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Modeling and optimization of nebulizers' performance in non-invasive ventilation using different fill volumes: Comparative study between vibrating mesh and jet nebulizers

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ABSTRACT

Backgrounds: Substituting nebulisers by another, especially in non-invasive ventilation (NIV), involves many process-variables, e.g. nebulizer-type and fill-volume of respirable-dose, which might affect patient optimum-therapy. The aim of the present work was to use neural-networks and genetic-algorithms to develop performance-models for two different nebulizers.

Methods: In-vitro, ex-vivo and in-vivo models were developed using input-variables including nebulizer-type [jet nebulizer (JN) and vibrating mesh nebulizer (VMN)] fill-volumes of respirable dose placed in the nebulization chamber with an output-variable e.g. average amount reaching NIV patient. Produced models were tested and validated to ensure effective predictivity and validity in further optimization of nebulization process.

Results: Data-mining produced models showed excellent training, testing and validation correlation-coefficients. VMN showed high nebulization efficacy than JN. JN was affected more by increasing the fill-volume. The optimization process and contour-lines obtained for in-vivo model showed increase in pulmonary-bioavailability and systemic-absorption with VMN and 2 mL fill-volumes.

Conclusions: Modeling of aerosol-delivery by JN and VMN using different fill-volumes in NIV circuit was successful in demonstrating the effect of different variable on dose-delivery to NIV patient. Artificial neural networks model showed that VMN increased pulmonary-bioavailability and systemic-absorption compared to JN. VMN was less affected by fill-volume change compared to JN which should be diluted to increase delivery.

1. Introduction

Modeling and optimization of multivariate and complex domains require use of sophisticated mathematical and statistical models and the results are not always easy to interpret. Artificial neural networks (ANNs) belong to data mining technology and are considered a powerful tool to model and optimize these kinds of data with fast and easy interpretation of the results. In many studies previously performed in the field of pharmaceutical development, data mining technology in the form of artificial neural networks and neurofuzzy logic were successfully applied for modeling, optimization and prediction of formulation and/or in-vitro/in-vivo performance of various dosage forms and medical devices [1–3]. It is well known that ANNs superseded conventional mathematical and statistical modeling methodologies, for their ability to model non-linear data and unnecessary establishment of equations to describe relationship between input and output variables [4]. In addition to the above advantages, ANNs can be used for evaluation of historical data and new models can be updated with added new experiments [5]. In similar studies, ANNs were also applied for building predictive models to evaluate the relationship between in-vitro aerosol characteristics and pulmonary bioavailability of inhaled drugs [6,7].

In our previously published work, data mining was employed in many area related to drug delivery e.g. dry powder inhaler delivery [8];

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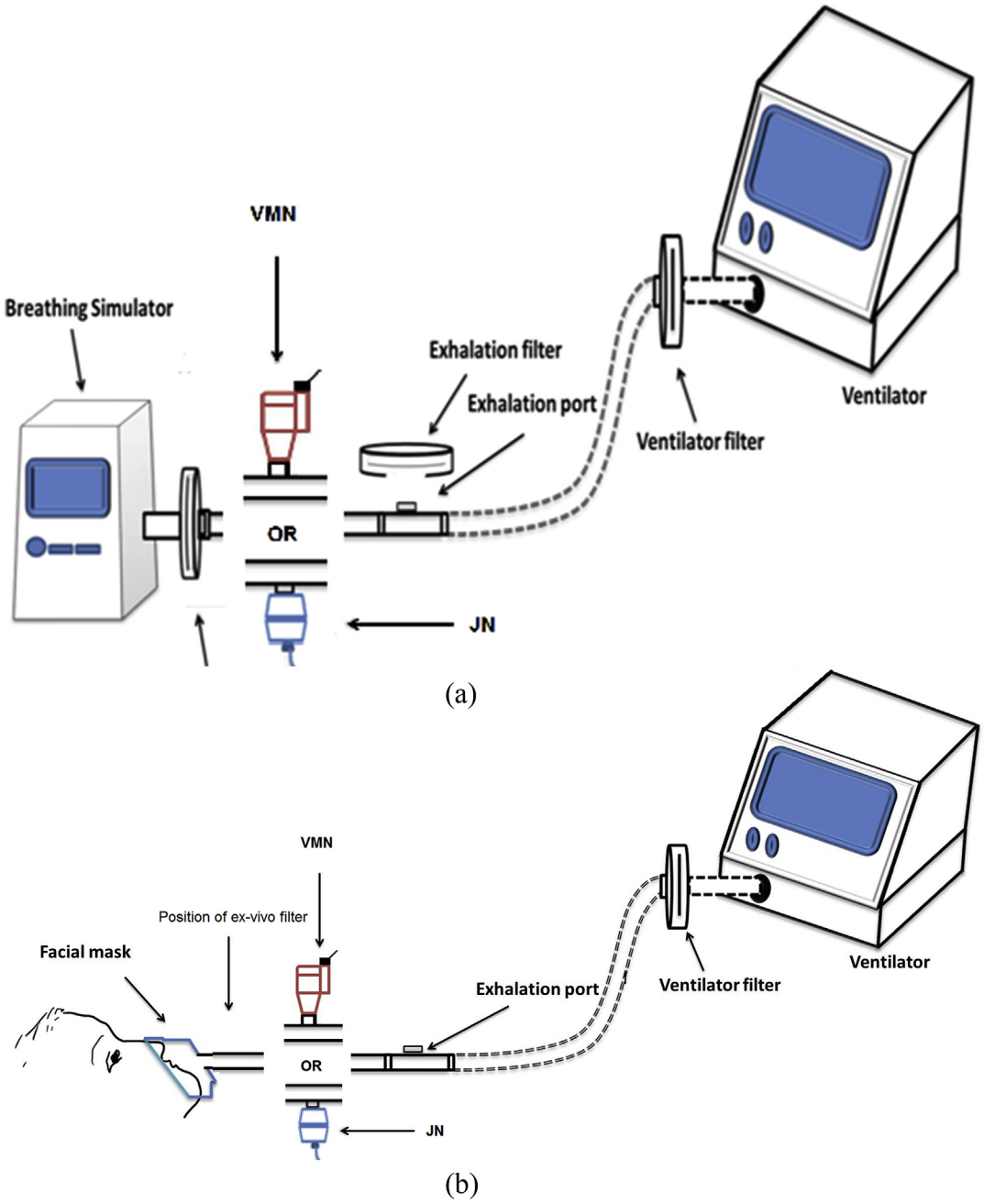

Fig. 1. Schematic design of the nebulizers positions within the non-invasive ventilation circuit. (A) In-vitro setting to determine the fate of the aerosolized dose. (B) In vivo and ex-vivo setting. The inspiratory filter was placed between the patient and nebulizer in the ex-vivo part of the study only.

