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Nebulizer calibration using lithium chloride: an accurate, reproducible and user-friendly method

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Nebulizer calibration using lithium chloride: an accurate, reproducible and user-friendly method. R.J Ward, D.W. Reid, R.F. Leonard, D.P. Johns, E.H. Walters. ©ERS Journals Ltd 1998.

ABSTRACT: Conventional gravimetric (weight loss) calibration of jet nebulizers overestimates their aerosol output by up to 80% due to unaccounted evaporative loss. We examined two methods of measuring true aerosol output from jet nebulizers.

A new adaptation of a widely available clinical assay for lithium (determined by flame photometry, LiCl method) was compared to an existing electrochemical method based on fluoride detection (NaF method). The agreement between the two methods and the repeatability of each method were examined. Ten Mefar jet nebulizers were studied using a Mefar MK3 inhalation dosimeter.

There was no significant difference between the two methods (p=0.76) with mean aerosol output of the 10 nebulizers being 7.40 mg·s^{-1} (SD 1.06; range 5.86–9.36 mg·s^{-1}) for the NaF method and 7.27 mg·s^{-1} (SD 0.82; range 5.52–8.26 mg·s^{-1}) for the LiCl method. The LiCl method had a coefficient of repeatability of 1.3 mg·s^{-1} compared with 3.7 mg·s^{-1} for the NaF method.

The LiCl method accurately measured true aerosol output and was considerably easier to use. It was also more repeatable, and hence more precise, than the NaF method. Because the LiCl method uses an assay that is routinely available from hospital biochemistry laboratories, it is easy to use and, thus, can readily be adopted by busy respiratory function departments.

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Jet nebulizers are used clinically and in research in the measurement of airway responsiveness [1–3]. Drug output from nebulizers has traditionally been calibrated by weighing the units before and after nebulization. Variations in the design of nebulizers means that aerosol output cannot be predicted by conventional weight loss calibration [4]. Several methods of measuring true aerosol output have been attempted [5–7] with some success, but these methods have tended to be confined to specialist research centres because of their complexity.

There are two components represented in the gravimetric method, evaporative loss of water vapour and aerosol. Of these two components only the aerosol carries drug and, therefore, it is only this component that is relevant for dose assessment. The gravimetric method, though easy to perform, overestimates true drug output by 25–85% [4–6].

DENNIS *et al*. [4] developed a chemical tracer technique using sodium fluoride (the NaF method) which measured true aerosol output. NaF solution was placed in a nebulizer and the nebulizer activated. The resultant NaF aerosol was collected onto a filter paper and recovered. The concentration of NaF was measured electrochemically with a fluoride electrode and the amount present represented the aerosol fraction of the concomitant weight loss. Solute tracer methods, in particular the NaF assay, have proved useful in research studies needing accurate assessment of nebulizer output. However, the NaF method is time consuming, difficult and requires sensitive specialist equipment and dedicated staff. This has resulted in only a few centres, with the appropriate resources, adopting the method.

We present a simple, repeatable method of nebulizer calibration which can be carried out either in the respiratory laboratory or in hospital biochemistry departments on routine analysers that employ low detection limits. The method is similar to the NaF method, but uses lithium chloride (LiCl) as the tracer. LiCl is ideal for this purpose because it is readily soluble, uncommon in the environment (unlike other salts such as Na^+ and K^+) and has a low molecular weight, maximizing the molar concentration of a weight/volume solution.

When introducing a new method it is necessary to validate it against an existing established method. We compared the NaF tracer method and the new LiCl tracer method using 10 Mefar jet nebulizers (Mefar, Brescia, Italy). These nebulizers were chosen for relative aerosol output assessment, because of the international importance they have achieved through selection for use in the European Community Respiratory Health Survey (ECRHS) [1].

Methods

Nebulizers and dosimeter

Aerosol output and weight loss from a Mefar dosimeter (driving pressure 180 kPa) was assessed for each of two

batches of five Mefar jet nebulizers. Aerosol output was measured in triplicate using both the LiCl and NaF methods. The batches were calibrated twice with each tracer method, with 5 days separating each calibration session. Between assessments each nebulizer was thoroughly washed and dried.

