Engineering Honors Capstone Final Report

Exploration of Protein-Specific Physical and Electrical Properties for the optimization of Electrohydrodynamic Jetting for Synthesis of Protein-Based Nanoparticles

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Table of Contents

Introduction

Electrohydrodynamic Jetting

The process used by the Lahann Lab, within the Biointerfaces Institute at the University of Michigan, to synthesize nanoparticles is known as Electrohydrodunamic Jetting (EHDJ). EHDJ is a complex process in which the charge of the fluid induced by the electric field influences the momentum flux of the fluid. It is done by supplying an electric charge to a solution which, at an induced flow rate, results in the solution "spraying" onto an oppositely charged base. It is often also called electro-spraying, electrospinning, or electro-printing. A schematic showing the equipment setup for this process is shown in Figure 1 below.

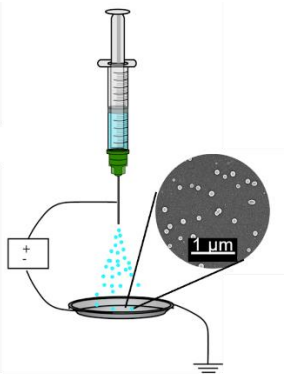

Figure 1. *Schematic showing the equipment setup to synthesize nanoparticles using EHDJ within the Lahann Lab*

A nanoparticle can loosely be defined as any particle of matter which is between 1 nm to 100 nm, however it is often used for particles up to 500 nm. As we typically use protein nanoparticles for the biological purposes within this lab, our jetting solution typically consists of a protein (most commonly Human Serum Albumin or HSA) as well as other antibiotics or bioactive components, dependent on its research purposes. The spraying mechanism results in droplets of protein solution precipitating as it travels from the needle, in which it is originally housed, to the metal pan, as well as evaporating solvent thus forming solid nanoparticles.

The Taylor Cone

The high electric potential between the capillary tip and the counter electrode creates an electrostatic force on the fluid, and the electric tangential force induces the formation of a Taylor cone. The Taylor cone can be observed at the interface between the needle and the material 'spray' or jet. This can be seen in Figure 2 below.

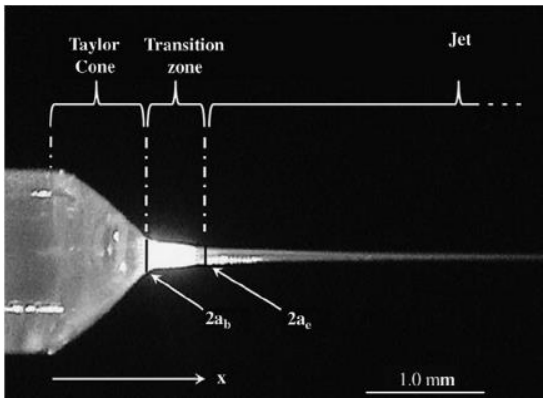

Figure 2. *Image showing the formation of a Taylor Cone at the interface between the needle and the 'jet'.*

The Taylor cone, despite it's name, can take on different physical representations dependent on the stability of the jetting process. Figure 3 below shows a summary of the possible jetting modes, dictated by the supplied parameters for the jetting process. As shown in Figure 3, increasing electric potential results in changes to the jetting mode. As Electric Potential can be defined as the potential energy per unit charge, this quantity is a factor of the supplied energy due to both flow rate and voltage. To achieve a stable jet, we typically aim to observe the 'Cone-Jet' shown in Figure 3.