Table 1
Mean (SD) demographic data of the 4 groups.

	Age (Years)	Wight (kg)	Height (cm)
Group 1	62.3 (6.0)	70.7 (7.9)	173 (4.7)
Group 2	65.0 (5.3)	74.4 (8.5)	170.6 (7.2)

different formulation preparation and optimization [9,10] and they proved to be an effective tool. We extend the use of such a methodology to optimize metered dose inhaler (MDI) delivery with spacers [2] and vibrating mesh nebulizers delivery [1] in non-invasive ventilation (NIV) and again they proved to be an effective and good tool. The modeling study of the VMNs recommended inclusion of other variables in the NIV circuit to optimize the model e.g. nebulizer type and fill volume of the respirable solution.

Hence, this study aimed to evaluate the effects of nebulizer type, fill volume of the respirable solution, placed in the nebulization chamber, on effective drug delivery. The modeling and optimization was carried out using ANNs and neurofuzzy logic based data mining technology.

2. Materials and methods

2.1. Experimental method

Study consisted of three models (in-vitro, ex-vivo and in-vivo) using two type of nebulizers, vibrating mesh nebulizer (VMN, Aerogen Solo, Aerogen Limited, Ireland) and the Oxycare jet nebulizer (JN, Ceren Uretim A.S., Istanbol, Turkey) attached to a compressor (PortaNeb, Philips Respironics, UK) set at 6 L/min. The three parts of study was

Table 2
Fate of emitted dose across compartment in μg (SD) with total fill volumes of 1, 2 and 4 mL with vibrating mesh nebulizer (VMN) and jet nebulizer (JN); n = 10.

Mean (SD)	VMN			JN		
	1 mL	2 mL	4 mL	1 mL	2 mL	4 mL
Inhalation filter	1691.1 (266.3)	1922.9 (207.4)	1946.0 (157.7)	685.2 (381.8)	1095.8 (166.9)	1338.8 (42.1)
Exhalation filter (fugitive aerosol)	2175.4(342.6)	2473.5(266.8)	2503.4(202.9)	881.5(491.2)	1409.6(214.6)	1722.2(54.2)
T-piece and ventilator tubing	314.7(100.4)	665.5(332.4)	862.3(147.2)	523.3(259.0)	564.3(444.8)	686.4(348.4)
Nebulization chamber	529.0(352.4)	257.2(76.9)	252.5(128.4)	2927.9(887.7)	2259.1(348.1)	1630.1(243.1)
Nebulization time	2.33 (0.07)	4.29 (0.07)	9.61 (0.30)	4.55 (0.30)	13.55 (0.30)	20.67 (5.87)

Table 3
Mean (SD) USAL0.5, USAL24 and TID in μg and nebulization times in minute using different fill volume.

Nebulizer type	Fill volume	USAL 0.5 (μg)	USAL 24 (μg)	Ex-vivo (μg)	Time (minute)
JN	1 ml	28.3(7.6)	146.3(42.3)	792.9(130.7)	3.42(0.08)
	2 ml	47.9(10.6)	429.5(106.9)	1395(152.2)	6.86(0.42)
VMN	1 ml	107.9(28)	542.1(88.4)	1480.9(116.5)	2.29(0.08)
	2 ml	134.6(44.7)	599.6(60.9)	1690.8(156.0)	4.22(0.7)

Table 4
Sample of modelling input and output variables for the *in-vitro* model.

Run	Nebulizer type	Fill volume (mL)	Inhal. filter (μg)	Exhal. filter (μg)	Tubing	Amount in nebulizer (μg)
1	0.1	1.0	1345.55	1730.90	379.99	611.50
2	0.1	1.0	1443.69	1857.15	214.34	1051.25
3	0.1	1.0	1402.89	1804.66	313.41	981.18
4	0.1	1.0	1569.59	2019.10	224.22	960.11
[a]5…	0.1	1.0	1762.46	2267.22	142.17	467.75
1	0.2	1.0	347.39	446.88	749.18	1968.56
2	0.2	1.0	337.63	434.32	614.81	1467.17
3	0.2	1.0	683.10	878.73	773.24	1569.84
4	0.2	1.0	576.02	740.99	817.26	3412.54
5…	0.2	1.0	501.41	645.01	759.11	3604.50
1	0.1	2.0	1646.24	2117.71	1206.21	419.77
2	0.1	2.0	1443.70	1857.16	1117.56	337.71
3	0.1	2.0	2081.70	2677.88	703.20	195.64
4	0.1	2.0	2008.73	2584.01	473.34	263.63
[a]5...	0.1	2.0	2029.16	2610.29	895.01	295.80

[a] The number of records is continuous to 10 runs for each type of nebulizer and fill volume.

Table 5
Sample of modeling input and output variables for the *Ex-vivo* model.

