

Aerosolization of Protein Solutions Using Thermal Inkjet Technology

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ABSTRACT

Vapotronics Inc. is developing the thermal inkjet (TIJ) technology used extensively in the printer industry to create a digital aerosol inhaler for the inhalation of therapeutics for local and systemic delivery. The operation of thermal inkjet printers requires generation of high temperatures and vaporization of the liquid formulation to effect droplet ejection. A study was conducted to develop formulations that would permit the generation of aerosols of therapeutic proteins without damage to the inkjet system or degradation of the proteins. Two proteins, human growth hormone and insulin, were formulated and aerosolized. The aerosol was collected and subjected to assays to compare the physicochemical and biological activities of these proteins before and after aerosolization. In each case, there was no significant changes to the proteins as a result of the aerosolization, providing evidence that TIJ can be used for aerosolizing solutions of protein therapeutics.

Key words: inkjet, aerosol, protein therapeutics, growth hormone, insulin

INTRODUCTION

THE SHORTCOMINGS of currently available aerosol drug delivery systems ("puffers," nebulizers, dry powder inhalers) have created intense interest in developing alternative technologies. Improvements in delivery technology aim to provide accurate dose delivery, reliable bioavailability for systemic drug applications, and digital control for patient compliance. Several different technologies have been proposed to achieve these advances. An aerosol system based on mechanical extrusion of liquid is currently in advanced stages of clinical trials in the United States.^{1,2} Another prototype has been developed based on piezo-electric principles.³ Other aerosol

systems using dry powders are also well advanced in their development.^{4–6} A novel technology based on the thermal inkjet (TIJ) technology for aerosol generation has recently been developed by Vapotronics Inc. (San Diego, CA).

Thermal inkjet is one of several inkjet technologies that have been developed to provide a low cost printing technology for home and office use. Various electronic droplet delivery systems were researched in the 1940s and 1950s. Naiman of the Sperry Rand Corporation proposed the Sudden Steam Printing system in 1962.⁷ This idea was not realized until the late 1970s, when the development of personal computing with its demand for high-speed electronic printing accelerated the drive to commercialize inkjet technology.

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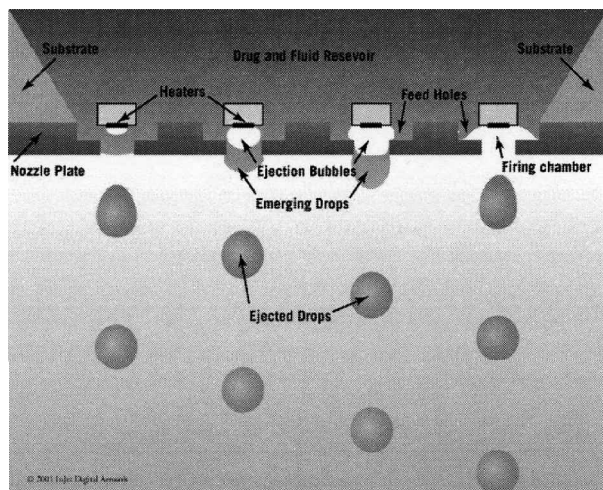


FIG. 1. Thermal inkjet ejection process.

Canon were the first company to demonstrate the "bubblejet" printer in Tokyo in 1981, and now TIJ printing has become widely commercialized by Canon and Hewlett Packard.

TIJ is a simple digital pump (Fig. 1). The volume of each droplet is governed by the geometry of the chamber from which it is fired. The liquid flows into the chamber by capillary action, and on demand, the heater heats the surface of the heater for 1–2 μ sec and reaches a surface temperature of up to 300–400°C before the superheated surface spontaneously boils. The vapor bubble created pushes the liquid out through the nozzle before the cooling surface allows the bubble to collapse and the chamber to refill. This droplet ejection process can be repeated up to 25 kHz in conventional printheads. Since this activity comprises all that is required for TIJ operation, it is very small, and an array of nozzles can be configured into an area of a few square millimeters.

Control systems for modern inkjet cartridges include on-head intelligence to monitor when a nozzle is not firing, allowing the printhead to redirect another nozzle to take its place in the printing event. This combination of intelligence and drop control allows for very accurate volume delivery. There are no moving parts in the TIJ system, and its operation is completely digital. It is therefore predicted that drug delivery control can be made realtime for dose decisions, including recording of dose and control of dose repetition to prevent overdosing.

