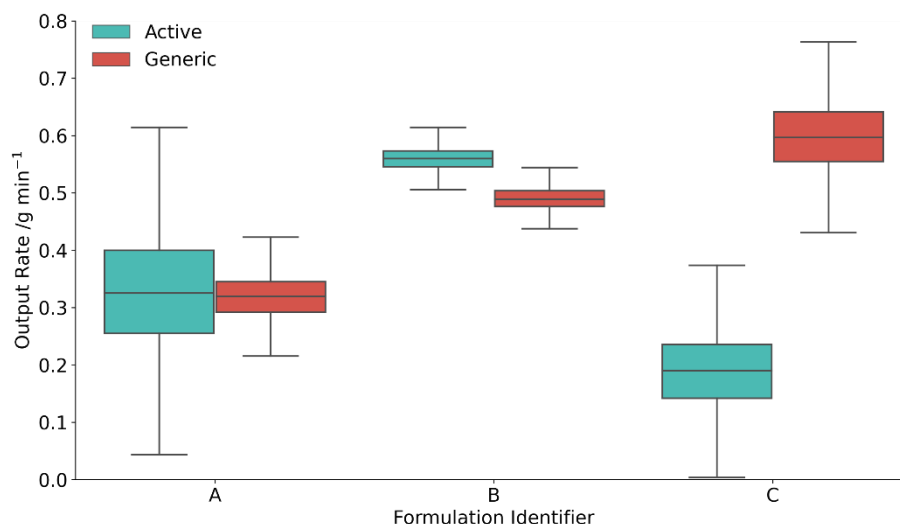


Figure 2: Output Rates for active (blue) and generic (red) formulation pairs

A possible explanation arises if the interfacial tension at the mesh/formulation interface is considered. As the surface tension is measured dynamically, the equilibrated surface of the SM active is not represented by the model and the mesh/mixture interfacial tension is not considered as a factor. As such, the model mathematics would be lacking in the necessary information required to model formulations in which a wetting or non-wetting behaviour may cause deviations in nebulisation performance. In practise, such deviations may arise when using an organic-based solvent to improve solvation or a modifier that changes orientation of molecules at the liquid/mesh interface, altering the surface composition at equilibrium. These deviations should be acknowledged when attempting to compare the nebulisation of multi-component formulations, as it is apparent the OR of one formulation (with specified physicochemical properties) may not be useful in guiding the development of a second formulation with differing components.

Conclusion

A predictive model, developed with a DoE methodology, can parameterise a generic formulation with four components in terms of three physicochemical properties: surface tension, conductivity, and viscosity. This model was used to successfully mimic the physicochemical properties of three active formulations and further development will be a useful tool in exploring nebulisation characteristics and deviations thereof. The generic formulations were able to replicate output rate, a key factor in assessing nebulisation success, in two of three instances. One active formulation was poorly represented by its generic equivalent and the measured physicochemical properties were postulated to be aliasing more complex interfacial interactions. These interactions may be better characterised by measuring properties, such as interfacial tension at the formulation/mesh interface. This model also illustrates how formulations with differing components should be compared with caution, especially in formulation development, where modifications for the purposes of stability or solubility can drastically alter nebulisation performance regardless of similarities between the selected physicochemical properties. An alteration of this model, encompassing excipients employed in innovated inhaled pharmaceutical products as the components, may allow for increased efficiency for formulation development for further complex formulations for nebulisation.

References

- 1 T. C. Carvalho and J. T. McConville, *J. Pharm. Pharmacol.*, 2016, 68, 556–578.
- 2 R. Respaud, D. March and C. Parent, *mAbs*, 2014, 6, 1347–1355.
- 3 T. Ghazanfari, A. Elhissi, Z. Ding and Kevin. Taylor, *Int. J. Pharm.*, 2007, 339, 103–111.
- 4 M. Najlah, A. Vali, M. Taylor, B. T. Arafat, W. Ahmed, D. A. Phoenix, K. M. G. Taylor and A. Elhissi, *Int. J. Pharm.*, 2013, 456, 520–527.
- 5 A. Hibbitts, L. Tajber and R. MacLoughlin, in *Int Soc Aerosol Med. 19th International Congress*, 2013.
- 6 T. C. Carvalho, J. P. McCook, N. R. Narain and J. T. McConville, *J. Liposome Res.*, 2013, 23, 276–290.
- 7 M. Beck-Broichsitter, M.-C. Knuedeler, N. Oesterheld, W. Seeger and T. Schmehl, *Int. J. Pharm.*, 2014, 459, 23–29.
- 8 D. Montgomery, *Design and Analysis of Experiments*, Wiley, 8th edn., 2013.
- 9 Y. Buruk Sahin, E. Aktar Demirtaş and N. Burnak, *PAJES*, 2016, 22, 297–304.