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# Preparation and characterization of curcumin-loaded liposomes for delivery to the lungs

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## ABSTRACT

Curcumin-loaded liposomes comprising dipalmitoylphosphatidylcholine, cholesterol, and dioleoylphosphatidylethanolamine (72:8:20 mole ratio) were prepared by the thin-film hydration method, followed by probe sonication to achieve a mean diameter <200 nm. The properties of aerosols generated using from 4 mL optimized liposome preparation filled into an air-jet nebulizer were determined using a Fast Screening Impactor (FSI) pre-cooled at 2–8°C for at least 90 min at 15 L/min. Curcumin recovered from the impactor and nebulizer was quantified using a validated high-performance liquid chromatography method. Overall, 2.5% w/w curcumin-loaded liposomes was the optimal formulation based on the maximum encapsulation efficiency of approximately 87% and mean hydrodynamic diameter of 97 nm. This chosen curcumin concentration was effectively aerosolized with a fine particle fraction of 53.25%, and a fine particle dose of 53 µg being deposited on the lower stage of the FSI (cutoff diameter 5 µm).

**Keywords:** Air-jet nebulizer, cascade impactor, curcumin, Fast Screening Impactor, liposome

## INTRODUCTION

Curcumin, (1E, 6E)-1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a herbal substance extracted from *Curcuma longa*. This phytochemical compound has been used extensively as a traditional medicine, particularly in Asian countries such as China and India<sup>(1,2)</sup>, and has gained popularity in various therapeutic applications, resulting from, for example, antioxidant, antimicrobial, anti-inflammatory, and anticancer activities<sup>(3,4)</sup>. Curcumin has an inhibitory effect on cell growth and an apoptosis-inducing effect on human A549 lung cancer cells<sup>(4-6)</sup>. However, the oral bioavailability of curcumin is limited by its low aqueous solubility (0.6 µg/mL)<sup>(7,8)</sup>. Formulation approaches utilizing the pulmonary route have consequently been studied to overcome this major delivery challenge<sup>(2,9)</sup>.

Nanotechnology-based formulations, for example, liposomes, polymeric micelles, nanoparticles, and cyclodextrins, have been studied for curcumin delivery through inhalation for localized treatment<sup>(3,4,8)</sup>. Liposomes are considered appropriate lipid-based nanoscale drug delivery

systems due to their biocompatibility, biodegradability, and the ability to accommodate both hydrophilic and hydrophobic drugs<sup>(10,11)</sup>.

Dipalmitoylphosphatidylcholine (DPPC), a component of human pulmonary surfactant, is frequently used as the major phospholipid component in liposomal formulations intended for delivery to the lungs<sup>(10,12,13)</sup>. The delivery of curcumin using DPPC-based liposomes administered through the pulmonary route accordingly represents a rational and challenging strategy of local treatment in this study.

Nebulizers aerosolize liquid drug formulations into aqueous droplets for inhalation into the lungs. They are most frequently used in the hospital setting to deliver relatively large doses or volumes of medications<sup>(14)</sup>. Nebulizers require little or no coordination of breathing and actuation for effective use as compared to pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs) and therefore are particularly suitable for certain patient groups, such as infants and the elderly<sup>(10,15,16)</sup>. Nebulizers are classified into three main types; air-jet, ultrasonic, and vibrating mesh. Jet nebulizers are connected to air compressors using tubing, with compressed air flowing at high velocity through the liquid formulation

which is aerosolized with a droplet size distribution suitable for inhalation<sup>[17]</sup>. Atomization of liposomes using air-jet and vibrating-mesh nebulizers produces less disruption to liposomal bilayers during aerosolization compared with ultrasonic devices<sup>[11,18]</sup>. However, vibrating mesh devices have been reported to give a lower output efficiency than air-jet devices, due to liposome fusion/aggregation during aerosolization, causing particles to block the mesh pores<sup>[11,19,20]</sup>. Consequently, an air-jet nebulizer was used for liposomal delivery in this study.

The characteristics of nebulized aerosols are highly dependent on both the nebulizer and the properties of the fluid nebulized, with significant interbrand variability for different nebulizer/drug combinations<sup>[16,21,22]</sup>. Aerodynamic particle size distribution (APSD) is recognized as a critical quality attribute in the *in vitro* characterization of orally inhaled and nasal drug products<sup>[23-25]</sup>. The APSD of an aerosol cloud signifies where the particles or droplets in that cloud are likely to be deposited within the airways following inhalation and hence determines the percentage of the total emitted dose that actually gets into the peripheral lung during inhalation and which is thereby therapeutically effective for the user<sup>[22,26,27]</sup>. The effectiveness of inhaled therapeutics is generally dependent on the fine particle dose (FPD), (the particle mass below 5 micrometers), whilst fine particle fraction (FPF) is the fraction over the emitted dose (<5 micrometers) expressed as a percentage (%)<sup>[11,28]</sup>.

