

Evaluating Delivery of a Monoclonal Antibody using a Linear Lorentz-Force Actuated Needle-Free Injector

By Tiffany Jin

Submitted to the Department of Mechanical Engineering in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in Mechanical Engineering

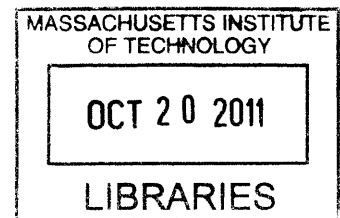
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ABSTRACT

The medical application of injection of monoclonal antibodies using a controllable auto-loading needle-free jet injector has been evaluated for two potentially limiting factors: viscosity of the formulation and shearing of the antibody during ejection. We used the Hepatitis B monoclonal antibody C86322M for its easy access and widespread usage. We used repeatability studies of glycerol at up to 200 m/s and 200 μ L delivery volume to demonstrate precision at viscosities up to 21.6×10^{-3} Pa·s. We determined that viscosity alone would not limit the jet injector's performance. Additionally, we evaluated the integrity of the antibody post-ejection using the enzyme-linked immunosorbent assay (ELISA) and gel electrophoresis methods. Using the ELISA method, we compared the ability of the antibody to bind to its specific antigen, HBsAg, both before and after ejection at multiple speeds. Changes in molecular size and charge of the monoclonal antibodies were evaluated by gel electrophoresis, more specifically with SDS polyacrylamide gels in reducing and non-reducing situations, native gel electrophoresis, and IEF gel electrophoresis. Most of these techniques revealed little to no change between pre-ejectate and ejectate migration, indicative of an unchanging molecular size and overall charge. However, with IEF gel electrophoresis, we observed two extra residues around a pI of 6.8. A change in charge due to alterations of protein side-chains may affect the stability of the molecule, and so this result is worth further pursuing on a quantitative basis. Despite this possibility, overall we have demonstrated that at 1 g/L, no significant aggregation or degradation results from jet ejection.

Thesis Supervisor: Ian Hunter
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Table of Contents

List of Figures	5
1. Introduction.....	6
1.1 Needle-free jet injector.....	6
1.2 Monoclonal antibodies	6
1.3 Jet injector application	7
2. Background.....	8
2.1 Needle-free jet injector.....	8
2.1.1 Linear Lorentz-force actuation	8
2.1.2 Software-based control system.....	9
2.1.3 Competitive advantages of jet injector	10
2.2 Monoclonal antibodies	12
2.3 Hepatitis B monoclonal antibody	13
2.4 Applications of jet injection	15
2.5 Major concerns	16
3. Experimental Methods.....	18
3.1 Pre-calibration	18
3.2 Ejection studies to evaluate the repeatability of delivery volumes	20
3.2.1 Repeatability of increasing concentrations of glycerol	20
3.2.2 Repeatability of MAb buffer formulation	22
3.2.3 Repeatability of buffer formulation containing MAb	22
3.3 Viscosity measurements.....	23
3.4 Evaluation of MAb integrity following ejection	24
3.4.1 MAb integrity as determined by ELISA	25
3.4.2 MAb integrity as determined by gel electrophoresis.....	27
3.4.2.1 SDS polyacrylamide gel electrophoresis	27
3.4.2.2 Native gel electrophoresis.....	30
3.4.2.3 IEF gel electrophoresis	30
4. Results and Discussion	31

4.1 Ejection studies to evaluate the repeatability of delivery volumes	31
4.1.1 Repeatability of increasing concentrations of glycerol	31
4.1.2 Repeatability of MAb buffer formulation	34
4.1.3 Repeatability of buffer formulation containing MAb	35
4.2 Viscosity measurements.....	36
4.2.1 Viscosity of increasing concentrations of glycerol	36
4.2.2 Viscosity of MAb buffer at variable temperature.....	37
4.3 Evaluation of MAb integrity following ejection	38
4.3.1 MAb integrity as determined by ELISA	38
4.3.2 MAb integrity as determined by gel electrophoresis.....	39
4.3.2.1 SDS polyacrylamide gel electrophoresis	39
4.3.2.2 Native gel electrophoresis.....	42
4.3.2.3 IEF gel electrophoresis	43
5. Analysis.....	46
6. Conclusions and Future Work	47
Acknowledgements.....	52
References.....	53