The objective of the present study was to show

the feasibility of using the TIJ system to deliver aerosolized protein molecules.

MATERIALS AND METHODS

The TIJ device

The TIJ operates with two major components: the cartridges and the drive circuits. Cartridges were supplied by Hewlett Packard (San Diego, CA). Cartridges defined as 45 units were selected to deliver an aerosol with volume median aerodynamic diameter (VMAD) approximately 40 μ m, while the 48 units and the 48(8) units were delivering 20- μ m and 8- μ m aerosol, respectively as estimated by laser diffraction. The 45 and 48 units were commercially available inkjet cartridges normally used for high resolution printing. The 48(8) units were modified from the conventional 48 design by modification of the nozzle design with the intention of reducing the VMAD of the emitted aerosol. Novel R&D cartridges were selected to deliver a 3- μ m VMAD aerosol. All cartridges were acquired empty from HP and filled by syringe with selected formulations.

Drive circuits for the cartridges were constructed by Defiant Pty. Ltd. (Brisbane, QLD, Australia) and Vaptoronics Inc. The circuit assembly consisted of three components, a harness for the cartridge connections, a control box containing hardware and software required to effect the operation of the cartridge and a laptop computer using the "Hyperterminal" function in Windows 98 to read the status of the device soft-

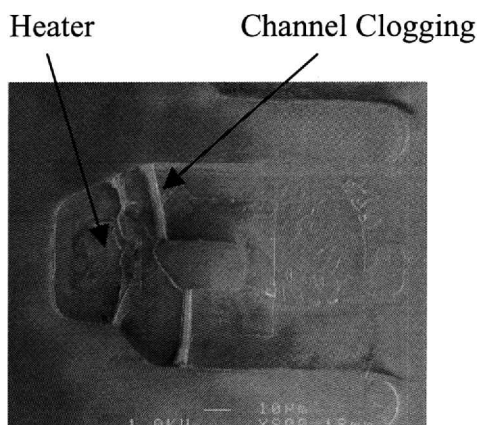


FIG. 2. SEM photo of denatured HGH from type 48 cartridge.

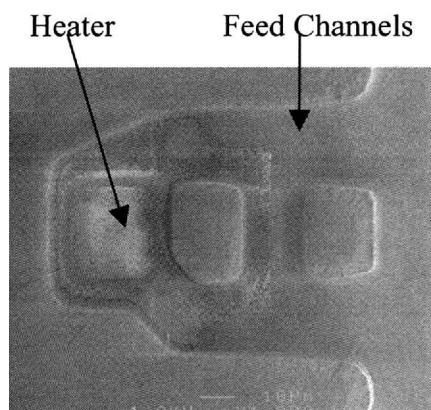


FIG. 3. SEM photo of protected HGH from type 48 cartridge.

ware and enter commands to it. The harness for the 45 units was obtained from a HP printer. The harness for the 48 and 48(8) units was a prototype inhaler that included capability to measure inspiratory air flow rates. The device software allowed selection of firing parameters such as firing duration and frequency and these parameters were set at 1 second duration firings at 10 kHz throughout this study.

Aerosol measurement

Aerosol particle size distribution was performed using a Malvern Mastersizer S laser diffraction (Worc, U.K.). The measurement was made by positioning the aerosol in the expanded laser beam across a 35-mm lens with minimum vignetting or interference from the cartridge. The instrumental presentation used to calculate the particle size distribution was: refractive index 1.33 for the aqueous droplets and 1.00 for the air dispersant.

Dose rates determination

Dose rates were estimated gravimetrically by capturing aerosol in a 50-mL Container.

Scanning electron microscopy of the cartridge

Microscopy was conducted with a Jeol 6400 Field Emission scanning electron microscope (SEM; Tokyo, Japan) at the University of Queensland Centre for Microscopy and Microanalysis to examine the surface of the TIJ resistors for deposition and degradation.

Bioactivity and physical stability of proteins

Two proteins were used for the study: human growth hormone (hGH; Breasgen, Adelaide, South Australia) and insulin (Bovine pancreas, I-5500; Sigma, St. Louis, MO).