Patient	Nebulizer type	Fill volume (mL)	Inhalation filter (μg)	Ex-vivo (μg)
1	0.1	1.0	1691.08	1454.32
2	0.1	1.0	1691.08	1553.44
3	0.1	1.0	1691.08	1450.44
4	0.1	1.0	1691.08	1668.10
*5..	0.1	1.0	1691.08	1459.97
1	0.1	2.0	1922.86	1698.50
2	0.1	2.0	1922.86	1695.62
3	0.1	2.0	1922.86	1637.25
4	0.1	2.0	1922.86	1763.99
5..	0.1	2.0	1922.86	1798.69
1	0.2	1.0	685.21	586.90
2	0.2	1.0	685.21	893.81
3	0.2	1.0	685.21	699.81
4	0.2	1.0	685.21	866.72
5..	0.2	1.0	685.21	706.10

conducted in dry non-humidified ventilation circuit. Placing the nebulizer near the subject between the face-mask and the expiration port was previously shown to produce a greater aerosol delivery with less aerosol loss through the expiration port [11,12]. Therefore, we placed both nebulizers in the above-mentioned position.

The schematic design of the in-vitro bench model setting and nebulizers positions within the non-invasive ventilation circuit are presented in Fig. 1 A. For in-vitro model JN and VMN filled with 3 different volumes (1, 2 and 4 mL, with normal saline as diluent) containing 5000 μg salbutamol respirable solution (Farcolin respirator solution, 5000 μg ml^{-1}; Pharco Pharmaceuticals, Egypt), nebulizers were attached to its T-piece which was attached from one side to mechanical ventilator (Nippy2, B&D Electromedical, UK), set in spontaneous mode with a peak inspiratory pressure (PIP) 20 cmH_2O and a peak expiratory pressure (PEP) 5 cmH_2O, and a 180 cm single limb NIV circuit connected to a breathing simulator (5600i, Michigan Instruments, Germany) from the other side, with inhalation to exhalation ratio 1:3, 15 breaths per minute, tidal volume 500 mL. An inhalation filter (Pari GmbH, Germany) was placed before breathing simulator to collect amount of drug that would be delivered to patient lung, also an exhalation filter placed 4 cm from expiratory port for collection of expired amount of drug.

Each fill volume was tested 10 times (n = 10). Amount of salbutamol was collected from inhalation, exhalation filters, T-piece and nebulizer chamber. Amount of salbutamol quantified using high performance liquid chromatography (HPLC) [1].

The schematic design of the in-vivo and ex-vivo model setting and nebulizers positions within the non-invasive ventilation circuit are presented in Fig. 1 B. The study was conducted in accordance with amended Declaration of Helsinki. Local institutional review boards and independent ethics committees approved protocol, and written informed consent was obtained from all patients. Subjects were ineligible to be included in this study if they had taken part in research study during previous 6 months, had known hypersensitivity to salbutamol, systolic blood pressure of < 100 mmHg or severe renal impairment defined as Creatinine Clearance or eGFR of < 20 ml min^{-1}.

For in-vivo model a 24 (12 females) NIV patients were included in the study and divided to two groups (12 each). For each group, one nebulizer of the two nebulizers studied was used with two fill volumes (1 and 2 mL, with normal saline as diluent) containing 5000 μg salbutamol respirable solution in days one and three of the study. Urine samples were collected 30 min (as an index of lungs deposition) after nebulization and urine pooled for next 24hr (as an index of systemic absorption) [13].

Ex-vivo study was carried out in day two, using the same experimental setting with, by placing an inhalation filter before face mask for collection of salbutamol before reaching patient lung. All in-vivo and ex-vivo samples were analyzed using HPLC [1].

Table 6
Sample of modeling input and output variables for the *in-vivo* model.

Patient	Nebulizer type	Fill volume	Inhalation filter	D/urine 30min	D/urine 24 h
1	0.1	1.0	1691.08	88.55	427.16
2	0.1	1.0	1691.08	108.67	597.08
3	0.1	1.0	1691.08	153.99	431.16
4	0.1	1.0	1691.08	91.54	511.85
*5..	0.1	1.0	1691.08	96.68	487.87
1	0.1	2.0	1922.86	105.25	672.38
2	0.1	2.0	1922.86	120.13	703.29
3	0.1	2.0	1922.86	131.89	572.63
4	0.1	2.0	1922.86	211.62	646.24
5..	0.1	2.0	1922.86	85.09	566.19
1	0.2	1.0	685.21	18.73	120.78
2	0.2	1.0	685.21	36.17	114.26
3	0.2	1.0	685.21	28.61	84.94
4	0.2	1.0	685.21	28.12	227.64
5..	0.2	1.0	685.21	24.12	104.32

2.2. Modeling of the data using artificial neural networks

In this study modeling and optimization of the in-vitro, ex-vivo and in-vivo data were undertaken using artificial neural networks (ANNs)-Genetic algorithm software package (INForm V3.71, Intelligensys Ltd., UK) [14]. The experimentally collected data set for each model (in-vitro, ex-vivo and in-vivo) was divided into training records (80%), testing records (10%) and validation records (10%) for model training testing and validation, respectively. Model success in predictability was evaluated using the correlation coefficient (R^2) values computed automatically during training and testing as well as validation steps. High R^2 values closer to unity indicate appropriate predictability of the trained model and confirm its trustability [10].

The value of R^2 describes how much of the variance of the dependent variable is accounted for in the model. The root mean squared errors (RMSE) were also calculated and compared for model training, testing and validation. The relationship between each of the independent variables (inputs) and each of the dependent ones (outputs) were explained by 3D response surface plots [15]. The in-vitro performance of the nebulizers was modeled using 60 data records. The input variables included; nebulizer type, run number (1–10), drug respirable solution fill volume (1, 2 or 4 mL). The output properties included; dose collected on the inhalation filter (Inhal-fiter in μg), the exhalation filter (Exhal-filter in μg), dose precipitated in Tubing (μg) and dose remained inside the nebulization chamber (μg). Each nebulizer was given a numeric code to be included in the modeling and differentiate the two types as follows; VMN (0.1) and JN (0.2).

For ex-vivo evaluation of the nebulizers, the amount of salbutamol collected on the filter before reaching the face mask of the patients (ex-vivo) was modeled as the output property. The input variables included; patient number, nebulizer type and average collected emitted dose from the nebulizer on the inhalation filter (Inhal-filter). The ex-vivo study for patients (n = 24) using two nebulizers and 2 fill volumes (1 and 2 mL) reached 48 records. The same codes 0.1 and 0.2 were given to the VMN and JN, respectively, as mentioned above.

