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An assessment of jet and ultrasonic nebulisers for the delivery of lactate dehydrogenase solutions

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Abstract

The aim of this study was to investigate the suitability of commercial jet and ultrasonic nebulisers for effective delivery of the model hydrophilic protein lactate dehydrogenase (LDH). Two jet nebulisers (Pari LC Plus and Pari LC Star) and two ultrasonic nebulisers (Sonix 2000 and Omron U1) were used to nebulise LDH solutions and the effects on protein activity and protein concentration determined. The size distribution of the aerosols produced, measured by laser diffraction analysis, temperature changes during nebulisation, the time to atomise a 5 ml dose volume and the mass output of the four nebulisers were compared. A twin impinger (TI) was used to collect the nebulised protein, which was assayed for total and active protein content. There was a large variation in the median size and size distribution of the aerosols produced by each of the nebulisers from LDH and Sørensen's modified phosphate buffer, and in the time taken to reach the sputtering phase of aerosolisation. During use, the concentration of LDH increased in the Omron U1 nebuliser, but did not change significantly in the others. The temperature of the protein solution decreased by approximately 8 °C during jet nebulisation but increased by 3 and 10 °C in the Omron U1 and Sonix 2000 nebulisers, respectively. Denaturation of LDH within the nebuliser reservoir, occurred in the order Sonix > Pari LC Plus > Pari LC Star > Omron U1, whilst the deposition of active and total protein within the stages and throat of the TI was a function of the particle size of the aerosols generated and the specific device used.

Keywords: Jet nebuliser; Ultrasonic nebuliser; Protein aerosol

1. Introduction

There have been great advances in therapeutic protein and peptide production within the last decade due to the advent of techniques such as recombinant DNA technology (Banga, 1995). The vast majority of these products are administered

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parenterally due to their poor oral bioavailability. Nebulisation has been used as a non-invasive alternative for the delivery of these therapeutic agents to the airways.

Jet nebulisers have been used successfully to generate protein aerosols. For instance, the activity and structural integrity of recombinant human deoxyribonuclease (rhDNase) was maintained during jet nebulisation (Cipolla et al., 1994). However, an irreversible time-dependent loss of lactate dehydrogenase (LDH) activity occurred on jet nebulisation, due to physical disruption of the protein structure, resulting from shear stress and surface effects, which were dependent on the volume of fluid and rate of aspiration (Niven and Brain, 1994).

As an alternative to the more common jet nebulisers, ultrasonic nebulisers may be employed for aerosol generation. These devices use only a small fraction of the available energy from a vibrating piezoelectric crystal in the production of droplets. Excess energy is converted to heat causing the temperature of the liquid within the nebuliser to increase, such that the temperature of drug solutions in medical ultrasonic nebulisers have been reported to increase by up to 20 °C above ambient temperature during use (Phipps and Gonda, 1990; Taylor and Hoare, 1993). They would thus appear less suitable than jet nebulisers for the generation of protein aerosols because of the heat sensitivity of such materials. When two ultrasonic nebulisers were investigated for the delivery of rhDNase there was evidence, with both, of thermal denaturation of the enzyme towards the end of nebulisation, when the volume of liquid was minimal and its temperature the greatest (Cipolla et al., 1994). The maximum temperature of the rhDNase solution was 58 °C, close to the thermal transition temperature (approximately 65 °C) of rhDNase (Cipolla et al., 1994). Similarly, recombinant consensus α-interferon and LDH have been reported to denature during nebulisation in commercial ultrasonic nebulisers (Ip et al., 1995; Niven et al., 1995).

Ultrasonic nebulisers have some advantages over jet devices since they generally produce higher mass outputs, reducing the duration of nebulisation and are quieter in operation (McCallion and Taylor, 1999). Additionally, the denaturation of proteins during ultrasonic nebulisation may be device-dependent. Flament et al. (1999) reported that aerosolisation of α1 protease inhibitor in the SAM LS ultrasonic nebuliser did not reduce its antielastase activity. This was attributed to design features of the nebuliser which minimised temperature changes during use.

In this study, four modern, commonly used, jet and ultrasonic nebulisers, having different operating and design characteristics, were studied for their suitability to effectively deliver model protein (LDH) solutions.