LiCl calibration

Four millilitres of 0.24 M (1% (weight/volume)) solution of LiCl was added to each nebulizer reservoir. Each nebulizer was activated five times by the Mefar dosimeter (Brescia, Italy) for 1 s each time. During activation, ambient air was drawn through at a rate of 20 $L \cdot min^{-1}$, by means of a vacuum pump and T-piece through a 47 mm Whatman (Maidstone, UK) glass fibre filter paper onto which the resultant aerosol was entrained and impacted. The paper was positioned 5 cm from the nebulizer head and held in a filter holder. The LiCl filters were transferred into 10 mL screw capped sample tubes and eluted by the addition of 5 mL of reverse-osmosis purified water and left overnight. LiCl was simply quantified using a flame photometer [8] (Instrumentation Laboratories, Watertown, MA, USA) and calibrated against a commercial standard of 1 mM lithium carbonate (Instrumentation Laboratories). Five LiCl standard solutions were also prepared as follows: 25, 50, 100, 150, 200 µL aliquots of 0.24 M LiCl solution were added to five 10 mL sample tubes, each containing 5 mL of purified water. These five standards were used to generate a standard curve from which the concentration of LiCl in the samples was calculated. This was repeated on each study day. It is important that the same solution of LiCl is used in the standards and nebulizers as LiCl is deliquescent and the exact concentration of the solution may vary between preparations. Inclusion of a standard curve is, therefore, necessary each time the method is used.

NaF calibration

Four millilitres of 0.24 M (1% w/v) NaF solution was added to the nebulizer reservoir. The method of nebulizer activation and aerosol collection was identical to that used for LiCl. The filters were then removed and placed in 30 mL bottles. The filters were left overnight and eluted by the addition of 20 mL of 50% total ionic strength adjusted buffer (TISAB; BDH, Poole, UK). The amount of NaF was measured electrochemically as described by DENNIS *et al*. [4]. A Jenway 3045 ion meter (Jenway, Dunmore, UK) was used with an NaF specific electrode and calomel reference electrode (Ionode, Brisbane, Australia). The methodology outlined by DENNIS *et al*. [4] was followed exactly.

Weight loss

Each nebulizer reservoir was filled with 4 mL of purified water and weighed on an analytical balance (sensitivity ±0.0001 g). Five activations at 6 s intervals were performed, the nebulizer was re-weighed and the measured loss divided by five to obtain output per individual activation. Measurement of weight loss was repeated in triplicate for each of the 10 nebulizers and the mean taken.

Given that the concentrations of the tracers (LiCl, NaF) used were only 0.24 M (1% w/v) a direct comparison of weight loss and aerosol output can be made assuming that 1 mg = 1 µL.

Statistical analysis

The repeatability of each method of nebulizer calibration was examined using the coefficient of repeatability as described by BLAND and ALTMAN [9]. This assumes that 95% of observed differences will lie within two standard deviations of the mean [9]. The coefficient of repeatability is approximately twice the standard deviation of the measured mean differences between two sessions. One-way analysis of variance was used to assess the agreement between the two methods. The standard curves were assessed using linear regression and the r^2 value.

Results

The measurements of mean aerosol output by the two methods are presented in table 1. There was no significant difference (p=0.76) in the mean output of the 10 nebulizers measured by the NaF and LiCl methods: 7.41 mg s^{-1} (SD 1.06; range 5.86–9.36 $mg \cdot s^{-1}$) and 7.30 $mg \cdot s^{-1}$ (SD 0.82; range 5.52–8.26 $mg \cdot s^{-1}$), respectively. The relationship between the two methods is illustrated in a BLAND and ALTMAN plot [9] comparing the two solute tracer methods (fig. 1). Plotted NaF and LiCl values are derived from the mean calibration value of days 1 and 2. The points represent the mean output of each nebulizer as measured by the two methods.