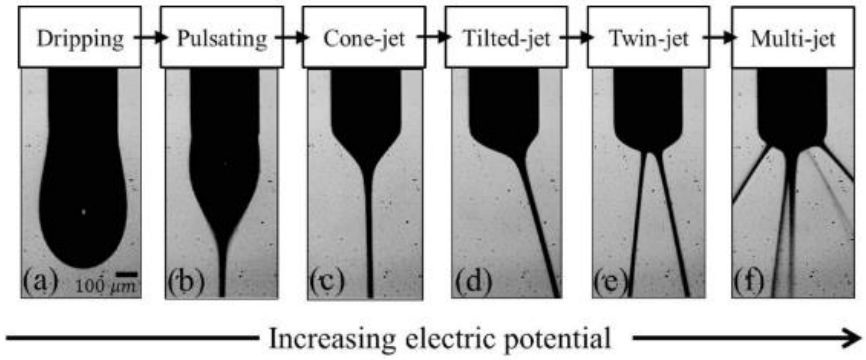

Figure 3. *Possible Jetting modes with respect to Electric Potential*

The problem which this project aims to evaluate is members of the Lahann Lab typically solely use human observation to determine whether the process has achieved stability of not. It is usually assumed to be stable once, upon shining a low intensity laser at the needle, the Taylor cone ‘looks conical’. In an effort to achieve this, members of the lab try to adjust the supplied voltage without any standard process or desired range, until the desired effect is achieved. This results in variability between researchers and could potentially lead to wasted resources as the particles cannot be observed for validity of use until after the entire jetting process is complete. The success of this process is therefore not based on any previously explored or optimized parameters.

The purpose of this project will be to create a basis for the parameter input ranges and solution properties that result in a stable Taylor cone and thus result in a successful jetting process. To do this we plan to explore the effects physical and electrical properties, such as viscosity, surface tension, permittivity, and conductivity, of commonly used protein solutions such as Human Serum Albumin on Taylor Cone stability and potentially how these impact critical aspects of protein nanoparticles such as size and morphology.

Primary Objective

To determine the optimum parameter ranges of voltage and flow rate necessary to achieve desired process stability and nanoparticle properties given a characterized jetting solution.

Research Questions

1. At what upper and lower voltage and flowrate bounds does the taylor cone begin to destabilize for a given protein solution?
2. Do process parameter bounds vary based on the jetting solution used?
3. Can knowledge of the physical and electrical properties of jetting solutions be used to predict parameters that ensure process stability?

Key Skills

Skills needed: Data analysis, objective comparison, iterative analysis

Skills to be gained: modelling of 3-d shapes, experimental design, data presentation and analysis

Methods

To achieve our project objectives, we evaluated jetting solutions on their physical and electrical properties which affect the jetting process. Our methods follow the initial scheme as dictated by the article titled *"Optimization of Experimental Parameters To Determine the Jetting Regimes in Electrohydrodynamic Printing"* by Lee, et al. This paper outlines their evaluation on the chemical properties relevant to EHDJ as well as how they defined dimensionless numbers to represent the jetting process.

Relevant Physical and Electrical Properties

As described by Lee et al, the physical and electrical properties which are relevant to the jetting process are:

- ρ - density
- γ - surface tension,
- ε_0 - permittivity of free space,
- ε' - permittivity of fluid,
- K - conductivity,
- η - viscosity,
- d -nozzle diameter,
- L - distance between nozzle and electrode

For the purposes of our project, we plan to evaluate protein solutions commonly used in our lab as well as equipment specifications to quantify each variable listed above.

Density

To evaluate this property, we used the following method:

1. Measure the weight of a 1 mL syringe using a balance.
2. Fill the syringe with 1 mL of the protein solution being evaluated.
3. Measure the weight of the 1 mL syringe containing solution.
4. Determine the weight of the solution as the difference between the 2 measured weights.
5. Divide the obtained weight by the volume within the syringe to obtain the density in units of or derived from g/mL.

Surface Tension

To evaluate this property we used the *Ramé-Hart Contact Angle Goniometer* coupled with the Ramé-hart syringe adapter and a 1 mL Hamilton Syringe. The steps to evaluate this property are as follows:

1. To start a new experiment, go to file and click on "New Experiment Wizard"
    - The software might send an error message saying that a specific method file cannot be found. Just click the "OK" button.
2. Select "Surface Tension – Pendant", then click next.
3. Fill in the name of the file with the following format: "year month day – sample rep number". Then click next.
4. In the next screen, user will select "Droplet phase", "External phase", and "Solid phase" which corresponds to the sample material, the material on which the sample is suspended (mainly air), and the material of the needle being used to dispense the sample. Once appropriate materials are selected, click next.
    - Note that if a material that has not been used before needs to be tested, user will need to add this material to the software. See the steps below to use the Phase Editor.
5. Wizard will prompt the number of total measurements. Select at least 10 and space them by 5 seconds each and select "Finish".
6. After exiting the wizard, software will ask user if experiment should be started. This will not immediately start the experiment, it will simply prompt the experiment set up. Select "yes".
7. Once experiment is started, a small screen called "Set cursor position" will pop up. This will be used to indicate the edge of the dispensing needle. Now the instrument and software are ready to measure the surface tension.
8. Place the syringe adapter in the syringe holder, the arm sticking out over the small metal stage.
9. Fill the Hamilton syringe with 1 mL of testing material. Then place the syringe in the syringe adapter.
10. Adjust height of syringe holder so the needle is at the center of the picture shown in the software and located on the top third of the screen. Make sure the needle is vertical and not at an angle.
11. If the metal stage is near the needle, move the stage down to make space for the needle and the sample droplet. The stage should be at least 4 inches from the needle, otherwise the droplet will be attracted to the stage.

12. Carefully, depress the syringe plunger to dispense a small droplet. The droplet should span the rest of the screen, but still be in view. This means that the bottom of the droplet should still be on screen, otherwise the measurements will not be accurate.
13. Return to the software and locate the edge of the needle. Double click the center of the needle at the edge where the droplet starts. This will align the crosshair so that the vertical line is going up and down the center of the needle, while the horizontal line goes across the needle opening.
14. Press "Measure".

Permittivity of Free Space

This quantity was taken as a constant which reflects the ability of electrical field to pass through a classical vacuum. The constant is approximately equal to 8.85E-12 F/m (farad per meter).

Permittivity of Fluid

This quantity was analyzed using the permittivity reader within the Kamcev Lab. The steps to evaluate this property are as follows:

1. Place an O-ring separator on the first metal plate.
2. Using a transfer pipette, add a few drops of solution on the plate with an O-ring separator.
3. Place the second plate above solution. At this point the solution should be 'sandwiched' between 2 plates. Remove excess solution that was displaced above the second plate.
4. Ensure all relevant wires are connected between the plates and permittivity reader. Connect red, white and blue wires to matching colors.
5. Open permittivity measurement software.
6. Measure for 30 minutes.

Conductivity

This quantity was analyzed using the conductivity meter within the Kamcev Lab. The steps to evaluate this property are as follows:

1. Turn on the conductivity meter and computer and open the conductivity measurement software.
2. Choose a channel for conductivity reading. The channels are labelled from 1-4 for each conductivity meter. Any channel except 4 is fine.
3. Place adequate solution in the channel container to completely cover the open end of the conductivity reader. 10 mL or more is fine.
4. Measure.

Viscosity

This quantity was measured using the Rheosense m-VROC viscometer. The steps to evaluate this property are as follows:

1. Log into the computer and start the Rheosense Control Software before turning on the instrument.
2. Turn on m-VROC using the switch behind the instrument. Turn on Thermocube chiller using the switch on the left of the instrument.
3. Allow instruments to boot and wait until software identifies instruments. The "Status" screen on the top of the software interface will indicate if instruments have been connected to the software or not. Once the light next to "m-VROC" and "Bath" turn green, the system is ready to go.
4. Now that the software is ready, user can start preparing sample. Attach Rheosense filling adapter by screwing it to the Hamilton syringe. Dip the tube onto the sample reservoir and draw the sample into syringe. Make sure to remove any bubbles from the syringe.
5. Once sample is in syringe, remove the filling adapter, safely place the syringe on the counter, and prepare instrument for syringe.
6. The right section of the m-VROC works like a syringe pump. During operation, the pusher block shown in Figure 1 depresses the syringe plunger to move sample from the syringe into the VROC cell. The pusher block needs to be locked during testing, however, make sure it is unlocked when loading the syringe with sample. To unlock the drive nut knob, pull the knob towards you and twist it. Note that it might be necessary to play with the knob to free it. Once unlocked, a gap will be seen between the nut and the side of the pusher block, which will expose a brass rod.
7. At this point, the syringe can be attached to the VROC inlet. If closed, open the syringe jacket by undoing the thumb screw and lift the lid. Figure 5a shows a stock image of the VROC chip with the inlet valve. The inlet valve fits on the syringe jacket. The valve handle protrudes out and serves as a support. As shown in Figure 5b, carefully lift the VROC chip and inlet valve (they are connected to each other), unscrew the black thumb cap from the inlet valve, and screw the syringe. Place the syringe in the jacket and fit the valve handle in the appropriate cutout. Note that valve handle should be closed. Leave syringe jacket open.