2. Materials and methods

2.1. Nebulisation of LDH solutions and Sørensen's modified phosphate buffer

Two jet nebulisers, the Pari LC Plus and the Pari LC Star (Pari Medical Ltd. Surrey, U.K) with a Pari Turboboy compressor (US equivalent Pari Proneb; Pari Medical Ltd. Surrey, U.K), and two ultrasonic nebulisers, Sonix 2000 (Medix, Clement Clarke International Ltd., Essex, U.K) and Omron U1 (Hutchings Healthcare Ltd., West Sussex, U.K), were used in these studies. The Pari LC plus was used as it is recommended for delivery of the protein recombinant human deoxyribonuclease (ABPI, 1999). The Pari LC Star is structurally very similar but contains an additional baffle to reduce the size of emitted aerosol droplets, such that it is particularly appropriate for drug delivery to the peripheral airways. The Sonix 2000 nebuliser has a high operating frequency (1.8 MHz), whilst the Omron U1 operates at the much lower frequency of 66 kHz. The Sonix 2000 also has a removable baffle, used to modify aerosol output, by recycling large primary aerosol droplets. In all cases fill volumes of 5 ml were employed. LDH, Type II from rabbit muscle, was obtained from Sigma, U.K. in a stabilising solution of ammonium sulphate, pH 6. This was dialysed into Sørensen's modified phosphate buffer, pH 7.4. (SMPB) in accordance with the method of Harris and Angel (1989), and made up to a concentration of 25 μg/ml using SMPB. This

concentration was used in all experiments unless otherwise specified. The surface tensions of solutions were measured using a Wilhelmy plate method (Nima Technology, UK), and viscosity measurements using a size A Ostwald U tube viscometer (BDH, UK).

2.2. Sputtering time and mass output determination for the nebulisers

In clinical practice nebulisers are usually run to 'dryness', at which point all aerosol generation ceases, although some fluid remains in the nebuliser chamber as the 'dead' or residual volume (Clay et al., 1983). Prior to dryness, there is a point, the 'sputtering time', at which the production of aerosol becomes intermittent and variable (Kradjan and Lakshminarayan, 1985; Smye et al., 1990). The sputtering times for the four nebulisers when SMPB and LDH solutions were nebulised was measured using a stopwatch. Nebulisers were weighed prior to nebulisation, half way through nebulisation ($T_{50\%}$) and at the sputtering time ($T_{100\%}$). The mass of solution remaining in the nebuliser reservoir at these times was calculated for all nebulisers.

2.3. Mean size and size distribution of aerosols

The median size and size distribution of the aerosols produced by the nebulisers was measured using laser diffraction (Malvern 2600c, Malvern Instruments, UK). The nebulisers were clamped at a distance of 2.5 cm from the laser beam so that the aerosol traversed the beam 1.5 cm from the 63 mm lens. Measurements were taken every 30 s from the start of nebulisation to sputtering time. The instrument's software expresses particle size as the volume median diameter (VMD) and the size distribution is expressed as a span value [(90% undersize − 10% undersize)/50% undersize].

2.4. Concentration and temperature changes during nebulisation

LDH solution in SMPB was placed in the nebuliser reservoir, and the system was nebulised to dryness. Samples of solution, taken from the fluid in the nebuliser reservoir, were assayed for protein concentration before and after the nebulisation process using the Bradford Assay (Bradford, 1976).

SMPB was used in all experiments to determine changes in the temperature of the nebuliser fluid during nebulisation. An REK-2 thermocouple probe (HANNA Instruments, UK) was inserted into the nebulisation reservoir such that it was coiled around the wall of the chamber with its tip at the bottom of the chamber. The probe was attached to an electronic thermometer (HANNA Instruments, UK) and readings were taken every 30 s during nebulisation up to the sputtering time.

2.5. Activity of LDH during nebulisation

LDH solution was placed in the nebuliser reservoir. The solution was assayed for protein activity at 3 min intervals from 0 min to dryness, using the assay described by Wroblewski and LaDue (1955).