Modeling of the in-vivo study also included 48 records with similar inputs used for the ex-vivo study and the outputs being amount of drug collected in urine after 0.5 h (D/Urine 30min) as an index of pulmonary bioavailability and after 24hr (D/Urine 24hr) as an index of systemic absorption [13].

2.3. Model optimization

The performance of the nebulizers in the three models (in-vitro, ex-vivo and in-vivo) was optimized using the software model optimization window after setting the desired values for each property and the model optimization function as “Tent”.

Table 7
In-vitro model training and testing ANOVA statistics for each output property.

Output property	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	[a]RMSE	Computed f ratio
Inhalation Filter (μg)	Model	12914800.00	21.00	614991.00	784.21	19.79
	Error	1025490.00	33.00	31075.30	176.28	
	Total	13928400.00	54.00			
	Covariance term	Sum of Errors				
	11887.90	2.15				
	Train Set R^2	92.64%				
	Test Set R^2	94.36%				
Exhalation-Filter (μg)	Model	21357500.00	21.00	1017030.00	1008.48	19.84
	Error	1691890.00	33.00	51269.30	226.43	
	Total	23048800.00	54.00			
	Covariance term	Sum of Errors				
	654.24	4.20				
	Train Set R^2	92.66%				
	Test Set R^2	94.54%				
Tubing (μg)	Model	4247470.00	21.00	202261.00	449.73	3.97
	Error	1682380.00	33.00	50981.10	225.79	
	Total	5930000.00	54.00			
	Covariance term	Sum of Errors				
	148.109	2.59632				
	Train Set R^2	71.63%				
	Test Set R^2	32.11%				
Nebulizer (μg)	Model	64395400.00	21.00	3066450.00	1751.13	42.32
	Error	2391150.00	33.00	72459.10	269.18	
	Total	66764300.00	54.00			
	Covariance term	Sum of Errors				
	22195.5	0.99				
	Train Set R^2	96.42%				
	Test Set R^2	94.77%				

[a] RMSE: Root mean squared error.

Table 8
Model training and testing ANOVA statistics for the ex-vivo and in-vivo models.

Output property	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	[a]RMSE	Computed f ratio
Ex-vivo (μg)	Model	5101260.00	19.00	268487.00	518.16	14.63
	Error	440470.00	24.00	18352.90	135.47	
	Total	5651250.00	43.00			
	Covariance term	Sum of Errors				
	109523	32.76				
	Train Set R^2	92.21%				
	Test Set R^2	77.25%				
Drug in urine/30min (μg)	Model	88247.10	19.00	4644.58	68.15	4.18
	Error	26697.50	24.00	1112.40	33.35	
	Total	115000.00	43.00			
	Covariance term	Sum of Errors				
	55.13	0.26				
	Train Set R^2	76.78%				
	Test Set R^2	95.76%				
Drug in urine/24hr (μg)	Model	1471090.00	19	77426	278.26	10.34
	Error	179655	24	7485.62	86.52	
	Total	1651230.00	43			
	Covariance term	Sum of Errors				
	482.72	0.97				
	Train Set R^2	89.12%				
	Test Set R^2	77.70%				

[a] RMSE: Root mean squared error.

Table 9
Model validation ANOVA statistics for the in-vitro performance of the nebulizers.

Output property	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	*RMSE	Computed f ratio
Inhalation Filter (μg)	Model	1011050.00	21.00	48145.20	219.42	7.36
	Error	111182.00	17.00	6540.11	80.87	
	Total	1503560.00	4.00			
	Covariance term	Sum of Errors				
	381332.00	255.83				
	Validation R^2	92.61%				
Exhalation-Filter (μg)	Model	1671010.00	21.00	79572.00	282.09	7.13
	Error	189696.00	17.00	11158.60	105.63	
	Total	2488100.00	4.00			
	Covariance term	Sum of Errors				
	627394.00	328.78				
	Validation R^2	92.38%				
Tubing (μg)	Model	68021.50	21.00	3239.12	56.91	8.18
	Error	6733.80	17.00	396.11	19.90	
	Total	67818.60	4.00			
	Covariance term	Sum of Errors				
	6936.68	4.8013				
	Validation R^2	90.07%				
Nebulizer (μg)	Model	8356350.00	21.00	397922.00	630.81	91.45
	Error	73972.70	17.00	4351.34	65.96	
	Total	8245600.00	4.00			
	Covariance term	Sum of Errors				
	184729.00	194.21				
	Validation R^2	99.10%				

3. Results and discussions

The design and performance of the inhalation device should be evaluated based on its ability to systematically deliver uniform doses and maximize the inhaled drug bioavailability [16]. Nebulizers are widely used devices for aerosol deliver during NIV [17]. Low efficacy of nebulized aerosol delivery during NIV is still an obstacle to researcher and healthcare members [18]. This problem is fundamentally about the delivery of low percent of nebulized drugs to patient lungs (5–10%) [1,11,19–22]. There are many factors contributing to this problem e.g. type of aerosol generating device, fill volume, humidification conditions and others [23–28]. There are three main types of nebulizers differ basically in their operation and droplet generation mechanisms (JN, VMN, ultrasonic). They differ in their delivery efficacy [24,26,29] and so they cannot substitute each other without dose adjustment [19,21,30–34]. Such substitution was proven to result in significant change in the clinical response [35]. Here we tried to build models that can be used to optimize substitution and adjust the fill volume of respirable solution used.

3.1. Modeling of in-vitro, ex-vivo and in-vivo data

The mean (SD) demographic data of the 2 groups are presented in Table 1. Table 2 shows the fate of emitted dose across ventilation circuit

Table 10
Model Validation ANOVA statistics for the ex-vivo and in-vivo models.