Nozzle Diameter

This quantity refers to the diameter of the needle attached to the syringe used for jetting. It is therefore a constant related to the equipment setup. This is known as 0.5 mm.

Distance Between Nozzle and Electrode

This quantity refers to the distance between the tip of the needle used for jetting and the metal pan electrode. This is related to the Lahann Lab jetting practices and is taken as 60 mm.

Dimensionless Parameters

As previously mentioned, the variable parameters which affect the stability of the jetting process are the supplied flow rate and supplied voltage. As defined in Lee et al, two dimensionless quantities can be defined to simplify analysis of these parameters. These are the dimensionless flow rate (α) and the dimensionless voltage (β). These quantities can be defined as follows:

- **Dimensionless flow rate (α)** – the ratio of the supplied flow rate (Q_s) and the critical flow rate (Q_c).

$$\textbf{Equation 1:} \quad \alpha = {}^{Q_s}/_{Q_c}$$

- **Dimensionless voltage (β)** – the ratio of the applied voltage (V_a) and the critical voltage (V_c).

$$\textbf{Equation 2:} \quad \beta = {}^{V_a}/_{V_c}$$

Exploring ratios of these dimensionless numbers will allow us to systematically determine the appropriate ranges of supplied/applied parameters to achieve the desired jetting mode.

Critical Quantities

As mentioned above, the dimensionless numbers are ratios of the supplied/applied parameter to its critical quantity specific to a given solvent system. These critical quantities of flow rate and voltage were determined to be the minimum process specifications necessary to achieve cone-jet formation. These quantities can be defined and evaluated as follows:

1. **Critical Flow Rate (Q_c)** – the specific flow rate that causes electrical stress to strip off (or shear) the surface charge of the fluid.

$$\textbf{Equation 3:} \quad Q_c = \frac{\gamma \varepsilon_0 \varepsilon'}{\rho K}$$

2. **Critical Voltage (V_c) –** the voltage that supports the meniscus on the capillary tube of diameter d.

$$\textbf{Equation 4:} \quad V_c = \sqrt{\frac{\gamma d}{\varepsilon_0}}$$

As critical quantities are solution specific, they must be re-evaluated for all desired jetting solutions.

Results

We successfully evaluated two protein jetting solutions on the properties previously discussed in the *Methods* section. The solutions evaluated are:

- 7.5% wt% HSA in 80:20 v/v% Water:Ethylene Glycol
- 3.75% wt% HSA in 80:20 v/v% Water:Ethylene Glycol

These solutions are commonly used by members of the Lahann Lab to synthesize multi-functional nanoparticles. Measured and calculated results for each solution system are discussed in subsections below.

7.5% wt% HSA in 80:20 v/v% Water:Ethylene Glycol

The physical and electrical properties discussed within the *Methods* section, were successfully evaluated and can be seen in Table 1 below.

Table 1. Measured physical and electrical properties of a 7.5% wt% HSA jetting solution in 80:20 v/v% Water:Ethylene Glycol

Measured Variables				Unit Conversions	
Quantity	**Symbol**	**Unit**	**Value**	**Unit**	**Value**
Density	ρ	g/ml	1.052	$kg \cdot m^{3}$	1052
Surface Tension	γ	$mN \cdot m^{-1}$	60.71	$N \cdot m^{-1}$	0.06071
Viscosity	η	mPa·s	2.8	Pa·s	0.0028
Conductivity	K	$uS \cdot cm^{-1}$	402.41	$S \cdot m^{-1}$	0.040241
Permittivity	ε'	-	114.91	-	114.91

Using the quantities above, the critical values previously discussed can be calculated. These can be seen in Table 2 below.