2.6. Total and active protein deposited within a twin impinger

LDH (1 mg/ml) solutions were nebulised to dryness. A higher concentration was used in this part of the study to permit the unavoidable dilution of protein within the aerosol collection device, whilst maintaining sufficiently high concentrations for accurate determination of total and active protein. The aerosol generated by each nebuliser was collected by directing the aerosol into a two-stage (twin) impinger (TI) (Copley Instruments, Nottingham, U.K.), a device used routinely for the characterisation of aerosols (Hallworth and Westmoreland, 1987), with SMPB as the collection fluid and the aerosol drawn through at 60 l/min by means of a vacuum pump. The contents of the throat (T), upper impinger (UI) and lower impinger (LI) were collected and made up to volumes of 5 ml, 20 ml and 50 ml respectively with washings using SMPB. The samples were then assayed for total protein using the Bradford Assay (Bradford, 1976), and for active protein using the Wroblewski and LaDue (1955) assay.

Table 1
The sputtering times of nebulisers for SMPB and LDH solutions

Nebuliser	Sputtering time (min)	
	SMPB	LDH
Pari LC Plus	14.4 ± 0.7	13.3 ± 1.3
Pari LC Star	19.5 ± 2.8	21.0 ± 1.1
Omron U1	22.3 ± 1.2	20.1 ± 1.2
Sonix 2000	8.8 ± 0.5	10.8 ± 0.9
Sonix (no baffle)	5.9 ± 0.6	6.6 ± 0.8

Mean ± s.d.; $n = 4$.

3. Results and discussion

3.1. Sputtering time

The sputtering times for each nebuliser, when atomising 5 ml of SPMB and LDH solutions, are shown in Table 1. In all experiments statistical analysis was performed using analysis of variance (ANOVA). There was no significant difference in the sputtering time of the nebulisers using the two formulations, except that the time to sputtering was significantly longer for SMPB than LDH for the Omron U1 ($p < 0.05$). When considering inter-nebuliser differences, the four nebulisers exhibited significantly different sputtering times for the two solutions ($p < 0.05$). Overall the range of delivery times for the nebulisers was between 6 and 22 min, which has clinical implications in terms of duration of dosing and patient acceptability. Conventional ultrasonic nebulisers, having operating frequencies of 1 to 2 MHz, have higher mass outputs and reduced nebulisation times than jet nebulisers (McCallion and Taylor, 1999). Consequently, the Sonix had a shorter time to sputtering than either the Pari LC Plus or Pari LC Star. The presence of a baffle (Sonix) or additional baffle (Pari LC Star) increases the proportion of the primary aerosol generated within the nebuliser that is recycled rather than emitted from the device, and hence the sputtering time is increased over the respective devices without baffles, namely Sonix (no baffle) and Pari LC Plus. The low operating frequency of the Omron U1 results in poor mass output, and hence the longest time to nebulise solutions to sputtering.

3.2. Mass output of nebulisers

Delivery profiles of LDH and SMPB were similar for each of the nebulisers. The profile for SMPB is shown in Fig. 1. The Omron U1 nebuliser showed the smallest percentage mass released from the chamber up to $T_{50\%}$ and the smallest

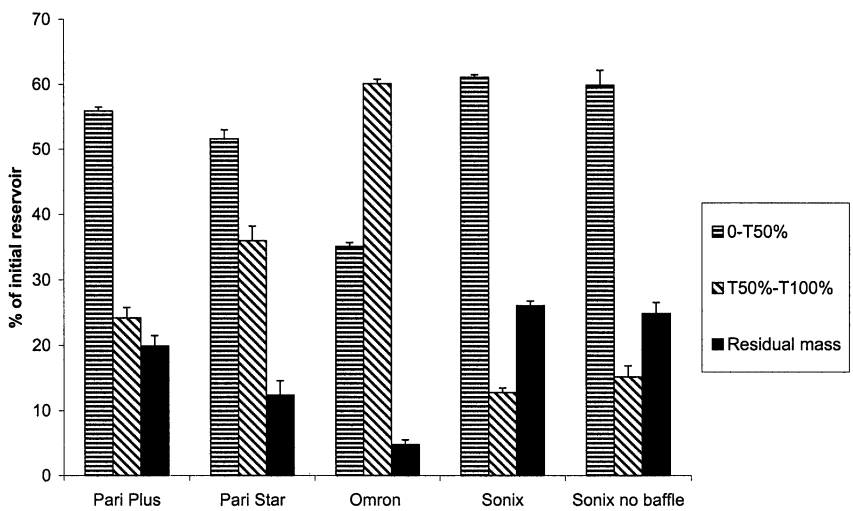

Fig. 1. The percentage mass delivered up to half way through the nebulisation time ($T_{50\%}$) and at sputtering time ($T_{100\%}$) and the residual mass on nebulisation of SMPB. Mean ± s.d.; $n = 4$.