Output property	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	*RMSE	Computed f ratio
Ex-vivo (μg)	Model	511450	13	39342.30	198.35	3.31
	Error	118691	10	11869.10	108.95	
	Total	447603	3			
	Covariance term	Sum of Errors				
	182538.00	70.65				
	Validation R^2	73.48%				
Drug in urine/30min (μg)	Model	6306.13	13	485.09	22.02	9.85
	Error	492.488	10	49.25	7.02	
	Total	4835.59	3			
	Covariance term	Sum of Errors				
	1963.03	34.36				
	Validation R^2	89.82%				
Drug in urine/24hr (μg)	Model	126860	13	9758.42	98.78	7.36
	Error	13255.6	10	−1325.56	36.41	
	Total	188003	3			
	Covariance term	Sum of Errors				
	47887.60	17.64				
	Validation R^2	92.95%				

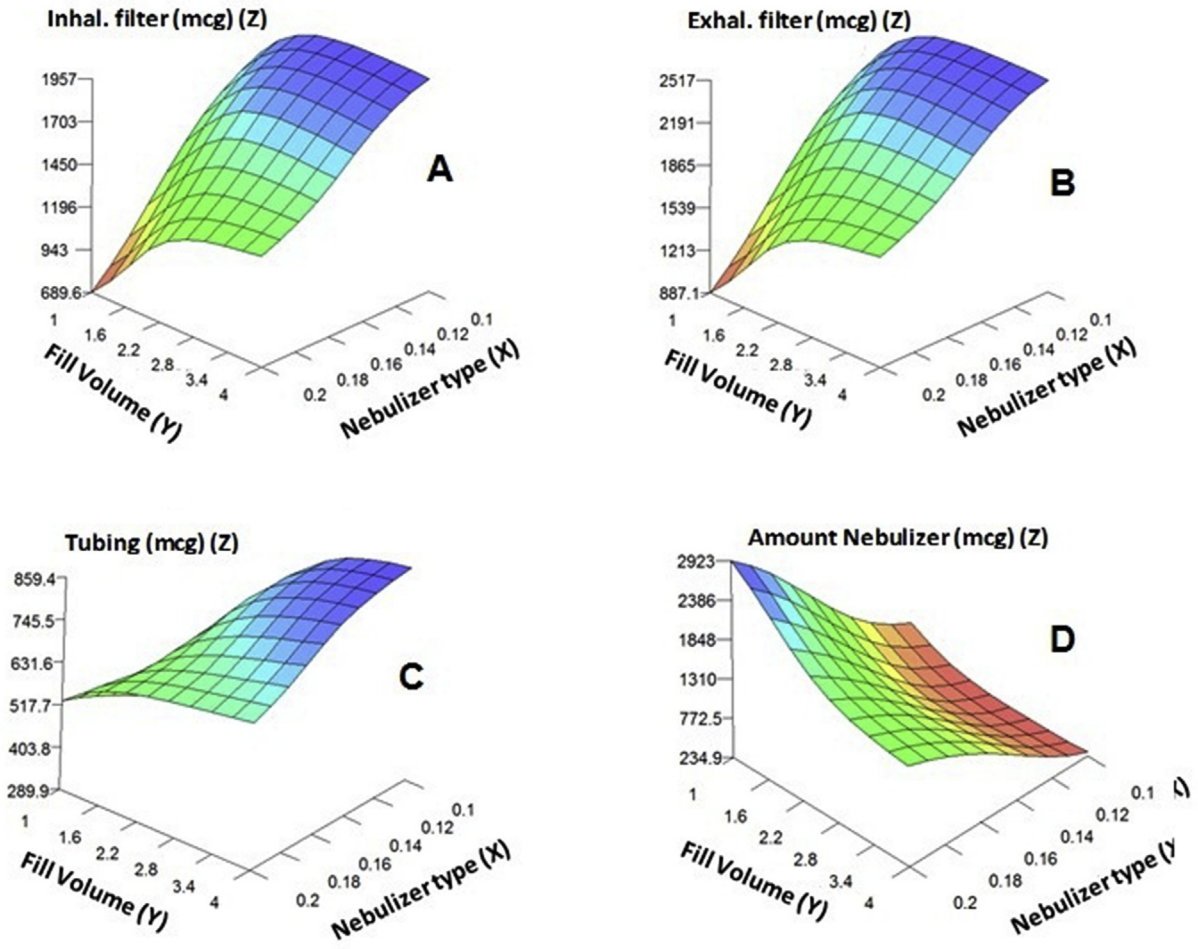

Fig. 2. Response surface plots showing effects of nebulizer type and fill volume on (A) amount of drug collected on the inhalation filter, (B) exhalation filter, (C) tubing and (D) remaining inside nebulizer chamber.

compartment in μg (SD) in the in-vitro study. Mean (SD) 30 min urine (USAL0.5) and pooled urine up to 24 h (USAL24) from the in-vivo study and the amount salbutamol deposited on the inhalation filter in the ex-vivo study (TID) are shown in Table 3.

The input and output variables for the in-vitro, ex-vivo and in-vivo data are summarized in Tables 4–6. The modeling process carried out using in-vitro data showed highly predictive model as indicated by its excellent ANOVA statistics including; high training and testing R^2 (> 90%) and F values as shown in Table 7. The only exception is the model obtained for predicting amount of drug remaining in the T-tubes