The standard method for measuring the APSD of inhaler products is the cascade impactor, included in international pharmacopoeias, which can differentiate the aerodynamic particle size measurement of the active pharmaceutical ingredient (API) in the presence of other components by chemical (usually high-performance liquid chromatography [HPLC]) analysis of deposited products<sup>[23,29]</sup>. Cascade impactors comprise a series of collection plates or cups set under jets with successively decreasing diameters<sup>[30,31]</sup>. As aerosol is drawn into the impactor through an induction port, particles with sufficient inertia, dependent on their aerodynamic diameter (a factor of size and particle density), will impact on a stage collection plate<sup>[24,29]</sup>. However, using conventional multistage impactors are time consuming and labor intensive, especially when they are used for quality control and early stage of product development<sup>[23,32]</sup>. They also require a high degree of skill and care on the part of the analyst to avoid error<sup>[33,34]</sup>. Furthermore, quantitative analysis for pharmaceutical formulations with low drug contents is challenging when small quantities of potent APIs are deposited across all the stages of the full cascade impactor. Instrument manufacturers are now developing faster methods of APSD determination, leading to the advent of abbreviated impactor measurement (AIM)<sup>[11,35,36]</sup>.

AIM enables faster throughput from a reduced number of impactor stages in the impactor and is simpler to use compared to its full resolution cascade impactor counterpart, making it useful in accelerating the process of aerosol characterization of inhaler products<sup>[23,27,36]</sup>.

The Fast Screening Impactor (FSI) is an abbreviated impactor. Used with a standard induction port, it comprises only two stages with a cutoff diameter of 5 µm between stages,

allowing quick determination of FPF/FPD by segregating the emitted dose into two fractions: Coarse particle dose and FPD<sup>[32,37]</sup>. The FSI yields accurate and reproducible results with lower internal wall losses with reduced chance of analyst errors compared to the full impactor<sup>[29,32,35]</sup> and can be used for a more rapid analysis of formulations during early product development, before full resolution studies are conducted on promising candidates, and for more accurate quantification for formulations with low drug concentrations<sup>[11,26,37]</sup>.

AIM has been little studied for characterization of nebulized aerosols with the previous studies focusing primarily on DPI and pMDI formulations<sup>[35,38]</sup>. The FSI was previously found to be a useful alternative impactor to the full, 8-stage Next Generation Impactor for aerosol characterization of nebulized liposomes coloaded with erlotinib and genistein<sup>[11]</sup>. In this study, the FSI was consequently the main impactor for aerodynamic characterization during the early-stage formulation development of curcumin-loaded liposomes for nebulizer delivery.

## MATERIALS AND METHODS

### Materials

Curcumin was sourced from Alfa Aesar (USA). Methanol (99.9%, HPLC gradient grade) was purchased from Sigma-Aldrich (Pooled, UK). The reagents and solvents used for the HPLC analysis: Acetonitrile (99.9%, gradient grade for HPLC), HPLC grade water and trifluoroacetic acid (TFA, >99.0%) were supplied by Sigma-Aldrich (Pool, UK).

For liposomal formulations, DPPC (1,2-dipalmitoyl-sn-glycerol-3-phosphocholine) and dioleoylphosphatidylethanolamine (DOPE) (1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine) used as major and helper phospholipids, respectively, were acquired from Lipoid (Ludwigshafen, Germany). Cholesterol used as the liposomal bilayer stabilizer, and the solvent system of absolute ethanol and chloroform (99.8%, analytical grade) was obtained from Sigma-Aldrich; Poole, UK.

### Preparation of Curcumin-loaded Liposomes

Multilamellar vesicles (MLVs) were prepared using the conventional thin-film hydration method<sup>[11,39,40]</sup>. Lipid components; DPPC, cholesterol, and DOPE (72:8:20 mole ratio) were dissolved in absolute ethanol and chloroform (4:1 v/v) together with curcumin (2.50% w/w to 10% w/w drug in total lipids). The solvents were then removed under low pressure for 20 min at 60°C to obtain a thin film of curcumin/lipids using a rotary vacuum evaporator (RC 900, KNF Neuberger GmbH, Germany). The dry thin film was subsequently flushed with nitrogen gas and hydrated with 20 mL HPLC grade water. This was followed by gentle rotation using the rotary evaporator in the water bath at 60°C for 30 min to form MLVs<sup>[11]</sup>. The final concentration of phospholipids was 2.5 mg/mL.