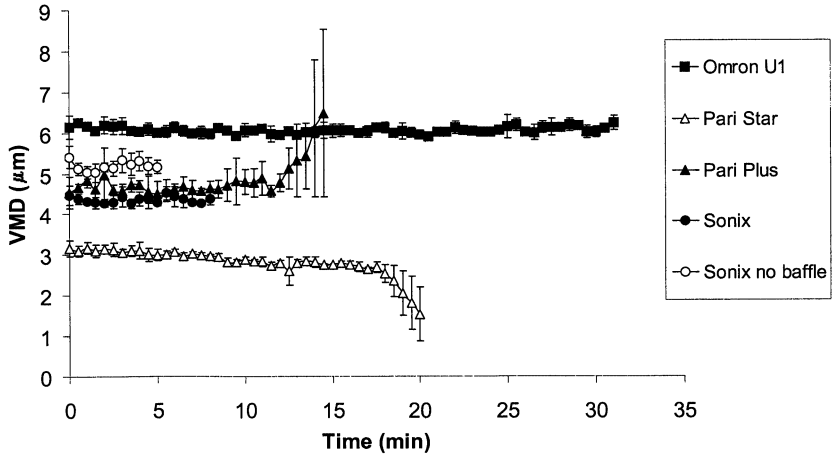

Fig. 2. VMD of the aerosols produced from SMPB up to sputtering time for the ultrasonic nebulisers and to dryness for the jet nebulisers. Mean ± s.d.; $n = 4$.

percentage residual mass. The Sonix with and without the baffle gave similar results but the variability, as shown by the standard deviation of these results, was greater without the baffle. The Sonix 2000 released the greatest percentage of its mass at $T_{50\%}$ but also had the largest residual mass at $T_{100\%}$ compared to the other nebulisers. The Pari LC Star, with its additional baffle, had a greater residual mass at $T_{50\%}$ and a smaller residual mass at $T_{100\%}$ than the Pari LC Plus. These results demonstrate that the release of drugs from nebulisers is not proportional to nebulisation time and that there was great variation between the nebulisers in their ability to efficiently deliver the solutions. In terms of minimising wastage and optimising delivery of an expensive peptide product, the Omron U1 would appear to be the nebuliser of choice, though the prolonged period of nebulisation up to sputtering with this device (Table 1) might mitigate against its use clinically.

3.3. Mean size and size distribution of the aerosols

The many commercially available nebulisers vary widely in the size of aerosol they generate (Waldrep et al., 1994). Fig. 2 shows the median size of aerosols generated by the four nebulisers from SMPB. There was a significant difference in the mean VMD of the aerosols produced by the four nebulisers ($p < 0.05$), determined for the plateau region from the initiation of aerosol generation to the point at which aerosol generation became intermittent, with a large change in measured size. All the generated aerosols had a mean VMD less than 6.5 μm, i.e. within the range likely to be effective for inhalation therapy. The Omron U1 gave the largest mean droplet size, 6.1 to 6.2 μm (Table 2). Aerosols of this size would be predicted to access the lower respiratory tract, but are not sufficiently small to penetrate to the alveoli (Stahlhofen et al., 1980). The Pari LC Star, specifically designed for drug delivery to the peripheral regions of the lung, gave the smallest droplet size (approximately 3 μm) over the nebulisation period (Table 2). Measured aerosol sizes should be interpreted with care, being to an extent determined by the method of measurement. Laser diffraction often gives a measured droplet size for nebulised aerosols larger than that determined by methods based on cascade impaction, during which solvent evaporation can occur. Median sizes determined by laser diffraction do correlate well with pulmonary deposition (Clarke, 1995).