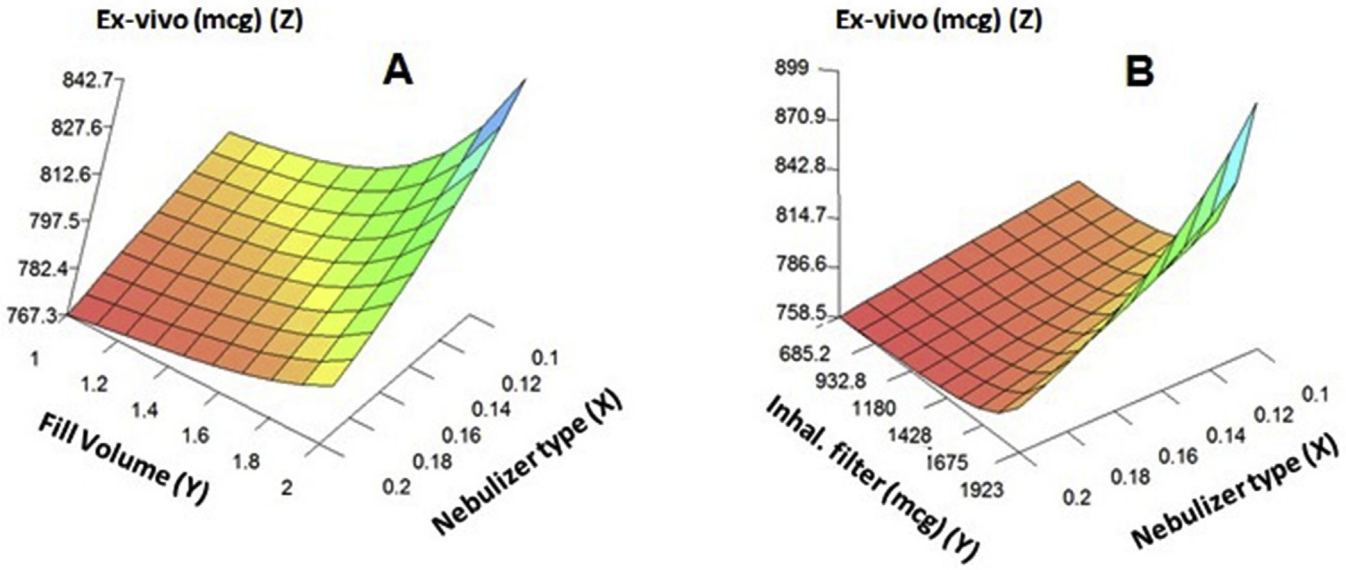

Fig. 3. Response surface plots showing effects of nebulizer type and (A) fill volume and (B) Inhalation filter on the amount of drug collected on the face mask.

(tubing) which showed a reasonable training R^2 but poor testing R^2 values due to limited scatter of the data. However, in general the obtained results were comparable to the good predictive models mentioned in our previous work and the literature in which, high R^2 values represent the high efficiency of the models to account for variability in the data [1,2]. The root mean squared errors (RMSE) values were also found to decrease from training to testing indicating that the obtained in-vitro models are trustworthy with low variability in predictions [1,2].

The model generated for the ex-vivo data (Table 8) also demonstrated good training and testing R^2 (92.21% for training and 77.25% for testing) and F values (14.63) indicated a well trusted model.

For the in-vivo models, D/Urine 30min showed good training R^2 (76.78%) and low RMSE values for model training and testing (68.15 and 33.35 respectively). D/Urine 24 h model demonstrated 89.12% R^2 and 278.26 and 86.52 RMSE values for model training and testing respectively as shown in Table 8 indicating better prediction capability of the second model.

The ex-vivo and the in-vivo models were much better than our previous models in NIV for MDI with different spacer [2] and different vibrating mesh nebulizers [1] since more variables are used here (different nebulizers and different fill volumes).

Models validation was carried out to check the ability of the generated models to predict unseen data (10% of the records). The results of validation for each of the three models demonstrated high R^2 and F values and low RMSE values, indicating validity and trustability of the models (Tables 9 and 10). These results also strongly support further use of the models in the optimization of the output properties to get the desired optimum in-vitro, ex-vivo and in-vivo attributes of the nebulization process through model suggested ideal values of nebulizers' variables [36]. However, even though the models here advise that the in-vitro bench model testing could really reproduce what would happen when NIV patient receive nebulized aerosol, the in-vitro, ex-vivo and in-vivo testing resulted in a bit different models. This would be due to the additional variables introduced in the ex-vivo and the in-vivo testing e.g. the variability in the patients' inhalation and exhalation profiles which was much higher than the breathing simulator [1,2,6,8]. Hence, introduction of actual patients' inhalation and exhalation profiles in the in-vitro testing methodology is required to decrease these variables [33,37].

3.2. Response surface plots and contour lines

The explanation of the cause-effect relationship between input and output variable are demonstrated by the response surface plots. The plots display the interaction between two variables on the output property at average levels of the third input variable.

The 3D plots obtained for the in-vitro model indicated that the amount of drug collected on the inhalation and exhalation filter were increased by increasing the nebulizer fill volume with both nebulizers but nebulizer encoded 0.1 (VMN) showing higher values at the same fill volume while level of increase of emitted drug detected for encoded 0.2 (JN) was higher than VMN as shown in Fig. 2A and B. This suggests that the fill volume change affect the JN more than the VMN. Hence, when using JN, it is better to dilute the respirable solution to deliver more drugs [24,38]. This recommendation is not valid for VMN which had a very low level of change in delivery with changing fill volume due to its low residual volume [24].

For the amount in tubing the fill volume 1 mL was better (minimum amount) for nebulizer 0.1 (VMN). It increased with 2 and 4 mL for nebulizer 0.1 (VMN) (Fig. 2 C). This could be because increasing the fill volume increased the nebulization time of the VMN which in turn increased time for aerosol to pounce and condensate on the tubing [24]. This was not seen in JN since its T-tubing was above the JN nebulization chamber so any aerosol condensates would return to the nebulization chamber to be renebulized [24].

The amount remaining in nebulizer was inversely proportional to the fill volume with nebulizer 0.2 (JN) demonstrating higher amounts than VMN (Fig. 2 D). Again this was due to the large residual volume of the JN making any dilution of the respirable solution affect the remaining drug amounts [23–26,33,34].

The ex-vivo and the in-vivo models were similar to in-vitro one but with low response. Again this would be due to the additional variables introduced in the ex-vivo and the in-vivo testing e.g. the variability in the patients' inhalation and exhalation profiles which was much higher than the breathing simulator [1,2,6,8]. Hence, introduction of actual patients' inhalation and exhalation profiles in the in-vitro testing methodology is a must to decrease these variables [33,37].

In the ex-vivo model, the amount of drug collected on the filter placed before the face mask was affected most by the fill volume, with 2 mL showing better value than 1 mL, especially with JN, and VMN being the best, and no interacting effects of the amounts collected on the inhalation filter was detected (Fig. 3A and B).