The mean aerosol output measured by each tracer method was significantly different (p=0.001) from that measured by weight loss (table 1). Aerosol output as a percentage of

Table 1. – Mean nebulizer output by weight loss, NaF and LiCl methods for two batches of Mefar nebulizers

Nebulizer No.	Calibration method		
	Weight loss	NaF	LiCl
1	10.14±0.32	7.30±0.29 (72.0)	7.09±0.30 (69.9)
2	12.35±0.48	6.68±1.56 (54.1)	6.84±0.34 (55.4)
3	9.35±0.39	5.86±0.63 (62.7)	5.52±0.35 (59.0)
4	11.87±0.05	7.34±0.05 (61.8)	7.24±0.10 (61.0)
5	10.61±0.16	6.42±0.22 (60.5)	6.57±0.01 (62.0)
Mean of batch 1	10.87±1.20	6.72±0.62 (62.2)	6.65±0.68 (61.4)
6	11.75±0.18	7.33±2.84 (62.4)	7.80±0.68 (66.4)
7	13.19±0.19	6.91±1.86 (52.4)	8.02±0.40 (60.8)
8	12.46±0.05	8.56±1.52 (68.7)	7.66±0.39 (61.4)
9	10.87±0.94	8.32±0.92 (76.5)	7.78±0.51 (71.6)
10	12.41±0.06	9.36±0.68 (75.4)	8.26±0.84 (66.6)
Mean of batch 2	12.14±0.87	8.10±0.98 (67.1)	7.90±0.24 (65.3)
Mean of batches 1 and 2 combined	11.5±1.21	7.41±1.06 (64.5)	7.30±0.82 (63.4)

Values are presented as mean±SD, with the "true percentage" aerosol output, based on each method and weight-loss data, in parenthesis. LiCl and NaF values are means of the two calibrated sessions (days 1 and 2).

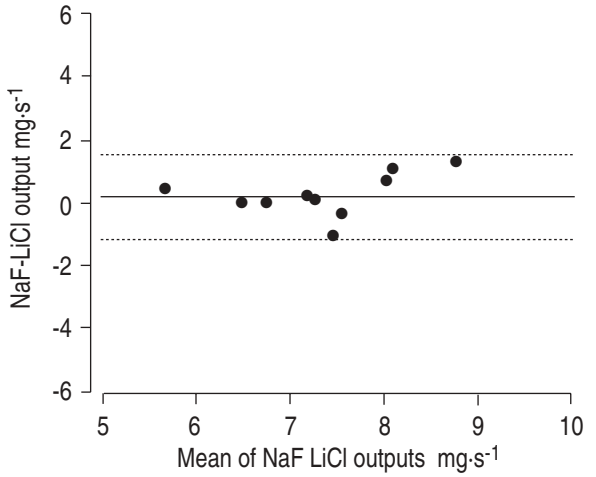

Fig. 1. – Bland and Altman plot [9] comparing the two solute tracer methods. NaF and LiCl values derived from the mean calibration value of days 1 and 2. The points represent the mean output of each nebulizer measured by the two methods. The solid line represents the mean difference between the two methods. The dashed lines represent the limits of agreement (mean difference±2 SD).

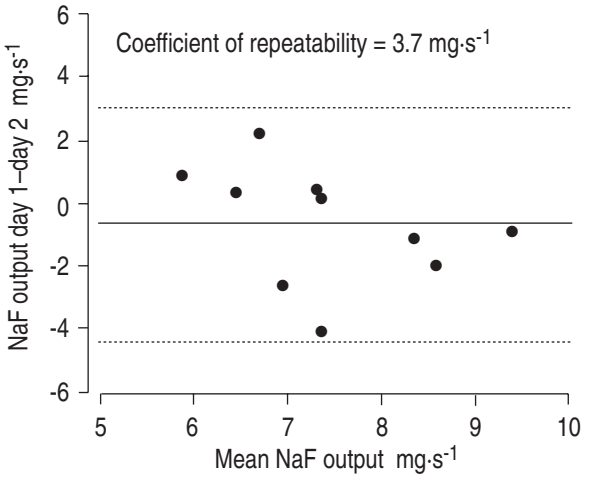

Fig. 2. – Bland and Altman plot [9] comparing the repeatability of NaF nebulizer calibration on two separate days. Each point is derived from the mean of triplicate calibrations on each nebulizer. The points represent the mean output of each nebulizer measured by the two methods. The dashed lines represent the limits of agreement (mean difference±2 SD).