The function of baffles in nebulisers to remove large droplets from the primary aerosol, prior to delivery to patients, is seen in Fig. 2 where the absence of a baffle using the Sonix 2000 resulted in a higher mean VMD. The full profile over the nebulisation period including sputtering has been

shown in Fig. 2 for the jet nebulisers in order to demonstrate that the standard deviation of the aerosol size increased greatly after the sputtering time. The time at which sputtering occurs varies with the same initial fill volume for different nebulisers. The span values showed minimal variation up to the sputtering time, at which point increased variability was observed (data not shown). The Pari LC Plus, Pari LC Star and the Sonix without a baffle produced aerosols with similar spans (Table 2). The Omron U1 and Sonix 2000 produced aerosols with a smaller span than the jet nebulisers and Sonix without a baffle.

3.4. The influence of formulation on aerosol size characteristics

Table 2 shows the VMD and spans of aerosols produced by the nebulisers from SMPB and LDH solutions, up to sputtering. Some small, but statistically significant differences were observed. In the Pari LC Plus, the SMPB formulation gave a significantly smaller VMD and a significantly greater span reading than LDH ($p < 0.05$) over the nebulisation period. The results for the Pari LC Star were similar to those for the Pari LC Plus. The Pari LC Star also showed that the VMD of the aerosol produced from SMPB was significantly smaller than that of LDH and the span of the aerosol produced by SMPB was significantly greater than that of LDH ($p < 0.05$). The VMD of the aerosol produced from SMPB using the Omron U1 was larger than that produced from LDH, whilst span values were the same ($p < 0.05$). The Sonix with a baffle showed a small but significant difference in VMD and span for the two fluids, with SMPB aerosols having the smallest VMD and span values ($p < 0.05$). The Sonix without a baffle produced aerosols with the same mean VMDs and spans for the two formulations. The observed differences may result from differences in the surface tension and viscosity of the two solutions (McCallion et al., 1995). Surface tension was 68.4 ± 0.14 and 58.58 ± 0.10 mN/m and viscosity was 0.528 ± 0.002 mm^2/s and 0.528 ± 0.002 mm^2/s for SMPB and LDH respectively. The differences in VMDs and spans were small and unlikely to be of clinical significance.

3.5. The effect of nebulisation on LDH concentration

The aerosol output from nebulisers comprises drug solution and solvent vapour which saturates the outgoing air. This has been reported to result in an increase in the concentration of solute solutions in jet nebuliser reservoirs during use (Ferron et al., 1976). An increase in concentration may be particularly important with respect to protein formulation due to the tendency of proteins to aggregate in solution. The concentration of the protein before and after nebulisation is shown in Table 3. The Omron U1 ultrasonic device produced a significant increase in concentration of LDH in the reservoir fluid, post- nebulisation ($p < 0.05$). Small increases were observed with the jet nebulisers and the Sonix device, though these increases were not significant ($p < 0.05$). This is an interesting finding which requires further investigation. It is possible that the surface activity of the protein

Table 2
VMD and span for aerosols produced by nebulisers up to the sputtering time

	SMPB		LDH	
	VMD (μm)	Span	VMD (μm)	Span
Pari LC Plus	3.98 ± 0.26	2.33 ± 0.07	4.83 ± 0.15	2.13 ± 0.09
Pari LC Star	2.83 ± 0.12	2.42 ± 0.07	3.25 ± 0.10	2.12 ± 0.04
Omron U1	6.18 ± 0.12	1.57 ± 0.01	6.10 ± 0.12	1.58 ± 0.02
Sonix 2000	4.36 ± 0.08	1.70 ± 0.05	4.37 ± 0.05	1.74 ± 0.01
Sonix (no baffle)	5.20 ± 0.12	2.26 ± 0.03	5.19 ± 0.24	2.31 ± 0.06

Mean $\pm$ s.d.; $n > 10$.

Table 3
Percentage of initial concentration of LDH in the residual volume before and after nebulisation in jet and ultrasonic nebulisers

	% before	% after
Pari LC Plus	100 ± 2	102 ± 1
Pari LC Star	100 ± 5	104 ± 5
Omron U1	100 ± 4	110 ± 6
Sonix 2000	100 ± 15	102 ± 16
Sonix (no baffle)	100 ± 11	96 ± 12

Mean ± s.d.; $n = 4$.

may lead to localisation at the air/liquid interface of droplets, reducing water transport from the droplets, as has previously been described for phospholipids (Marks et al., 1983).