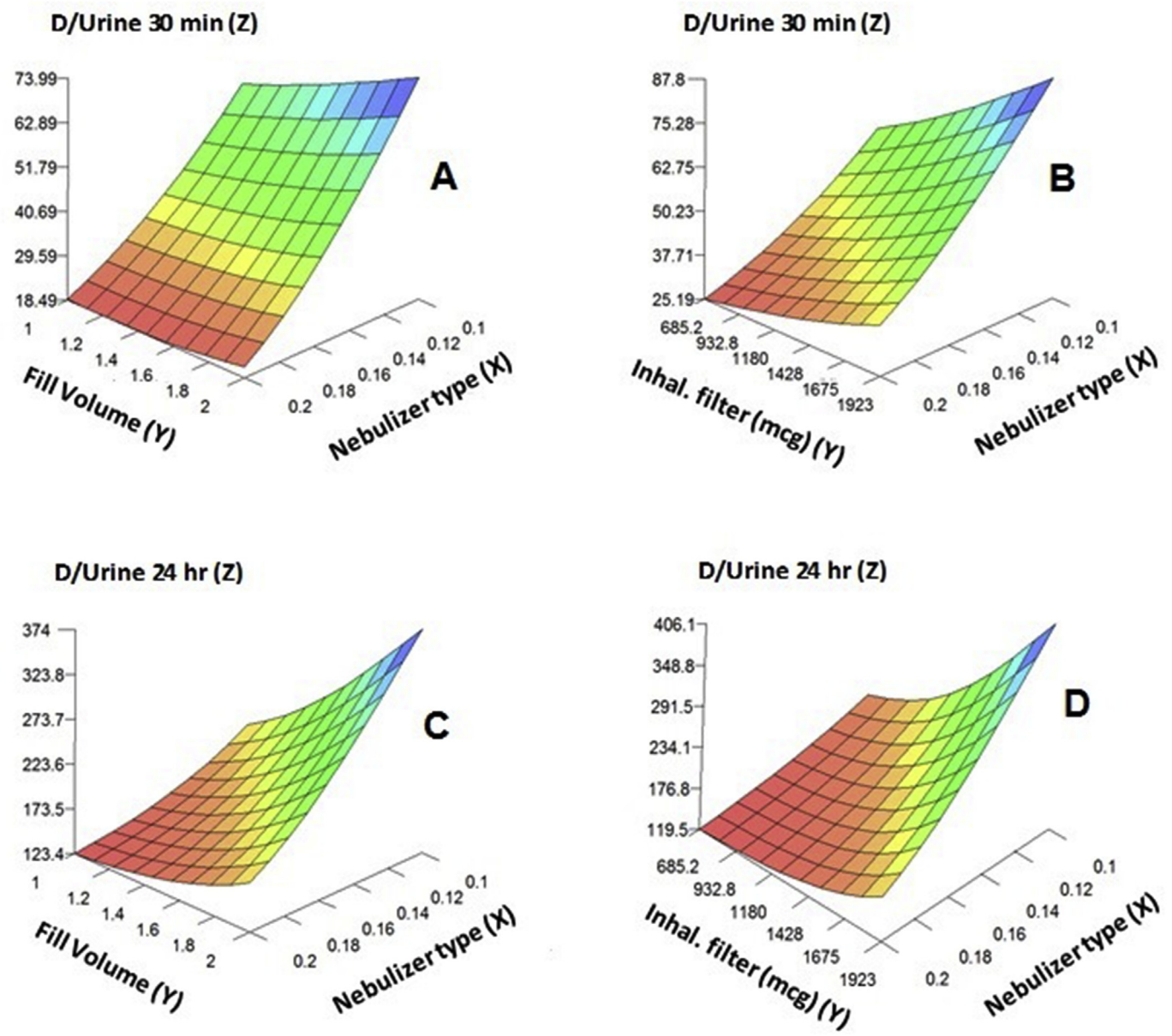

Fig. 4. Response surface plots showing effects of nebulizer type and (A) fill volume and (B) Inhalation filter on the amount of drug collected in urine after 30min and after 24hr (C & D).

The in-vivo model showed that the amount of drug in urine after 30min (D/Urine 30min) was increasing by increasing the fill volume with both nebulizers. But VMN (0.1) showed higher values than the JN (0.2) while level of increase of D/Urine 30min for JN was higher than VMN by increasing the fill volumes shown in Fig. 4A and B.

The generated response surface plots for the D/Urine 24 h demonstrated that at high fill volumes (2 mL) and high amounts of drug on the inhalation filter, maximum D/Urine 24 h was obtained showing an obvious difference between the two types of nebulizers in favor of the VMN (Fig. 4C and D).

In Fig. 5 A the contour lines for the in-vitro model demonstrated that higher amount of the dose collected in the inhalation filter were obtained with the VMN between 1 and 2 mL fill volumes. In Fig. 5 B, the ex-vivo collected dose was higher at 2 mL fill volume with the VMN. Similar to the ex-vivo results, the in-vivo model demonstrated high collected doses in urine after 30min and 24hr from the VMN at 2 mL fill volumes. These results indicate the presence of differences between the JN and VMN with favor of the VMN which was less affected by the fill volume changes. The data of the ex-vivo and in-vivo models also suggest that increasing the fill volume higher than 2 mL will bring no additional value.

3.3. Models' optimization

The optimization process was carried out using the software optimization window in which the desired levels of each output property were entered together with an optimization function (in this case the Tent function was used). In the first model (in-vitro performance) the desired range for the amount of drug collected on the inhalation filter was set at 1900–2000 μg, 2400–2800 μg for the exhalation filter, 200–300 μg for the tubing and 100–200 for the amount remaining in the nebulizer. In the second model (ex-vivo performance) the desired range of outputs included; 1500–1700 μg for the amount collected ex-vivo on the face mask. The in-vivo model desired range for drug collected in urine after 30min was set at 150–170 μg whilst for the 24hr samples it was set at 500–700 μg.