List of Figures

Figure 1a: Handheld jet injector.	9
Figure 1b: Lorentz-force motor	9
Figure 2: Injection waveform for delivering 150 μ L of water at 200 m/s	10
Figure 3: Immunoglobulin (antibody) structure	12
Figure 4: Velocity profile of fluid within ampoule.....	16
Figure 5: Water calibration of jet injector using different copper coils.....	19
Figure 6: Coil position, current, and voltage during ejection of 150 μ L of water at 200 m/s	20
Figure 7: Cone and plate rheometer.....	24
Figure 8: Procedure to evaluate the integrity of monoclonal antibody after ejection.....	25
Figure 9: Results from criss-cross ELISA	27
Figure 10: Setup for SDS-PAGE gel cassettes.	29
Figure 11: Injection volumes with increasing glycerol concentration.....	32
Figure 12a-f: Repeatability of delivery for 0, 1, 10, 30, 50, and 70 percent glycerol solutions....	32
Figure 13: Repeatability of 100 μ L buffer ejections.....	35
Figure 14: Repeatability of Hepatitis B MAb delivery.....	36
Figure 15a: Viscosities at increasing glycerol concentrations and shear rates	37
Figure 15b: Viscosities as a function of concentration.....	37
Figure 16: Viscosity of buffer formulation for MAb.....	38
Figure 17: Comparison of MAb after 50, 100, 150, and 200 m/s ejection with pre-ejectate	39
Figure 18: SDS-PAGE of Hepatitis B MAb under reducing conditions	40
Figure 19: Non-reducing SDS-PAGE of Hepatitis B MAb.....	41
Figure 20: Native polyacrylamide gel electrophoresis of Hepatitis B.....	43
Figure 21: IEF gel electrophoresis of Hepatitis B MAb.....	44

1. Introduction

1.1 Needle-free jet injector

The development of a controllable auto-loading needle-free jet injector using a Lorentz-force motor and software-based control system has opened the doors for many possibilities in the field of medicine. This platform technology, which uses a two phase injection profile consisting of a brief high pressure pulse followed by a longer, lower pressure follow-through, allows for robust and precise control over coil position and thereby over both the depth and volume of drug delivered. In addition, the bi-directionality of the system potentially provides a more efficient method of drug loading, re-loading, and reconstitution. Other benefits of the newly-prototyped jet injector are the eradication of needles that may cause accidental needle stick injuries, cross-contamination, the reduced person-time required for vaccination, and the potential to reduce both drug volume and therefore number of injections leading to a conservation of resources.

1.2 Monoclonal antibodies

Monoclonal antibodies (MAbs) are a broad class of bio-therapeutics that can be used to treat certain cancers and autoimmune diseases (Adams & Wiener, 2005; and van Vollenhoven, 2009). Because of their requisite high volume and high viscosity in order to be effective, these antibodies are often difficult to deliver. Currently, many MAbs must be delivered intravenously. While this presents as the fastest way to deliver medication throughout the body, delivery can be slow, there is the risk of infection, phlebitis, infiltration, and embolism. Delivery to the subcutaneous layer also carries a risk of infection and abscess at the site of injection. It is also problematic for people with belenophobia and contributes to reduced compliance. Several properties of the linear Lorentz-force actuated needle-free injector make it an attractive alternative delivery system for this class of bio-therapeutic.

1.3 Jet injector application

It has already been demonstrated that needle-free jet injection is a significant improvement over the traditional method of injection by needle and syringe for many reasons, including infection prevention, safety, and ease of use. In addition, the needle-free jet injector has potential applications in many different branches of medicine that previously utilized needles for injection. Herein we evaluate the feasibility of using a jet injector to deliver Hepatitis B monoclonal antibody (MAb). Hepatitis B MAb is one example of a broad class of bio-therapeutic compounds (Lynch *et al.*, 2009). With their development has come the need for more innovative and controllable delivery devices able to deal with issues of high volume, high viscosity, and variable drug state (*e.g.* lyophilized powder or particulate).

High viscosity due to formulation and/or MAb concentration could result in poor repeatability due to increased variability in the volume and/or depth of drug delivered while very high molecular weight protein or high viscosity has been shown, in specific cases, to cause high shear stress and resultant denaturation (Jaspe & Hagen, 2006). As such, this project intends to determine whether repeatability of delivery and/or shear-induced inactivation of Hepatitis B MAb preclude its being delivered using our Lorentz-force actuated needle-free jet injector.

2. Background

2.1 Needle-free jet injector

A jet injector platform has been developed that offers improvements over previous jet injectors through the use of a controllable linear Lorentz-force actuator and software-based control system. All jet injectors require a method of temporarily storing energy before transferring kinetic energy to a liquid drug. Currently, commercially available devices utilize compressed springs, compressed gases, or explosive chemicals for energy storage, all of which are relatively limited in terms of varying the jet velocity over the time-course of the injection. The control of the volume and depth of delivery was thus a primary consideration in the development of the new class of jet injectors outlined here.