3.6. Temperature changes during nebulisation

The two jet nebulisers showed virtually identical profiles for the change in the temperature of the fluid in the reservoir with time (Fig. 3). The temperature decreased sharply during the first 5 min of nebulisation, due to rapid solvent evaporation, as previously reported (Taylor et al., 1992), and was then relatively constant for the remainder of the nebulisation period. Overall, the temperature in the reservoir decreased by approximately 8 °C during nebulisation, as has been previously described (Clay et al., 1983).

The Sonix 2000, which with an operating frequency of 1.8 MHz is a conventional ultrasonic device, produces a large increase in solution temperature during operation. Removal of the baffle did not change the temperature profile. The temperature increase of approximately 10 °C above ambient, is similar to previous reports for similar devices (Phipps and Gonda, 1990; Taylor and Hoare, 1993). However, the measured temperatures did not exceed the denaturation temperature of LDH i.e. 56 °C (Stellwagen and Wilgus, 1974). The solution in the reservoir of the Omron U1 showed a slow, steady increase in temperature to approximately 3 °C above ambient during nebulisation. The difference in the temperature profiles of the ultrasonic nebulisers can be attributed to the Omron U1 operating at a much lower frequency (65 kHz) than the Sonix 2000.

3.7. The activity of LDH before, during and after nebulisation

The extent of denaturation of LDH within the nebuliser reservoir, occurred in the order Sonix > Sonix no Baffle > Pari LC Plus > Pari LC Star > Omron U1 (Fig. 4). The Omron U1 was the only nebuliser which did not cause significant denaturation of the protein during nebulisation ($p < 0.05$). However, LDH concentration increased during nebulisation in the Omron U1 (Table 3),

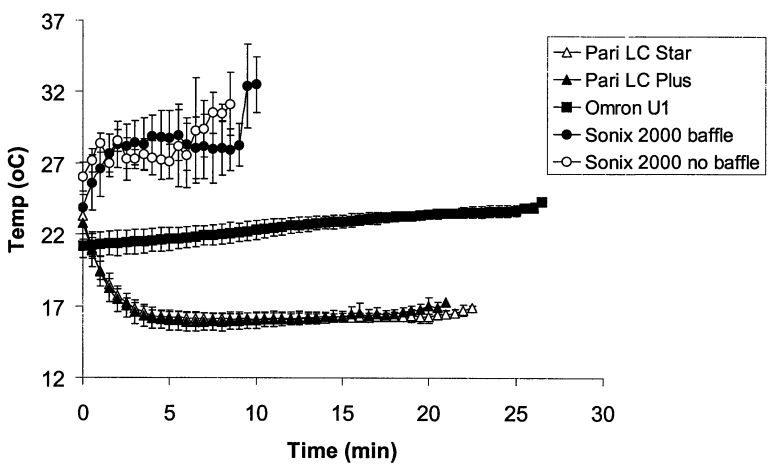

Fig. 3. Temperature of solution in the nebuliser chamber during nebulisation to dryness. Mean ± s.d.; $n = 4$.

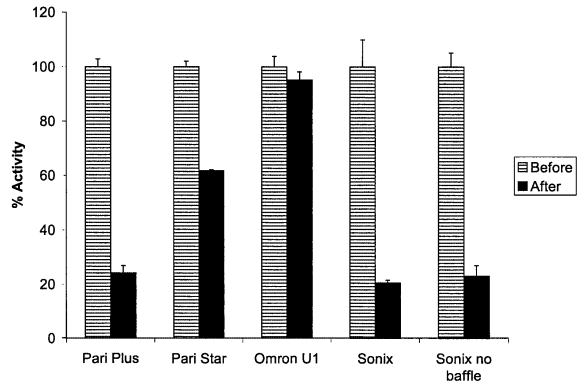

Fig. 4. The percentage activity of LDH in the nebuliser chamber before and after nebulisation. Mean ± s.d.; $n = 4$.

hence the effects of denaturation of the protein may to some extent be obscured.

Fig. 5 shows the activity of LDH sampled from the nebuliser at time intervals during nebulisation. By the end of nebulisation, the activity of the protein remaining in the nebuliser fluid was negligible for the Sonix 2000 ultrasonic nebuliser, with and without baffle and the Pari LC Plus jet nebuliser. The Sonix nebuliser decreased protein activity at a faster rate than the other nebulisers and this was greater when the baffle was removed. The Pari LC Plus showed a faster decrease in protein activity than the Pari LC Star. The Pari LC Star is identical in construction to the Pari LC Plus apart from the presence of an extra baffle. Thus, with both jet and ultrasonic nebulisers, presence of a baffle decreased the rate of denaturation, which may indicate that although the baffles provide a surface at which denaturation could possibly occur at the liquid/solid interface, their removal increases the volume of the nebuliser chamber, permitting greater denaturation at the liquid/air interface.