weight loss was calculated as (aerosol output/weight loss) ×100. The mean percentage aerosol output was 64.5% (SD 8.3%; range 52.4–76.5%) for the NaF method and 63.4% (SD 5.1%; range 55.4–71.6%) for the LiCl method.

Table 2 shows the between-session variability for the NaF method, which is shown graphically in figure 2. The coefficient of (between-session) repeatability for aerosol output by the NaF method was 3.7 mg·s^{-1}. Figure 3 shows the between-session variability for the LiCl method, with a coefficient of repeatability for aerosol output of 1.3 mg·s^{-1}.

Each aerosol tracer method detected small, but significant, differences between production batches of nebulizers (p=0.029 for the NaF method and p=0.005 for the LiCl method; table 1). For each method, batch 2 repeatedly produced more aerosol. However, within the batches of nebulizers, mean aerosol output was similar by each tracer method. Batch 1 had a mean aerosol output of 6.72 mg·s^{-1} (SD 0.6; range 5.9–7.3 mg·s^{-1}) for the NaF method and 6.6 mg·s^{-1} (SD 0.7; range 5.5–7.2 mg·s^{-1}) for the LiCl method. Batch 2 had a mean aerosol output of 8.1 mg·s^{-1} (SD 1.0; range 6.9–9.4 mg·s^{-1}) for the NaF method and 7.9 mg·s^{-1} (SD 0.2; range 7.65–8.3 mg·s^{-1}) for the LiCl method.

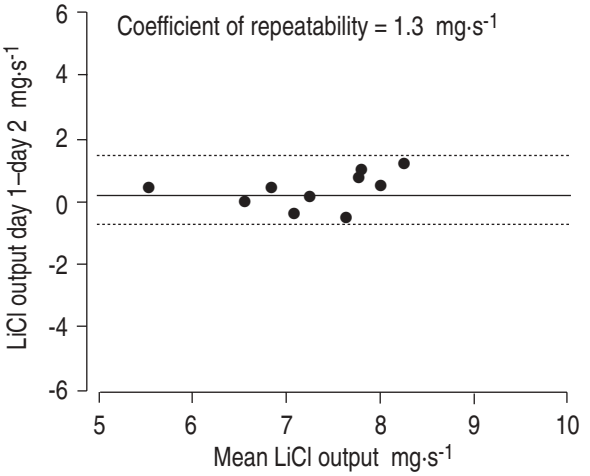

Fig. 3. – Bland and Altman plot [9] comparing the repeatability of LiCl nebulizer calibration on two separate days. Each point is derived from the mean of triplicate calibrations on each nebulizer. The points represent the mean output of each nebulizer measured by the two methods. The dashed lines represent the limits of agreement (mean difference±2 SD).

Table 2. – Aerosol output measured with NaF and LiCl methods between sessions

Nebulizer No.	Calibration method			
	NaF		LiCl	
	Day 1	Day 2	Day 1	Day 2
1	7.51±0.06	7.09±0.44	6.87±0.12	7.30±0.06
2	7.79±0.06	5.58±0.23	7.08±0.39	6.60±0.06
3	6.31±0.01	5.41±0.09	5.77±0.12	5.28±0.25
4	7.37±0.22	7.31±0.10	7.31±0.06	7.17±0.23
5	6.57±0.31	6.26±0.14	6.58±0.21	6.57±0.14
6	5.32±0.66	9.33±0.13	8.28±0.27	7.32±0.33
7	5.60±1.34	8.23±0.39	8.30±0.23	7.74±0.30
8	7.49±0.34	9.63±0.53	7.38±0.56	7.93±0.83
9	7.67±0.17	8.97±0.07	8.14±0.04	7.43±0.67
10	8.88±0.03	9.85±0.18	8.85±0.27	7.67±0.18
Mean	7.05±1.09	7.77±1.67	7.46±0.95	7.10±0.78

Values are presented as mean±SD, with the "true percentage" aerosol output, based on each method and weight-loss data, in parenthesis. LiCl and NaF values are means of the two calibrated sessions (days 1 and 2).