2.1.1 Linear Lorentz-force actuation

This novel jet injector uses a linear Lorentz-force (voice coil) motor, which is an electromagnetic actuator commonly used in audio speakers. When a voltage is supplied by a linear amplifier (AE Techron LVC 5050), the effective current passes through a coil of copper wire. This copper coil consists of six layers of copper wire tightly wound on an Acetal copolymer former, and provides an effective resistance of 11.3 Ω . The current generated causes changes in the magnetic field of an 8 mm portion of the copper coil, resulting in an axial force of up to 200 N. This force is applied to the 3.16 mm diameter piston, which then pressurizes and accelerates the fluid within the ampoule out through a small orifice 221 μm in diameter. These ampoules (Injex™ Ampoule, part #100100), which are commercially available, accommodate a volume of 300 μL over a 30 mm stroke length.

Using the three-position slide switch, the user can permit for manual loading of the ampoule, locking of position, and a ready-to-inject mode. A switch on the back of the injector

also allows for a slow retraction of the piston so that the ampoule can be easily reloaded under servo control. A photograph of the handheld jet injector and a cutaway model of the Lorentz-force motor can be seen in Figure 1 below.

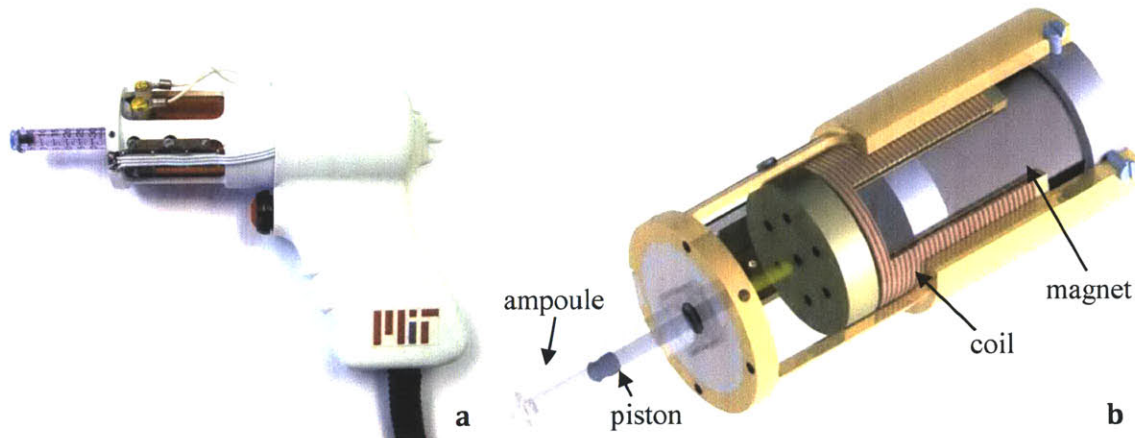


Figure 1: (a) Handheld jet injector, and (b) Lorentz-force motor.
Taken from Taberner *et al.*, 2010.

2.1.2 Software-based control system

The graphical user interface of the host application allows the operator to define and preview a jet injection waveform using four inputs: the desired initial jet velocity (V_{jet}), the time period for which V_{jet} is maintained (T_{jet}), a typically slower follow through velocity (V_{ft}), and the total injection volume. In adjusting the profile of applied voltage to the coil over time, we can control the force and position of the piston during ejection. These jet injectors have the ability to deliver injections using pre-defined pressure profiles (*i.e.* waveforms). The two phase injection profile, an example of which is seen in Figure 2, consists of a brief high velocity pulse (V_{jet}) required to penetrate the target tissue to a desired depth followed by a longer and lower-pressure follow-through (V_{ft}) that permits the bulk volume of drug to be delivered at this lower velocity. This injection profile reduces the potential for shearing while permitting absorption into the tissue. A linear slide potentiometer (750 V/m) measures the position of the coil. With this

measurement of the coil position and knowledge of the cross-sectional area of the piston ($1 \times 10^{-7} \text{ m}^3$), the swept volume throughout the time course of the ejection can be computed. This injection profile allows for more control over the depth at which an injection needs to be delivered and the volume of delivery.