Human growth hormone

The bioactivity of hGH was determined by a cell proliferation assay, where pro-B cells stably transfected with hGHR cDNA proliferate over 24 h in proportion to hGH concentration.⁸ Cell number is indicated by MTT substrate conversion to an insoluble formazan chromophore by endogenous mitochondrial dehydrogenases, measured at 595nm. The interassay coefficient of variation of the assay is less than 10%. The hGH solutions were also assessed for protein aggregation by high-performance liquid chromatography (HPLC) with UV detection (Shimadzu, Tokyo) with an Ultrahydrogel 250 (Waters, Milford, MA) column.⁹

Insulin

The aggregation of Insulin from TIJ was examined using HPLC (Waters) which consisted of a 515 pump, a 717 autosampler, and a 2487 UV detector. The aggregates were eluted from a Protein-Pak column 125 (Waters) using phosphate buffer (100 mM sodium phosphate and 500 mM sodium chloride, pH 6.6–6.8). Deamidation of in-

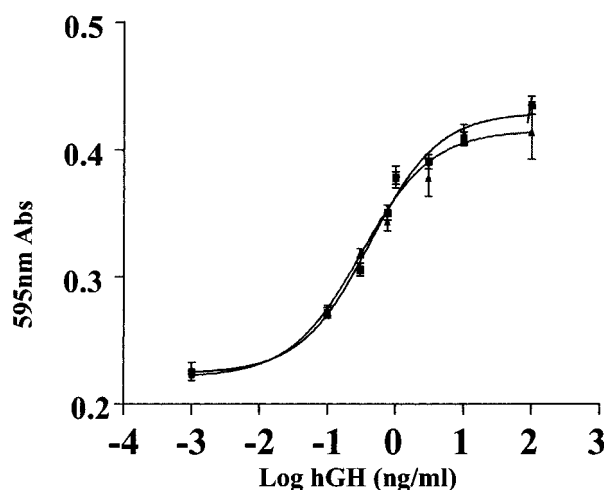


FIG. 4. hGH bioactivity. ■, preaerosolization; ▲, postaerosolization.

Overlaid Chromatograms of hGH

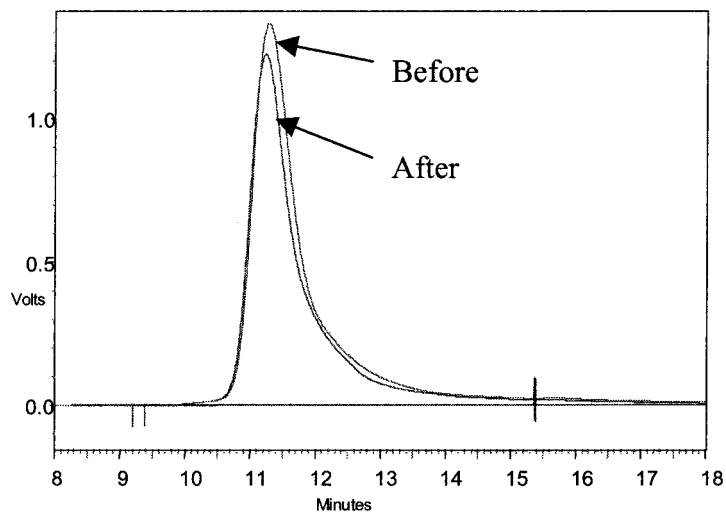


FIG. 5. Overlaid chromatograms of hGH.