The Omron U1, although an ultrasonic device like the Sonix 2000 which produced rapid denaturing of LDH, caused least protein damage. Indeed, there was an apparent increase in LDH activity during nebulisation, with protein activity returning to its initial value at the end of nebulisation. This is likely to result from the increase in protein concentration, occurring simultaneously with some slight denaturation during nebulisation. It should be noted, however, that the denaturation or otherwise of the protein in the nebuliser reservoir does not necessarily correlate with the denaturation of protein within the clinically useful delivered aerosol.

3.8. LDH deposition in the twin impinger (TI)

The total protein deposited on the stages and throat of the TI by each nebuliser is shown in Fig.

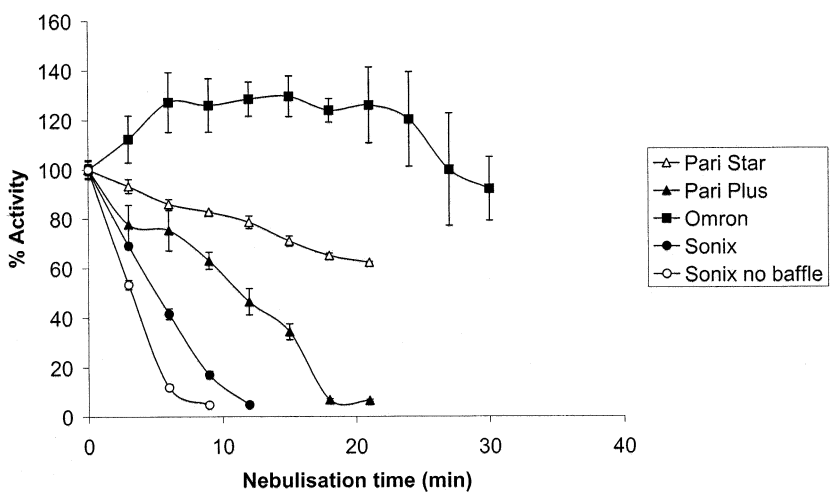

Fig. 5. The percentage activity of LDH in the nebuliser chamber over the time required to nebulise solutions to dryness. Mean ± s.d.; $n = 4$.

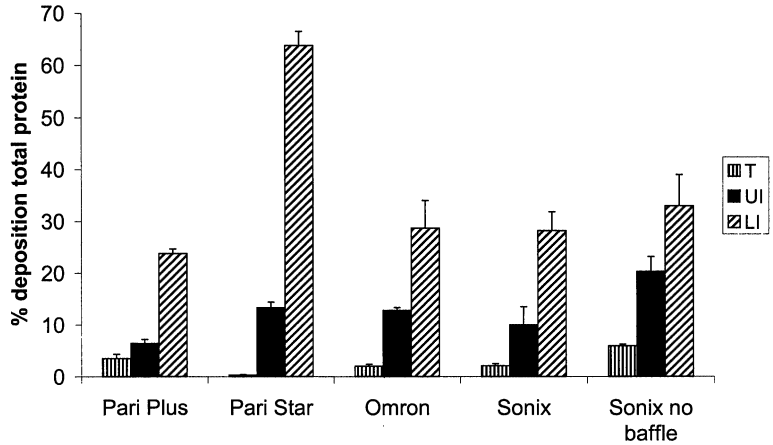

Fig. 6. The percentage deposition of total protein in the stages of the twin impinger for LDH 1 mg/ml. T, UI and LI represent the throat, upper impinger stage and lower impinger stage respectively. Mean ± s.d.; $n = 4$.