Following formation, the size of liposomes was reduced by probe sonication to achieve a mean diameter <200 nm. Nanocarriers with the mean particle size smaller than 200 nm are effective in alveolar macrophage avoidance, mucus

penetration, and are efficiently incorporated into aerosol droplets during nebulization<sup>[41-43]</sup>. Probe sonication was performed at 12 W constant output for 30 min (5 min/cycle and 1 min cooling at 4°C to prevent overheating)<sup>[44]</sup>. Non-incorporated curcumin was separated by syringe filtration through a 0.45 µm cellulose acetate membrane filters (Merck Millipore Ltd., UK) to yield curcumin-loaded liposomes<sup>[11]</sup>.

Empty liposomes were also prepared using the same procedure without curcumin to study the effect of curcumin incorporation on liposomal properties. Empty and curcumin-loaded liposomes were then characterized for particle size distribution, surface charge, encapsulation efficiency, and drug loading. All experiments were conducted in triplicate with results reported as mean value ± S.D.

## Characterization of Curcumin-loaded Liposomes

### Particle size distribution diameter and surface charge

Hydrodynamic diameter was determined by dynamic light scattering (DLS), and zeta potential measured from electrophoretic mobility, for a 1.0 mL sample of undiluted liposomes at 25°C using a Malvern Nano ZS Zetasizer (Malvern Instruments Ltd., UK).

The hydrodynamic diameter (Z-average) and the size distribution (polydispersity index, PDI) were measured. A PDI value <0.1 is indicative of a monodispersed system, a value <0.3 is considered a relatively monodispersed system, while a PDI value >0.7 represents a polydispersed system<sup>[44,45]</sup>.

### Encapsulation efficiency and drug loading

A 1.0 mL of formulation was diluted with absolute ethanol to a final volume of 10 mL before determination of encapsulation efficiency and drug loading, using a validated HPLC assay.

Samples were analyzed using an HPLC system equipped with an autosampler and UV/Vis detector (Agilent 1100 Series, Agilent, USA). A Synergi 4 µm Polar-RP 80°A HPLC column (250 × 4.6 mm × 4 µm; Phenomenex, UK) was used, with isocratic elution using 0.1% TFA and acetonitrile (50:50) as the mobile phase at a flow rate of 1 mL/min and detection at 420 nm. Each run was set for 15 min at 40°C with an injection volume of 10 µL. The HPLC method was validated for assay of curcumin according to the ICH Q2 guideline<sup>[46]</sup>. The lower limit of quantification was 3.00 µg/mL and the limit of detection was 0.64 µg/mL.

The entrapment efficiency (%EE) and drug loading (%DL) of curcumin in liposomes were calculated from the following equations:

$$\text{Encapsulation efficiency (\%EE)} = \frac{\text{mass of drug in liposomes}}{\text{mass of drug weight for encapsulation}} \times 100\%$$

$$\text{Drug loading (\%DL)} = \frac{\text{mass of drug in liposomes}}{\text{mass of phospholipids and drug}} \times 100\%$$

### Transmission electron microscopy

The morphology of the curcumin-loaded liposomes was determined using a transmission electron microscope (TEM,

Philips Electron Optics BV, Netherlands). A drop of liposomes was placed on a copper grid and excess liquid removed using a filter paper. After 2 min, the samples were negatively stained with 1% w/v uranyl acetate. The copper grid was dried under ambient conditions and observed with the TEM<sup>[47]</sup>.

## Aerodynamic Particle Size Characterization using the FSI

A PARI LC® Sprint Nebulizer (PARI Medical Ltd., Byfleet, UK) was used in this study, connected to a PARI Boy (PARI Medical Ltd., Byfleet, UK) air compressor. Nebulization time was 10 min for each experiment.