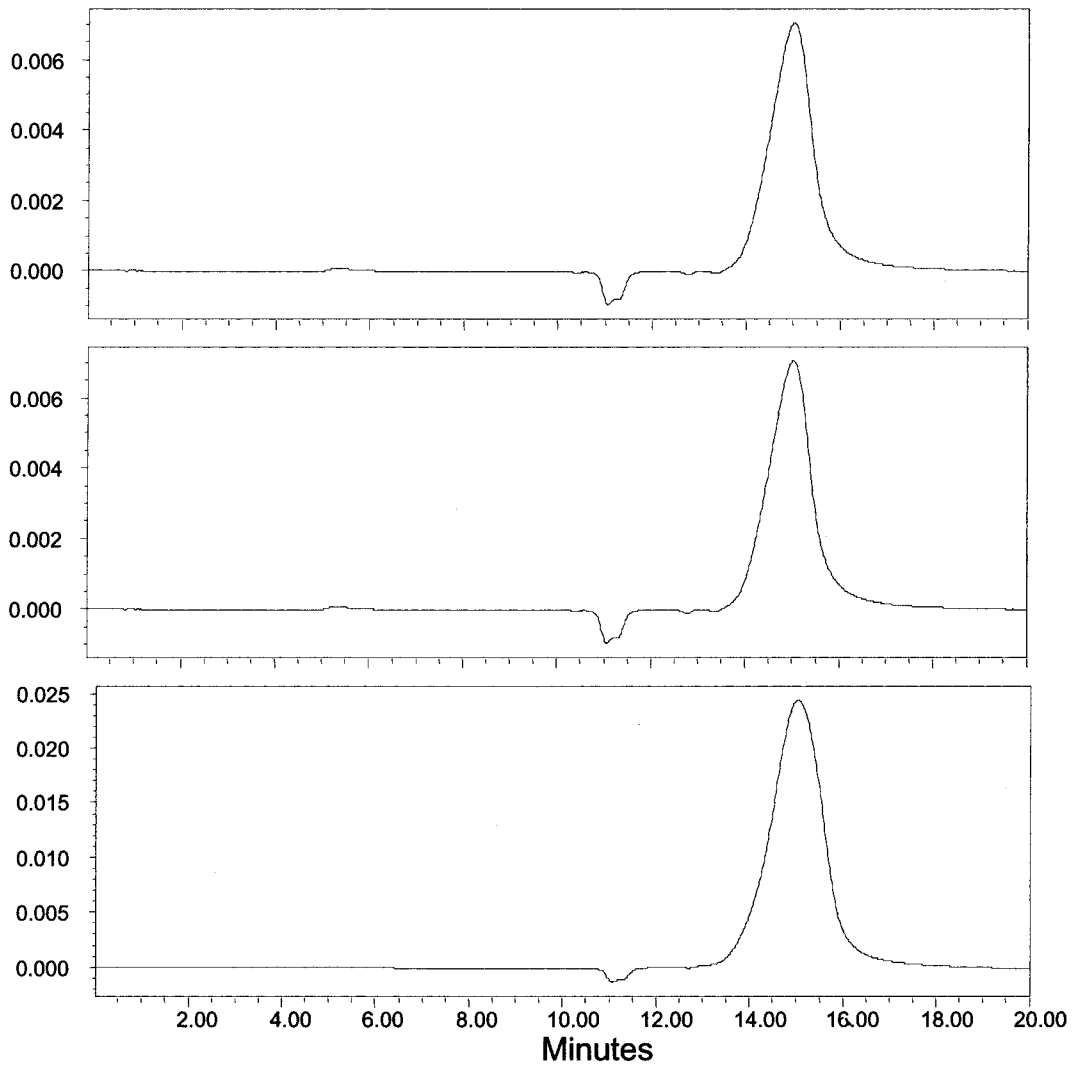


FIG. 6. Size exclusion HPLC of insulin reference solution (top), spray 75-100 of 5 mg/mL (middle), and spray 1-25 of 10 mg/mL (bottom) of insulin solution.

sulin was assessed by HPLC using a Symmetry 300 C18 column (Waters) eluting with a gradient from 26% to 33% acetonitrile (Mallinkrodt, Paris, KY; HPLC grade) containing 0.1% trifluoroacetic acid (Sigma; HPLC grade).

RESULTS

Human growth hormone

hGH was formulated at 2 mg/mL in deionized water and fired from type 48 cartridges. Dose rate

of aerosol indicated the cartridge was no longer functioning after 15 one second duration firings. The cartridge was disassembled and examined. Figure 2 shows the fluidic channels of the chamber blocked with protein precipitate. An hGH formulation containing 2% polyethylene glycol 8000 (Sigma) and 0.1% Tween 20 (Sigma) was prepared and the firing repeated. The cartridge continued to fire at an average of 20 μ L/sec for 100 1-sec duration firings. The aerosol particle diameter was estimated by laser diffraction to have a VMAD of 7.68 μ m with a span of 1.189 where the

