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Microaerosol and Nanoparticle Synthesis for Drug Delivery via Surface Acoustic Wave Atomization

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Abstract

We describe the fabrication of a surface acoustic wave (SAW) atomizer, and show its ability to generate monodisperse aerosol and particles for drug delivery applications. In particular, we demonstrate the generation of insulin microdroplets for pulmonary delivery, and polymeric nanoparticles for one-step rapid encapsulation using SAW devices with resonance frequencies of 8.6 MHz and 19.3 MHz. Insulin droplets around 4 μm were obtained, matching the optimum range for maximising droplet absorption in the alveolar region. Nanoscale polymer particles (130-220 nm in diameter) were obtained through a non-equilbrium evaporation and nucleation process, thereby presenting a quick and simple means for nanoparticle synthesis. These results exhibit the feasibility of SAW as a novel method for producing particles and droplets of controlled sizes.

Introduction

The applications of ultrasonic atomization for the generation of aerosol sprays are growing due to its capability for producing very small droplets with very narrow size distributions. The droplet size depends on the ultrasonic frequency, and hence it is possible to generate droplets in the micron range by using MHz-order driving frequencies. With higher frequencies, capillary waves are excited on the free surface primary drop at shorter wavelengths; destabilization of these waves then results in smaller atomized droplets. Nevertheless, ultrasonic atomizers are limited by a maximum frequency of around 1 MHz, due to the limitations of the piezoelectric material. It is however possible to circumvent this limitation by employing surface acoustic waves (SAWs), which are nanometer order amplitude elastic waves that propagate along the surface of a piezoelectric substrate, wherein it is possible to obtain working frequencies in the MHz-GHz range. Liquid atomization using SAW was first shown by Kurosawa *et al*. [5, 6].

In this work, we demonstrate the ability of SAWs for generating aerosol droplets with a relatively monodisperse size distribution for drug delivery applications. The production of aerosol sprays with droplet sizes between 1-5 μm are particularly interesting for the pulmonary delivery of therapeutic proteins and peptides, such as insulin or growth hormones, to induce systemic responses and control biological processes [1]. The pulmonary lung represents a suitable and effective alternative for drug delivery, due to its large surface area. However, optimum drug deposition and absorption is limited to a narrow droplet size range; particles smaller than 1 μm are mostly exhaled whereas the majority of particles larger than 5 μm are deposited in the tracheobronchial region. The 10 MHz order SAW resonance frequencies in this work allow the generation of aerosol droplets in this narrow size range. In particular, we use insulin as a model drug. The inhalation of regular insulin has been found to be a safe, painless and reliable option to the subcutaneous injected insulin [9]. As such, we present the SAW technology as a rapid and simple alternative for the development of a portable, low power pulmonary insulin delivery device.

In addition, we also show that the SAW atomization technique can also be exploited to synthesize 100 nm order polymeric particles if atomized droplets within which a polymeric excipient is dissolved undergo a non-equilibrium evaporation and nucleation process. The possibility of producing dry nanoparticles of proteins/peptides or polymer nanoparticles presents a significant opportunity for the encapsulation of drugs or therapeutic molecules into biodegradable polymer shells for controlled release drug delivery. The current conventional techniques for the production of polymer nanoparticles, such as solvent evaporation/extraction, spray drying, nanoprecipitation or emulsion photocrosslinking [2, 8, 12], typically require multi-step procedures and often result in a wide distribution of particle sizes. A rapid and straightforward single-step procedure such as that developed here using SAW therefore poses as an attractive alternative and advance to these techniques.

Experiments

The SAW atomisation device essentially comprises a single interdigital transducer (IDT) consisting of pairs of straight aluminium electrodes sputter-deposited onto a 127.68° *yx*-cut lithium niobate (LiNbO3) single crystal piezoelectric substrate. When a sinusoidal electrical signal matching the operating frequency is applied to the IDT, *x*-propagating Rayleigh waves are generated. A Rayleigh wave has an elliptical displacement on the surface due to the combination of its two mechanical acoustic components: a longitudinal component along the direction of propagation, and a transverse one perpendicular to the surface. Resonance frequencies of 8.6 MHz and 19.3 MHz were chosen to obtain insulin droplets 2-4 μm in diameter, the optimum size for droplet adsorption within the alveoli of human lung tissue. Figure 1 shows the fabricated SAW device and the Rayleigh wave generated on its surface measured with a 3D laser Doppler vibrometer (LDV).