The FSI (Copley Scientific, UK) was stored at 2–8°C for at least 90 min before each experiment to reduce inaccuracies in size measurement resulting from aerosol droplet evaporation during use<sup>[11,48,49]</sup>. An additional insert with six nozzles was positioned into the Coarse Particle Collector stage (Copley Instruments, UK), and three of the six nozzles were covered alternately with wet pieces of glass microfiber filter [Figure 1a] to obtain a stage cutoff diameter of 5 µm with the FSI operated at a flow rate of 15 L/min<sup>[11,48]</sup>. A 7 mm diameter glass microfiber filter was fitted in the filter holder and inserted securely into the filter housing. The induction port was used, with mouthpiece adaptor to connect the nebulizer to the induction port, as shown in Figure 1b. Nebulization was started within 5 min of removal of the FSI from the refrigerator<sup>[49]</sup>. The flow rate of the vacuum pump was set at 15 ± 5% L/min using a flow meter (Copley Scientific, UK). This flow rate is recommended in the European Pharmacopoeia for determining the APSD of nebulizer products using a full impactor, giving a good approximation of the normal tidal breathing conditions of an adult and minimizing solvent evaporation, which will reduce measured aerosol droplet size<sup>[49,50]</sup>.

The FSI was used with the air-jet nebulizer reservoir filled with 4 mL of 2.5% w/w curcumin-loaded liposomes (containing ~200 µg curcumin) and nebulized for 10 min. The amount of curcumin was quantified by collecting samples from the nebulizer reservoir, mouthpiece, mouthpiece adapter, induction port, additional insert of FSI housing, and glass microfiber filter using absolute ethanol followed by assay using HPLC analysis<sup>[38]</sup>.

Aerosolization parameters comprising % mass balance, % emitted dose (%ED), FPD, and % FPF were calculated as shown below and reported as mean value ± S.D.;

$$\text{Mass balance} = \frac{\text{Mass of drug from nebulizer to the filter}}{\text{Mass of drug initially placed into nebulizer}} \times 100\%$$

% Emitted dose (%ED) = Fine particle dose (FPD) is the amount/mass of drug deposited on the lower stage of the FSI.

$$\% \text{ Fine Particle Fraction (FPF)} = \frac{\text{Mass of drug from the filter}}{\text{Mass of drug from deposited drug on upper and lower stage of the FSI}} \times 100$$

The acceptance range of mass balance for full impactors in the European Pharmacopoeia is between 75% and 125%.

## Statistical Analysis

All measurements were carried out in triplicate and expressed as the mean value  $\pm$  standard deviation (SD). Data were statistically compared by Student's *t*-test and analysis of variance followed by Tukey's *post hoc* test using IBM SPSS Statistic 22 software.  $P < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

### Characterization of Coloaded Liposomes

DPPC, cholesterol, and DOPE at a mole ratio of 72:8:20% were considered appropriate in this study. This composition had previously resulted in optimal characteristics in terms of particle size distribution and drug entrapment for liposomes coloaded with erlotinib and genistein<sup>[11]</sup>. The latter drug has similar physicochemical properties (e.g., log*P* and aqueous solubility) to curcumin.

#### Effect of curcumin concentration on the properties of liposomes

Particle size distribution and surface charge  
DLS results showed that curcumin-encapsulated liposomes were small (~100 nm) with narrow size distribution for all preparations [Table 1]. No significant differences in the mean diameters were observed after curcumin loading ( $P > 0.05$ ) compared to the empty liposomes. Empty liposomes and formulations containing three concentrations of curcumin were approximately neutrally charged, due to the components of the formulation. Overall, loading of curcumin into the DPPC-based liposome formulation at the concentration range studied did not impact the key characteristics of liposomes studied, namely, mean hydrodynamic diameter, size distribution, and surface charge.

#### Encapsulation efficiency and drug loading

A large difference in encapsulation efficiency between 2.5% and 5% w/w curcumin-incorporated liposomes ( $P < 0.05$ ) was observed, whilst drug loading for both preparations showed no

significant difference ( $P > 0.05$ ) [Table 1]. When increasing the concentration of curcumin to 10%, there was a significant decrease in entrapment efficiency ( $P < 0.05$ ), though drug loading for 10% curcumin was similar to the other two formulations ( $P > 0.05$ ). These observations suggest that loading of curcumin into DPPC-based liposomes at the concentration of 2.5% w/w curcumin in total lipids was optimal, due to the maximum encapsulation efficiency combined with a suitable mean diameter for aerosolization (<200 nm). During liposomal preparation, additional curcumin higher than 2.5% w/w may not cause any further physically encapsulation into the available hydrophobic hydrocarbon region within the liposomal bilayers. This suggested that the highest curcumin incorporation had been established in the liposomal bilayers<sup>[51]</sup>.