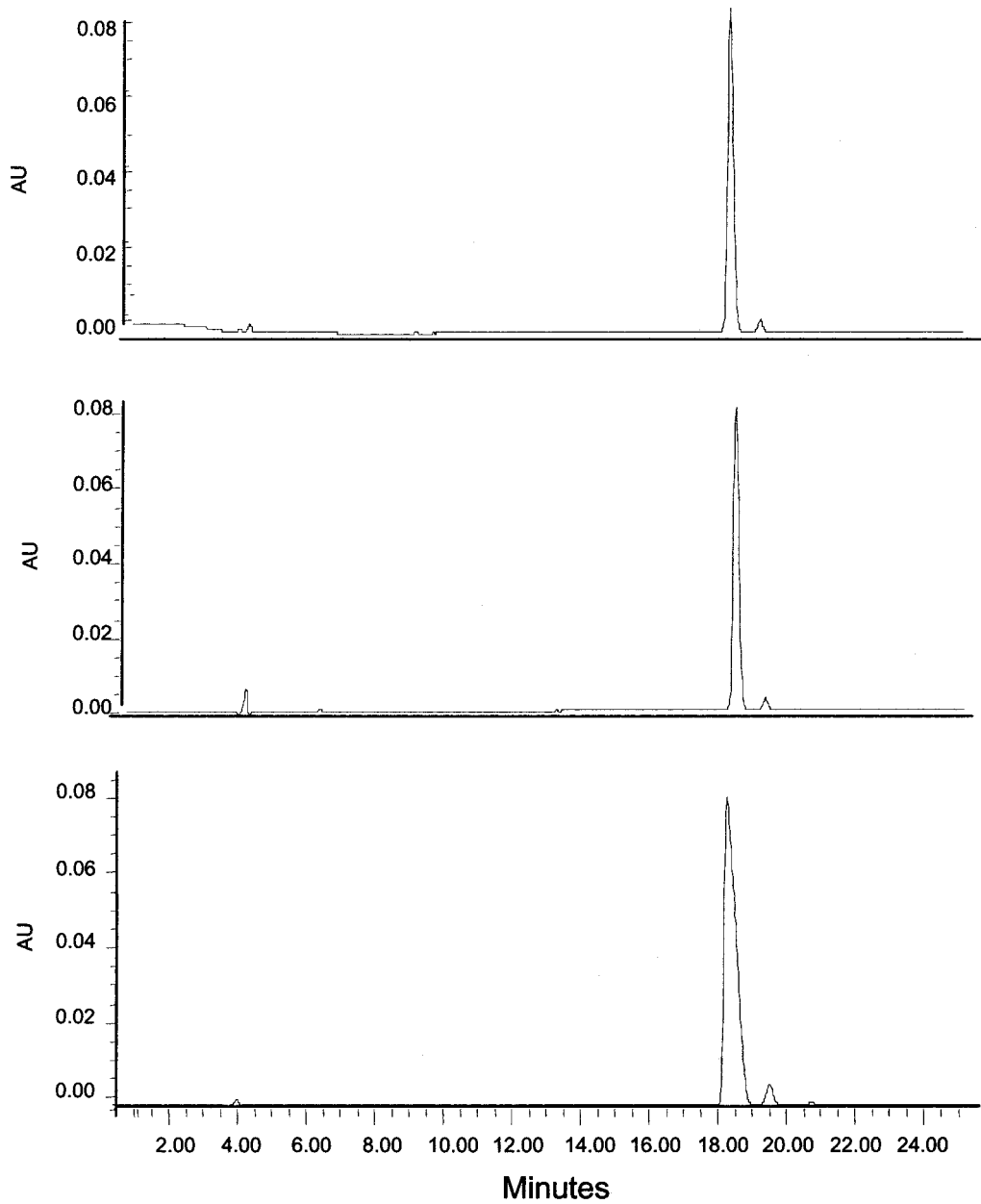


FIG. 7. Reverse phase HPLC of insulin reference solution (top), spray 75-100 of 5 mg/mL (middle), and spray 1-25 of 10 mg/mL (bottom) of insulin solution.

span is defined as the difference between the particle size of 90% cumulative mass and 10% cumulative mass divided by the median mass particle size. The cartridge was disassembled after the weight trial and examined by SEM. Figure 3 shows the fluidic channels of the chamber to be clear of any debris. This appearance was noted to be similar to a cartridge that had been used with deionized water.