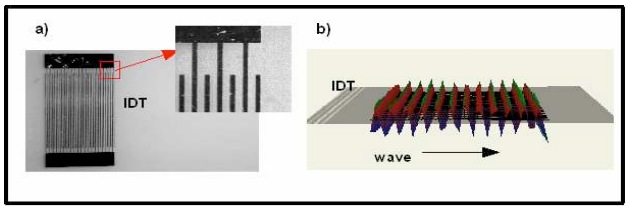

Figure 1. a) SAW device surface (close up of IDT); b) Example of wave propagation along the surface, measured with a 3D LDV.

In the experiments, three different solutions were placed on the SAW surface: distilled water, insulin and poly-ε-caprolactone (PCL). The insulin solution was prepared by dissolving 2 mg of insulin powder (Sigma, I5500) in 1 ml of DI water. Hydrochloric acid (1 M) was added in 2 µl increments until the insulin was completely dissolved. The final ph was adjusted to 5 by adding NaOH (1 M). The PCL polymer (Sigma-Aldrich Pty. Ltd., Australia) was dissolved in acetone (1% w/w).

When the crystal surface is in contact with the working solution, the transmission of acoustic energy into the liquid drop occurs through leaky SAW, in which longitudinal waves propagate into the liquid at the Rayleigh angle, defined as $\theta = \sin^{-1}(c_l/c_s)$, where c_l and c_s are the bulk velocities of the longitudinal wave in the liquid and in the substrate, respectively; for $LiNbO_3$ and water, the Rayleigh angle is 23°. As the leaky SAW propagates into the droplet, capillary waves are induced at the liquid-air interface due to excitation of lower-order vibration modes within the solution and at the liquid interface. With sufficient power input into the solution such that the acoustic stress overwhelms the capillary stress, destabilization of the interface occurs resulting in atomization of the liquid, thus producing a fine continuous mist of droplets. To visualize the atomization process, a high speed video camera (iSpeed, Olympus, Japan) was used. Figure 2 shows the atomization of water with a 19.3 MHz SAW device, being possible to observe the capillary waves produced on the liquid surface as well as the ejection of a droplet.

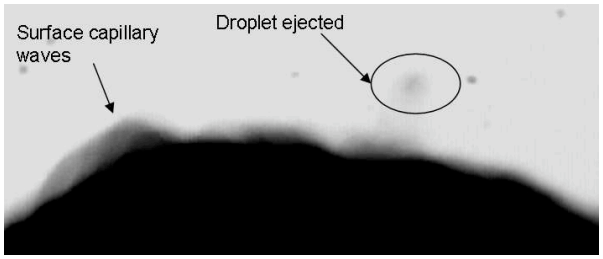

Figure 2. Atomization of a water droplet.

Insulin microdroplet generation

A condensation particle counter (CPC ; TSI Inc., model 3775) was first used to determine the minimum power input required to atomize the droplets and measure the number of droplets generated. The CPC system is based on the optical detection of aerosol particles/droplets whose size has increased due to condensation of a supersaturated vapour around the particle/droplet. The experiments were performed by atomizing a known volume of water into a small chamber. The aerosols generated were delivered to the CPC via 20 cm conductive tubing. With a 19.3 MHz device and an initial water volume of 4 µl, the minimum power needed was found to be ~0.3 W (13 V_{RMS}). The particle number density was observed to increase with the applied voltage, as shown in figure 3.

A continuous flow of insulin solution was then maintained over the SAW surface with the use of a syringe pump. The flow rate was adjusted such that the insulin solution forms a thin layer of liquid over the surface. Subsequent atomization of this thin liquid film then resulted in the formation of a continuous mist consisting of fine insulin liquid aerosol droplets over several minutes. The insulin atomization can be ensured by inspection of the substrate surface; if

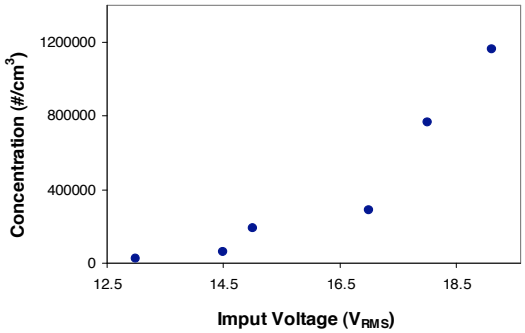

Figure 3. Particle number concentration as a function of the driving voltage.

the solvent in the drop was left to evaporate without atomization, a deposit of insulin particulates remained on the surface. On the other hand, a clean surface void of insulin particles was observed after atomization of the insulin drop.