#### Effect of filtration, used for removing untrapped drug, on the properties of liposomes

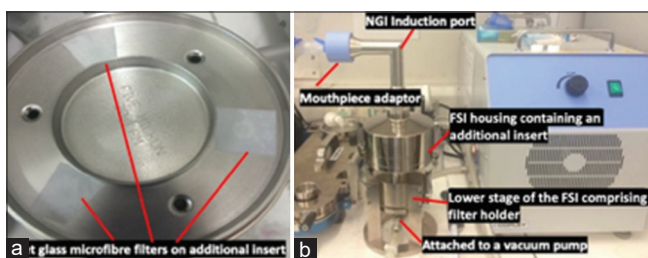
The mean particle size for all formulations was much smaller than the nominal pore size of 0.45  $\mu\text{m}$  hydrophilic cellulose acetate membrane filter used to remove untrapped curcumin from preparations [Table 2]. The mean size of 2.5% w/w curcumin-loaded liposomes was about 110 nm after 30 min probe sonication (pre-filtered liposomes), with a final mean particle size of 97 nm after removing untrapped curcumin (post-filtered liposomes), while the size of empty liposome was approximately 110 nm<sup>[11]</sup>. The particle size distribution (represented by the PDI) for the three preparations was similar, with a PDI of approximately 0.3 ( $P > 0.05$ ), representing a relatively homogeneous system. These results were in a good agreement with previous reports<sup>[11,39]</sup>. The entrapment efficiency and drug loading before and after passing sonicated curcumin-loaded liposomes through the syringe filter were not significantly different ( $P > 0.05$ ). All these observations suggested that method of separating non-incorporated insoluble drug using 0.45  $\mu\text{m}$  hydrophilic membrane filters was suitable, in accordance with the previous reports<sup>[11,18]</sup>.

#### Morphology

The morphology of curcumin-loaded liposomes prepared by thin-film hydration followed by 30 min probe sonication produced a population of spherical MLVs with a mean hydrodynamic diameter of approximately 100 nm [Figure 2]. These findings are similar to the previous reports of liposomes prepared and reduced in size using the same methods<sup>[8,10,52,53]</sup>.

### Aerodynamic properties of nebulized curcumin-loaded liposomes

The FSI allowed for a relatively fast determination of the properties of nebulized liposomal aerosols. The deposited



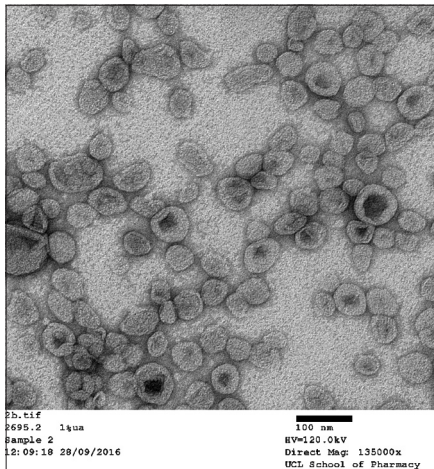
**Figure 1:** Fast Screening Impactor (a) three pieces of wet glass microfibre filters covered on additional insert and (b) the experimental setup

**Table 1:** Particle size distribution, zeta potential, entrapment efficiency, and drug loading of curcumin-loaded liposomes after passing through a hydrophilic membrane filter at various concentrations ( $n=3$ , mean $\pm$ S.D.)

Curcumin concentration	Hydrodynamic diameter (nm)	PDI	Zeta potential (mV)	%EE	%DL
2.5%	96.56 $\pm$ 11.68	0.30 $\pm$ 0.07	+0.39 $\pm$ 0.07	86.60 $\pm$ 3.72	2.12 $\pm$ 0.09
5%	128.95 $\pm$ 53.29	0.36 $\pm$ 0.04	-0.66 $\pm$ 0.05	55.17 $\pm$ 15.93	2.70 $\pm$ 0.78
10%	127.48 $\pm$ 31.11	0.41 $\pm$ 0.18	+2.17 $\pm$ 0.05	20.38 $\pm$ 4.81	1.86 $\pm$ 0.88
Empty liposomes	110.07 $\pm$ 3.69	0.30 $\pm$ 0.06	+0.30 $\pm$ 0.06	-	-

**Table 2:** Particle size distribution, zeta potential and entrapment efficiency of sonicated curcumin-loaded liposomes ( $n=3$ , mean $\pm$ S.D.)