The stabilized hGH formulation was aerosolized and recovered with the container being replaced every 20 firings. The solutions before firing and that collected from the last 20 firings were tested to determine their bioactivity. The result was interpreted by comparison of the ED50 of the dose-response lines, and indicated no measurable difference between the activities of the two solutions (Fig. 4).

The size exclusion results showed that the chromatograms for the formulations before and after aerosolization have indicated no increase in retention time for the hGH nor any higher molecular weight shoulders (Fig. 5).

Insulin

Insulin was formulated at 5 and 10 mg/mL and stabilized in the same manner as the hGH solution. The formulation was aerosolized, and each successive sample, containing a total of 25 firings, was collected and assayed for aggregation and deamidation of the protein. The results show no significant difference between the solution before aerosolization and after aerosolization in any of the insulin formulations and firings (Figs. 6 and 7). Aerosol characteristics using insulin were equivalent to that observed for hGH.

Latest TIJ design

A more recent TIJ design was tested by laser diffraction to determine whether a respirable aerosol could be generated. The cartridge was filled with a solution of 0.1% Tween 20 (Sigma) and fired with the same parameters as for the cartridges used above. The $D(v1,0.5)$ for the aerosol was measured as $3.01\ \mu\text{m}$ with a span of $0.945\ \mu\text{m}$. The distribution of particle size is shown in Figure 8.

DISCUSSION

The high temperatures used by TIJ to propel droplets from the chamber were shown to cause

precipitation of protein solutions that result in the malfunction of the TIJ system by preventing flow of liquid from the reservoir to the firing chamber as shown by electron microscopic examination of the chamber and fluid channels. The use of a humectant and a surfactant together were able to overcome the protein precipitation so that the device could continue to fire for 100 firings at 10 kHz or 1 million firing events per chamber without a decay in the delivery rate. Examination of the chambers after firing 1 million events showed the channels of the device to be free of debris or precipitate and the surfaces to be clear of visible charred material. The prevention of denaturation of protein by the addition of the excipients is believed to be the result of desolvation of the protein through hydrophobic association of the humectant with the protein. This association may inhibit the protein from structural modification during heating.

Evaluation of the bioactivity of the hGH solution that had been fired from the cartridge indicated there was no discernible difference between pre- and post-aerosolized solutions. Evaluation of the insulin also demonstrated stability of the protein structure. Both the size exclusion and reverse phase HPLC experiments showed equivalent profiles for the solutions before and after aerosolization, indicating that there was no detectable aggregation of the insulin or deamidation of the insulin during the aerosolization process.

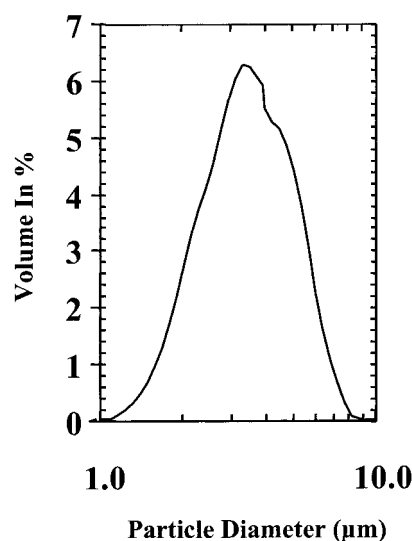


FIG. 8. Aerosol particle size distribution of a new TIJ aerosol measured by laser diffraction (Malvern Mastersizer).

Although the aerosol tested had a mean diameter of approximately 8 μm and is too large for inhalation, the experiments were designed to assess the protein stability during high thermal shock. Initial testing of a new TIJ design indicates it is possible to create a respirable aerosol with this technology and further testing will endeavor to combine the protein formulations with the new design.

CONCLUSION

Although TIJ is a high-temperature aerosolization system, it has been shown that formulations can be prepared that will allow labile proteins to be subjected to the aerosolization conditions without compromise of their structure or activity. It has also been shown that the technology can be configured to create a respirable aerosol, permitting the further development of the technology for pulmonary drug delivery.

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