For measuring the produced particle size, the insulin droplets were collected on a glass slide, initially covered with Teflon to avoid the hydrophilic effect of the glass over the droplets shape, and imaged in real time with the high speed video camera connected to a microscope (BXFM, Olympus, Japan). The distance between the SAW device and the glass slide was 1.8 mm to eliminate the evaporation effect on the particle size. The droplet size was determined using image processing software (ImageJ), for calculating the drop area. The droplets radius was obtained from this data, assuming spherical droplets. Figure 4 shows the size distribution obtained with this method. The particle mean size is about 4.5 µm, which match the range size needed for the optimal deposition of insulin in the pulmonary system.

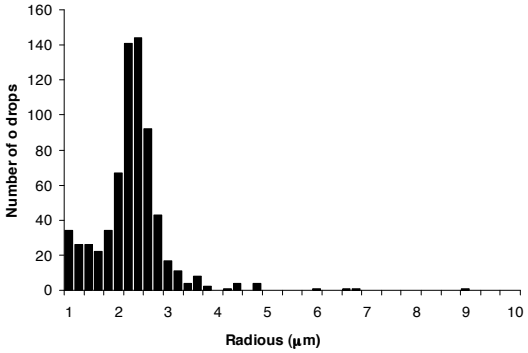

Figure 4. Particle size distribution.

The mean diameter, D of droplets generated by using low frequency ultrasonic vibrators can then be estimated from Lang's equation [7]:

$$D \sim \left(\frac{8\pi\gamma}{\rho f^2}\right)^{1/3}, \quad (1)$$

wherein γ and ρ are the surface tension and the density of the liquid, respectively, and f is the applied frequency. This equation has been likewise used for high frequency vibrators and SAW atomizers. Using this equation, the expected diameter of insulin droplets generated with a 19.3 MHz device, considering that the surface tension and the density are practically the ones from water, is ~ 1.7 µm, which is less than half of the measured size. A review of the

different works performed in this field reveal a discrepancy between the produced droplet size and the expected one. This could be due to the assumption of Lang's equation validity for high frequency atomizers, being necessary a review of this equation for these cases [10].

Depending on the delivery route chosen and the final application, insulin powder would be desired. The production of a fine insulin powder, instead of an aerosol, is straightforward. Once the insulin aerosol is produced, the evaporation of the carrier solvent, during the time in flight, will leave solid particles. A scanning mobility particle spectrometer (SMPS; TSI Inc.) was used to measure the solid insulin particles produced with this method. The SPMS use an Electrostatic classifier (TSI Inc., model 3080) and a condensation counter particle (TSI Inc., model 3775), for sizing particles in the range of 5nm–1μm. In this case, the insulin atomization was performed inside a chamber, connected to the SMPS inlet. Figure 5 shows an example of the measurements carried out with this technique. The statistics performed over several measurements give a size of 95 nm for the insulin particles. A lower second peak is generally visible, around 190 nm, probably due to the incomplete evaporation of some insulin droplets or the agglomeration of some droplets during the time in flight. Assuming an initial droplet size of 4.5 μm, the expected solid particle size should be around 600 nm if considering spherical particles and a density of dry particles of 1g/cm^3. The measured solid particles are smaller than the calculated value, probably due to an initial overestimation on the measured droplets size and loss of insulin during the atomization process (in the fluid system or even deposited over the SAW device).

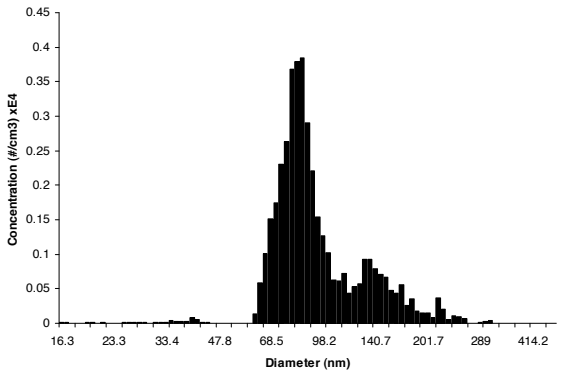

Figure 5. Aerodynamic diameter distribution.

On going experiments are being performed for measure the insulin concentration after the atomization process (for determining the insulin dosage), as well as the droplet assessment in an equivalent pulmonary lung, using a glass impinger.