The gravimetric method showed a similar trend, batch 1 had a mean output of 10.87 mg·s^{-1} (SD 1.20; range 9.35–12.35 mg·s^{-1}) and batch 2 had a mean output of 12.14 (SD 0.87; range 10.87–13.19 mg·s^{-1}) but the difference was nonsignificant (p=0.097).

Five-point standard curves for each method are shown in figures 4 and 5. The LiCl plot was linear over the concentration range studied (r^2=1.0).

The NaF calibration curve needed to be log transformed to be made consistently linear (r^2=1.0 for log transformed data.)

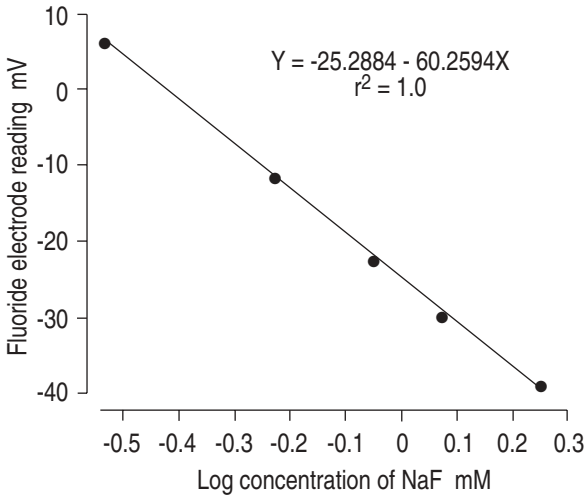

Fig. 4. – A five point calibration curve of the fluoride electrode. The curve was generated from 25, 50, 75, 100 and 150 µL aliquots of 0.24 M NaF solution added to 20 mL volumes of 50% total ionic strength adjusted buffer (TISAB) buffer. The resultant concentrations of NaF were 0.30, 0.60, 0.90, 1.19 and 1.79 mM. These concentrations were log-transformed as the relationship between NaF and voltage is log-linear.

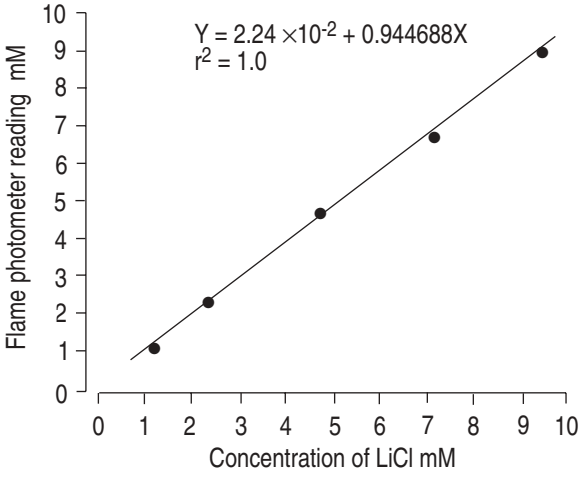

Fig. 5. – A five point calibration curve of the flame photometer. The curve was generated from 25, 50, 75, 100, 150 and 200 µL aliquots of 0.24 M LiCl solution added to 5 mL volumes of purified water. The resultant concentrations were 1.18, 2.36, 4.72, 7.08 and 9.44 mM. The regression equation was used to calculate nebulizer output values.