6. The TI has an effective cut-off diameter of 6.4 μm between the upper and lower stages, so the lower stage will contain what can be considered 'clinically useful' aerosol (Hallworth and Andrews, 1976). The VMD and span values for aerosols were not significantly different for any of the aerosols when LDH was nebulised at 1 mg/ml rather than 25 μm/ml (data not shown). Deposition in the throat was in the order: Sonix no baffle > Pari LC Plus > Sonix > Omron > Pari LC Star; deposition in the upper stage was in the order: Sonix no baffle > Pari LC Star > Omron > Sonix > Pari LC Plus; and deposition in the lower stage was in the order: Pari LC Star > Sonix no baffle > Omron > Sonix > Pari LC Plus. Thus, the Pari LC Star delivered the greatest percentage of total protein to the lower stage and gave the lowest deposition of protein in the throat. This is as might be predicted, as this nebuliser produced aerosols with the smallest droplet size (Table 2). The Sonix without a baffle gave the greatest percentage of total protein deposition in the throat and upper stage. The Omron U1 produced aerosols with the greatest particle size of the nebulisers investigated (Table 2), but with a narrow size distribution. The Sonix with the baffle had a smaller median size, but wider size distribution, which may explain why aerosols from this nebuliser had relatively greater deposition in the upper stages of the impinger.

The percentage of the initial active protein deposited in the throat was in the order: Sonix no baffle > Omron > Pari LC Plus > Sonix (Fig. 7). No active protein could be detected in the throat when the Pari LC Star was employed. In the upper stage of the impinger, deposition of active protein was in the order: Omron > Sonix no baffle > Pari LC Star > Pari LC Plus > Sonix, and in the lower stage, deposition was in the order: Sonix no baffle > Pari LC Plus > Omron > Pari LC Star > Sonix. Thus, the Pari LC Star delivered the largest quantity of total protein to the lower stage of the impinger, but when active protein was examined the percentage reaching the lower stage was relatively small. This indicates that the extent of LDH denaturation as well as the extent of deposition in the lower stage of the impinger is proportional to the aerosol size generated.

4. Conclusion

Two jet and two ultrasonic nebulisers were examined for their ability to aerosolise a model protein solution. The results may not be generalisable to other protein solutions or suspensions. These nebulisers showed variations in the size of aerosols produced, their sputtering times, their effect on the concentration and temperature of the solution being nebulised, degradation of LDH

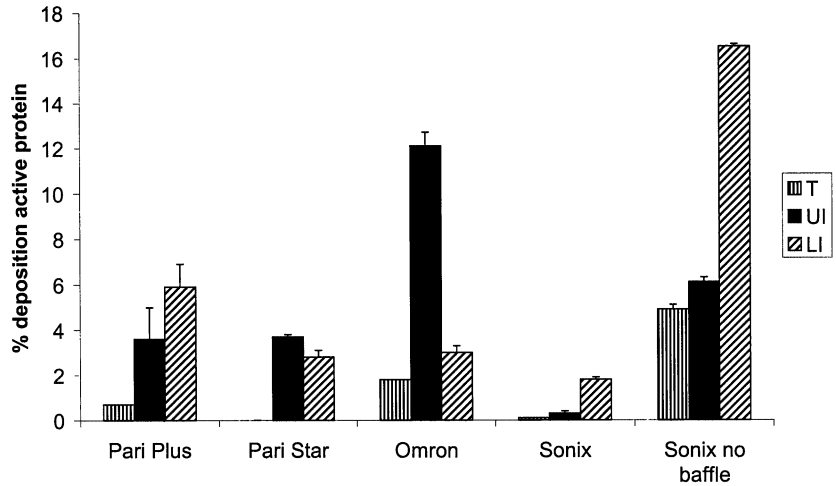

Fig. 7. The percentage deposition of initial active protein in the stages of the twin impinger for LDH 1 mg/ml. T, UI and LI represent the throat, upper impinger stage and lower impinger stage respectively. Mean ± s.d.; $n = 4$.

and their ability to deliver active protein. Predictions of the amount of active protein likely to be delivered to the lower airways cannot be based on simplistic evaluation of the size of aerosols generated by nebulisers, or the type (jet or ultrasonic) of nebuliser used, since degradation of LDH is associated with a number of factors including temperature (Niven et al., 1995) and shear stress and surface effects (Niven and Brain, 1994). The variations in the performance of the four nebulisers investigated, in particular the two ultrasonic devices, indicate that it is imperative for manufacturers to specify the appropriate nebuliser(s) to be used for delivering a particular protein formulation.

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