Table 2. Calculated critical values of a 7.5% wt% HSA jetting solution in 80:20 v/v% Water:Ethylene Glycol

Critical/Characteristic Values			
Quantity	**Symbol**	**Unit**	**Value**
Critical Flow Rate	Q_c	$m^{3} \cdot s^{-1}$	1.46E-12
Critical Voltage	V_c	V	1851.573
Time of Charge Transport	T_q	s	2.53E-08

With the evaluated critical values, the dimensionless numbers can be analyzed and observed for Taylor cone stability.

3.75% wt% HSA in 80:20 v/v% Water:Ethylene Glycol

The physical and electrical properties discussed within the *Methods* section, were successfully evaluated and can be seen in Table 3 below.

Table 3. Measured physical and electrical properties of a 3.75% wt% HSA jetting solution in 80:20 v/v% Water:Ethylene Glycol

Measured Variables				Unit Conversions	
Quantity	**Symbol**	**Unit**	**Value**	**Unit**	**Value**
Density	ρ	g/ml	1.087	$kg \cdot m^{3}$	1087
Surface Tension	γ	$mN \cdot m^{-1}$	64.24	$N \cdot m^{-1}$	0.06424
Viscosity	η	mPa·s	2.15538	Pa·s	0.002155
Conductivity	K	$uS \cdot cm^{-1}$	233.6	$S \cdot m^{-1}$	0.02336
Permittivity	ε'	-	114.91	-	114.91

Using the quantities above, the critical values previously discussed can be calculated. These can be seen in Table 4 below.

Table 4. Calculated critical values of a 3.75% wt% HSA jetting solution in 80:20 v/v% Water:Ethylene Glycol

Critical/Characteristic Values			
Quantity	**Symbol**	**Unit**	**Value**
Critical Flow Rate	Q_c	$m^{3} \cdot s^{-1}$	2.57E-12
Critical Voltage	V_c	V	1904.642
Time of Charge Transport	T_q	s	4.36E-08

With the evaluated critical values, the dimensionless numbers can be analyzed and observed for Taylor cone stability.

Discussion and Future Directions

While we have not yet evaluated the ranges of dimensionless parameters that result in a stable jetting process, we have successfully completed the framework by evaluating all relevant properties. When completing this project there were obstacles faced that prevented us from physically jetting to evaluate parameter ranges. A major obstacle experienced was our inability to create a stable humidity-controlled jetting station. We first planned to use the humidity-controlled room within the North Campus Research Complex (NCRC) but discovered that the humidity still experienced some variability. We then pivoted to using chemical driven methods to create a stable humidity-controlled environment, however, were not able to maintain the environment for durations long enough to complete a full jetting cycle (~30 minutes). We are continuing our efforts to maintain a stable environment, and, in the future, we hope to observe Taylor cone stability limits to determine the optimum parameter ranges of voltage and flow rate for both jetting solutions to achieve a stable cone jetting mode.

We also hope to extend our work to fully characterize other jetting solutions commonly in the Lahann Lab as well as characterize the impact of varying process parameters on nanoparticle properties such as size and morphology.

Conclusion

Throughout this project I learned the importance of a systematic approach and implementing creativity into my thought process towards achieving project goals. Hopefully in the future, upon continuing this project, members of the Lahann lab will be able to systematically apply the properties that result in a stable jetting process.

References:

[1] Electric energy and potential. (n.d.). Retrieved May 1, 2023, from http://physics.bu.edu/~duffy/PY106/Potential.html

[2] Electrospun metallic nanowires: Synthesis, characterization, and applications - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Image-showing-formation-of-Taylor-cone-Reproduced-by-permission-from□Han-et-al_fig2_259439266 [accessed 4 Apr, 2023]

[3] Lee, A., Jin, H., Dang, H.-W., Choi, K.-H., & Ahn, K. H. (2013). Optimization of experimental parameters to determine the jetting regimes in electrohydrodynamic printing. Langmuir, 29(44), 13630–13639. https://doi.org/10.1021/la403111m