Discussion

Airway responsiveness to methacholine (or other bronchoconstrictor agents) is frequently measured in the clinical laboratory and in epidemiological and pharmacological studies [1–3]. An accurate measurement of the cumulative dose of inhaled methacholine causing a 20% fall in forced expiratory volume in one second (PD20) depends upon knowing the precise amount of aerosolized methacholine administered with each nebulizer activation. The most commonly used weight-loss method of nebulizer calibration grossly overestimates true drug delivery and seriously compromises the power of any study that seeks to use PD20 in a precise way. Dennis *et al.* [4] tried to address this issue and, in a significant advance, recommended adoption of the NaF method of nebulizer calibration. However, although this method is more accurate, it is not widely applied because of difficulties with the assay. In our experience the method is poorly repeatable and depends upon the maintenance of a delicate fluoride electrode, which can be temperamental and has a tendency to drift. Our finding that the NaF method had more between-session variability than the LiCl method is consistent with this operational experience.

The data produced in this study compares well with published performance data on the Mefar dosimeter. Dennis *et al.* [5] found a mean aerosol output in two batches of nebulizers of 5.66 and 10.56 m·s^{-1} (aerosol output 51% and 75%), respectively. Our data is comparable and confirms the potential for between-batch variability in the Mefar system. This is of particular relevance given the use of the Mefar nebulizer and dosimeter in the ECRHS. A recent study by Chinn *et al.* [10], using the NaF method suggested that the between-batch aerosol output variability for the nebulizers used in the ECRHS was of limited magnitude, which was reassuring for the centres involved. The nebulizer calibrations were performed in a single centre, which found a disconcertingly low aerosol output of 43% (*i.e.*, mean aerosol output of approximately 4.7 m·s^{-1}) which seems inappropriate for a modern and well-designed nebulizer system [11]. We believe this is a cause for concern since the outputs obtained for the Mefar nebulizers were well below those observed by Dennis *et al.* [5] and ourselves.

Furthermore, Chinn *et al.* [10] also found a low between-batch coefficient of variability which is surprising in view of our current and past experience [12] of significant between-batch variability. They expressed airway responsiveness as log slope, which is less dependent upon a precise knowledge of nebulizer output than the more conventional PD20, but, as the authors pointed out [10], their method is acceptable only if there is small between-batch output variability.

We feel that, given the importance of between-centre reproducibility of nebulizer output in the ECRHS, the data presented by Chinn *et al.* [10] needs corroborating in other centres using aerosol tracer techniques.

Our data suggest that LiCl is simpler and preferable to NaF as a tracer for calibration purposes. The method described is easily applicable, utilizes preexisting and generally available hospital laboratory facilities for the assay and is highly accurate. The method makes standardization of calibration widely available and allows multicentre studies to be directly comparable and meaningful. Furthermore, we have shown that the LiCl method has a considerably lower coefficient of repeatability which means that its between-session precision is higher.

Practically, for PD20 measurement, the Mefar dosimeter is intended to produce 10 mg of aerosol per activation. Our data show that this can be achieved only by the use of an adjusted mean activation time for a batch of nebulizers once the true aerosol output is known. For instance, batch 1 in this study would require an activation time of 1.5 s to produce a mean output of 10 mg within 95% confidence limits of 8.7 and 11.3 mg. For batch 2, with a higher mean aerosol output, an activation time of 1.3 s would produce a mean aerosol output of 10.3 mg within the 95% confidence limits of 9.9 and 10.7 mg. For each nebulizer used in this study, the manufacturer had reported a uniform gravimetric output of 10 mg·s^{-1}. This is at variance with our data and that of Chinn *et al.* [10] and Dennis *et al.* [5].

MERKUS *et al.* [13] has shown with the weight loss method that repeated nebulizer use has little effect upon output provided the nebulizer is thoroughly cleaned between daily uses. In view of this, we recommend annual calibration of nebulizers, but weekly checks of the static output pressure from dosimeters, which may be a greater source of variability in our experience.

In conclusion, we believe that the lithium chloride method is an important advance in nebulizer calibration. Although it is generally accepted that gravimetric determination of solute output is inaccurate, the absence of a practical and easy alternative method has resulted in most centres, including those in involved in the European Community Respiratory Health Survey, persisting in its use. The lithium chloride method addresses this problem simply and accurately and is suitable for widespread laboratory use.

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