Polymeric nanoparticle synthesis

An extension to the generation of microdroplets through SAW atomization is the production of solid polymeric nanoparticles. PCL nanoparticles were produced from an initial PCL solution (1% w/w PCL in acetone). A continuous flow for maintaining a volume of 25 μl on the substrate was used. As the PCL droplets were ejected, the residual solvent in which the PCL is dissolved evaporates in-flight, leaving behind solid polymer particles. The dried particles were collected in a continuously stirred mixture of deionized water and two different surfactants, SDS (anionic) and Tween-20 (non-ionic), at a different distances from the atomizer [3]. Due to the hydrophobic nature of PCL, the particles tended to accumulate on the bath surface, forming large aggregates. Before measuring the particles size, the large aggregates were removed and the suspension was centrifuged at 7000 rpm for 10 minute. The *z*-average diameter of the suspended particles was measured using dynamic light scattering, DLS (Zetasizer Nano S, Malvern Instrument Ltd., UK). Table 1 shows the particle sizes obtained with the DLS system, depending on the travel distances and the surfactant used in the bath. A clear dependency in the particle sizes could be observed when using Tween-20, increasing the size when increasing the surfactant concentration. This dependency is not observed when using SDS. With this surfactant, the smallest particles (181 ± 4.9 nm) were obtained when they were collected at a distance 8 cm away from the atomizer in a sonicated 10 mM SDS solution. The particle size increase with the distance, maybe due to the agglomeration of the PCL particles in flight ones they get dry.

SDS	Tween-20	Distance	*z*-average dia.
mM	% v/v	cm	nm
1	—	8	162.3 ± 3.0
10	—	8	153.4 ± 4.0
40	—	8	185.4 ± 4.5
70	—	8	180.2 ± 13.6
100	—	8	177.1 ± 6.9
—	0.1	8	159.8 ± 1.7
—	1	8	169.9 ± 7.6
—	5	8	247.5 ± 3.5
10	—	6	167.3 ± 5.6
10	—	12	214.6 ± 2.5
10	—	16	236.0 ± 61.0
10*	—	8	181.4 ± 4.9

Table I. Particle sizes obtained with the DLS system.

To corroborate the polymer particle size, transmission electron microscopy (TEM) has been used. The particle sizes obtained with the light scattering technique are consistent with that observed through TEM, which show spherical particles with an average diameter of 131.0 ± 4.9 nm. As shown in figure 5, these nanoparticles are comprised of agglomerations of particles of far smaller diameter.

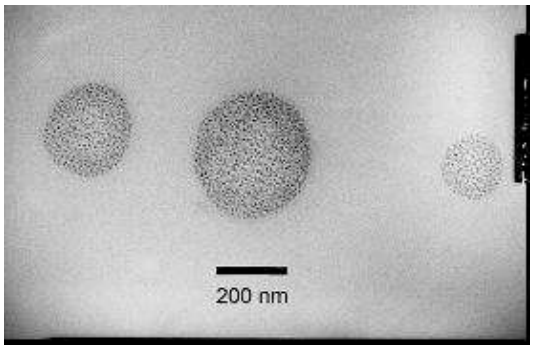

Figure 5. Transmission electron microscopy of the particles obtained by SAW atomization and solvent evaporation.

The particle formation can be explained via a twofold process: atomization of microdroplets due to destabilization of the drop interface under acoustic forcing, followed by a second process that occurs during in-flight evaporation of the solvent. Due to rapid quenching as the post-atomized droplet is ejected, the evaporation process is spatially non-uniform, resulting in a thermodynamic instability in which phase separation via spinodal decomposition takes place. This results in a metastable state in which both polymer-rich and solvent-rich regions exist. Since the polymer-rich region which typically appears adjacent to the droplet surface solidifies faster than the solvent-rich region inside the drop, a series of nucleation sites is created. Each nucleation site is then responsible for the clustering of PCL molecules [4, 11], further promoted by the hydrophilicity of the acetone solvent, until a critical size is reached. From classical nucleation theory, we have shown that this corroborates well with the sub-50 nm particulates that aggregate into the larger particle agglomerations seen in figure 5 [3]. Work is currently being undertaken to demonstrate encapsulation of active pharmaceutical ingredients within these nanoparticle agglomerates.

Conclusions

We demonstrate a straightforward and rapid atomization process driven by surface acoustic waves that is capable of continuously producing monodispersed distributions of about 4 μm aerosol droplets of insulin and 150-200 nm poly-ε-caprolactone (PCL) particle aggregates comprising agglomerations of sub-50 nm PCL molecule clusters. These results demonstrate the potential of the atomizer as a powerful tool in the development of miniaturized devices for protein inhalation delivery and the synthesis of nanoparticles from biodegradable polymeric excipients as a first step towards the encapsulation of active pharmaceutical ingredients for controlled release delivery by oral, transdermal, subcutaneous, or other routes.

Acknowledgements

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