Formulations	Hydrodynamic diameter (nm)	PDI	%EE	%DL
Pre-filtered liposomes	110.11 $\pm$ 14.89	0.42 $\pm$ 0.07	94.84 $\pm$ 4.93	2.27 $\pm$ 0.12
Post-filtered liposomes	96.56 $\pm$ 11.68	0.30 $\pm$ 0.07	86.60 $\pm$ 3.72	2.12 $\pm$ 0.09
Empty liposomes	110.07 $\pm$ 3.69	0.30 $\pm$ 0.06	-	-

**Figure 2:** Transmission electron microscope image of curcumin-containing liposomes prepared by thin-film hydration method, followed by probe sonication

curcumin was collected from the FSI for analytical quantification within approximately 30 min, potentially halving the amount of time taken per determination compared to a full cascade impactor<sup>[54]</sup>. The fewer stages and simple design of the FSI can also help to reduce any operator-dependent variation, such as technique for sample recovery from the impactor. The FSI was previously evaluated and shown to be particularly valuable compared to a full impactor where there is a small fill volume of nebulizer solution, or a formulation having small amounts of active substance, for example, due to small loading capacity of liposomes<sup>[11]</sup>.

The values of % mass balance of curcumin from the impactor were within the requirements of the European Pharmacopoeia<sup>[49]</sup>. About 100% mass balance is very difficult to obtain due to a loss of curcumin when manually collecting and rinsing the impactor to collect samples for analysis. Most of the liposomal curcumin emitted from air-jet nebulizer deposited on the lower stage of the FSI rather than additional insert within FSI housing.

Table 3 indicates that about 58% of curcumin goes in the impactor. Decreasing the particle size, surface tension, and viscosity of liposomal dispersions possibly can reduce the residual volume on nebulization as previously suggested, yielding higher FPD<sup>[55,56]</sup>. However, HPLC water was used as a vehicle in this preliminary study to obtain the simple formulation during formulation development. As compared to our earlier study of liposomes coloaded with erlotinib and genistein under the same experimental conditions<sup>[11]</sup>, air-jet nebulizer attached to the FSI showed significantly higher output efficiency in delivering curcumin-loaded liposomes in terms of FPF in this study. The FPF from the earlier study was approximately 42%. This may be due to the lower total lipid concentration and

**Table 3:** Aerosol properties of nebulized liposomal curcumin formulation determined using the FSI air-jet nebulizer ( $n=3$ , mean value $\pm$ S.D.)

Aerosol parameters	Emitted dose (%)	Fine particle dose ( $\mu$ g)	Fine particle fraction (%)
	58.91 $\pm$ 5.58	53.00 $\pm$ 8.41	53.25 $\pm$ 1.20

different properties of drug. However, the effect of changes in formulations merits further investigation using the FSI.

The developed curcumin-loaded liposomes were successfully nebulized using the air-jet nebulizer, with 53.25% of aerosolized curcumin being deposited in the lower stage of the FSI (cutoff diameter 5  $\mu$ m). The FPF was approximately 50% were well agreed with other studies using nebulizers for the delivery of curcumin-loaded nanoemulsions and polymer-glycerosomes to the lungs, indicating the predominant of the nebulized curcumin could be successfully reached the deep lung region<sup>[57,58]</sup>.

The findings demonstrate that most of the liposomes delivered from the nebulizer were deposited on the lower stage of the FSI, suggesting that these would be deposited in the respiratory airways of humans.

## CONCLUSIONS

A curcumin-loaded liposomal formulation for pulmonary delivery was successfully designed and developed. The liposomes prepared by thin-film hydration, followed by probe sonication for size reduction has been characterized for size distribution, surface charge, and drug entrapment. The FSI, based on AIM, demonstrated that the formulation aerosolized using a medical air-jet nebulizer had good aerosolization characteristics, generating high FPF and FPD. This is promising for future formulations and their preparation methods. Although very useful in this research and development study, it should be noted that the FSI has only two stages as opposed to the multiple stages of a full cascade impactor and hence it provides only limited data regarding the aerosol particle size distribution within the nebulized aerosol cloud as compared with the full cascade impactor. The FSI is subsequently suggested for aerosol characterization during the product development as well as quality control processes for new candidates of pharmaceutical products.

In summary, the developed curcumin-loaded liposomes in this study can be considered as promising nanocarriers for pulmonary delivery with desirable size, drug entrapment, and aerodynamic properties.

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## DECLARATION OF INTEREST

The authors do not have any competing interests with regard to the use of this nebulizer and impactor in this work.

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