The results of optimization for the in-vitro and ex-vivo models suggested a model proposed solution composed of a nebulizer of the

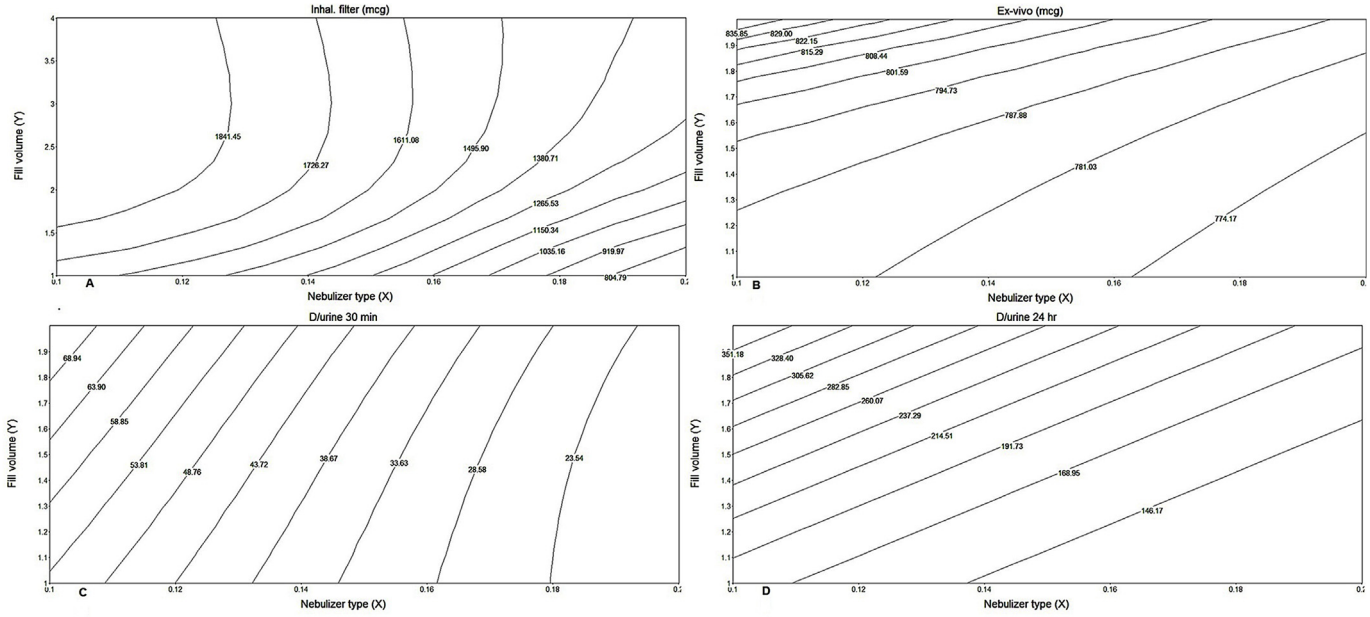

Fig. 5. Model generated contour lines for the in-vitro collected dose on (A) the inhalation filter, (B) the Ex-vivo collected dose, the in-vivo collected dose in urine (C) after 30min and (D) after 24hr.

Table 11
Model optimization results for the best performance of the three models.

In-vitro model	Desirability	X1	X2	X3	Y1	Y2	Y3	Y4
		Run	Nebulizer type	Fill volume (mL)	Inhal. filter (μg)	Exhal. Filter (μg)	Tubing (μg)	Amount/ Nebuliser (μg)
Solution	0.98	7.00	0.10	1.00	1903.12	2449.59	336.39	369.15
Ex-vivo model		**X1** Patients	**X2** Nebulizer type	**X3** Fill volume	**X4** Inhalation filter	**Y1** Ex-vivo (μg)		
Solution	1.00	8.00	0.10	1.00	1774.30	1506.10		
In-vivo model		**X1** Patients	**X2** Nebulizer type	**X3** Fill volume	**X4** Inhalation filter	**Y1** D/urine 30min	**Y2** D/urine 24hr	
Solution	1.00	12.00	0.1	2.00	1922.86	159.76	582.35	

VMN type (encoded 0.1) with fill volume 1 mL which could achieve almost all the desired ranges of in-vitro and ex-vivo output properties set out before optimization (Table 11). The proposed solution for the in-vivo model also suggested a solution with a nebulizer of the VMN (0.1) and a fill volume of 2 mL in order to achieve the desired D/urine 30min and D/urine 24hr (Table 11). These results demonstrate that the in-vivo model was affected by the same variables used with the in-vitro and ex-vivo models. Other suggested variables that could be used to improve the in-vivo model may include; patient's variability in inhalation and exhalation profile, lung capacity as well as the liver and kidney functions. These factors may play major roles in predicting the amount and fate of the inhaled dose and would be more helpful in modeling and optimization of an in-vivo data.

4. Conclusions

The modeling of aerosol delivery by JN and VMN using different fill volumes in NIV circuit was successful in demonstrating the effect of different variable on dose delivery to NIV patient. The ANNs model showed that VMN increased the lung deposition and systemic absorption compared to JN. VMN was less affected by the fill volume change compared to JN which should be diluted to increase delivery. The in-vitro results showed better model than the ex-vivo and in-vivo. Thus, more variables related to patients, e.g. the actual patient respiration profile, should be introduced in the in-vitro testing methodology to imitate the true aerosol delivery method in the patient.

5. Location of study

Teaching Hospital of Faculty of Medicine, Faculty of Medicine, Beni-suef.

University, Beni-suef, Egypt and the Clinical Pharmacy Department, Faculty of Pharmacy, Beni-suef University, Beni-suef, Egypt (analysis).

R&D Approval for patient study: Beni-suef Teaching Hospitals Research Ethics Committee approval number: FMBSU REC FWA#: FWA00015574.

Role of authors

Haitham Saeed: Experiment, data entry, writing.
Ahmed M. A. Ali: modeling, writing.
Ahmed A. Alberry: Concept, study design.
Abeer Salah Eldin: Concept, study design.
Hoda Rabea: Concept, study design.
Mohamed E. Abdelrahim: Concept, planning of study design, statistics, and writing.

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