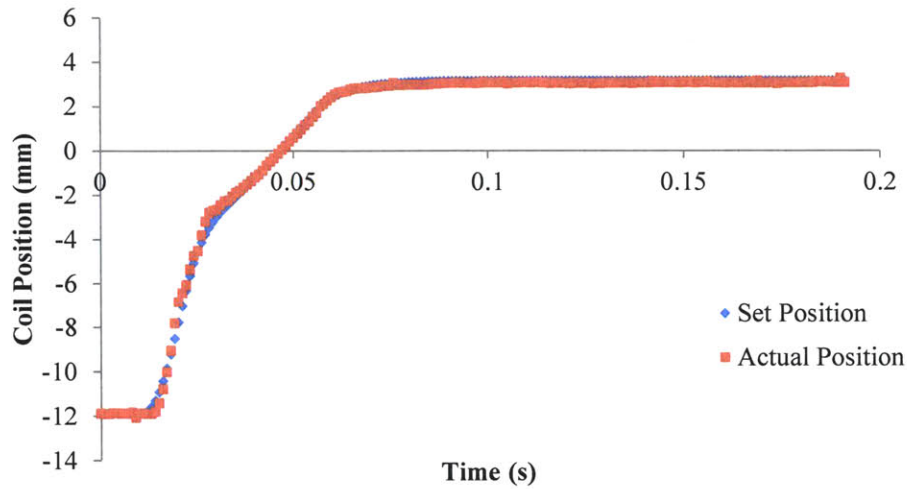


Figure 2: Injection waveform for delivering 150 μL of water at 200 m/s.

The software-based system takes advantage of the bi-directionality of the system by using its auto-reloading feature to increase efficiency during several successive ejections. This feature uses full real-time feedback control, drawing the piston back in small steps and using the position sensor as a reference. The drive power is modulated by the computer over the entire reloading, and the non-uniform friction is accounted for over the travel of the piston. The auto-reloading feature thus allows for relatively constant-velocity reloading, something difficult to do during manual reloading due to human capabilities and the nature of some of the more viscous compounds.

2.1.3 Competitive advantages of jet injector

The jet injector offers many improvements over the conventional needle and syringe. Even when handled by trained professionals, rate of delivery by hypodermic needles will vary.

Automation by the control system of the jet injector allows for consistent speed and time of delivery. Jet injection alleviates the two primary concerns associated with this current method of drug delivery by eliminating both needle stick injuries and the high cost associated with the need for sharps disposal. Additionally, the ampoule contains a smaller orifice of 200 μm in diameter compared with a typical 28 gauge needle with outer diameter of 362 μm . The decreased puncture size reduces recovery time and the potential for infection. Finally, the use of the jet injector increases acceptance and patient compliance, decreasing instances where a user or caregiver might deviate from a strict dosing regimen due to belonephobia (Hogan, 2011).

The software-based control system also has improved repeatability over previous jet injectors because of its ability to use a two-phase pressure profile during the entire time-course of delivery. The controllability of our jet injector becomes essential for two main reasons. First, it is able to regulate the depth and dose of the drug delivered, ensuring penetration of skin and then preventing excessive fluid from splattering. This control of the velocity allows for more efficient delivery of drug, reducing the potential for shearing while enabling absorption within tissue. This efficiency decreases the volume required for effective delivery, which also decreases the time of injection. Second, the four user-determined parameters (V_{jet} , T_{jet} , V_{ft} , and volume) allow for flexibility in injection depth and drug volume. The injection profile can thus be adjusted for a wide range of skin types and a wide range of medical applications.

Hemond *et al.*, 2006, has shown that the controllable jet injector is capable of delivering a specific volume of fluid to a specific depth based entirely on the four input parameters mentioned previously. Under auto-loading computer control, the ejected volumes match to within 3 percent tolerances, indicating high accuracy of the software system. Furthermore, Taberner *et al.*, 2010, has demonstrated the ability to both accurately and precisely control

delivery of up to 250 μL volume into acrylamide gels (for its analogous properties with living tissue) and post-mortem animal tissue. Despite variations in skin type due to stiffness and thickness, the jet injector can effectively and repeatably breach the skin and then deliver fluid volume to a pre-determined depth. The success of these experiments indicates that the controllable Lorentz-force actuated jet injector can be an attractive alternative drug delivery system.

2.2 Monoclonal antibodies

Monoclonal antibodies are characterized by their specificity to a given antigen. They are used extensively within the fields of biology and chemistry, and have some applications in the medical field as far as both diagnostic tests and therapeutic treatments. The structure of an antibody (Figure 3) contains heavy and light chains held together by a combination of non-covalent interactions and covalent disulfide bonds. The Y-shaped structure is bilaterally symmetric, and the two antigen-binding sites are located at the tips of the “Y” (Pierce).

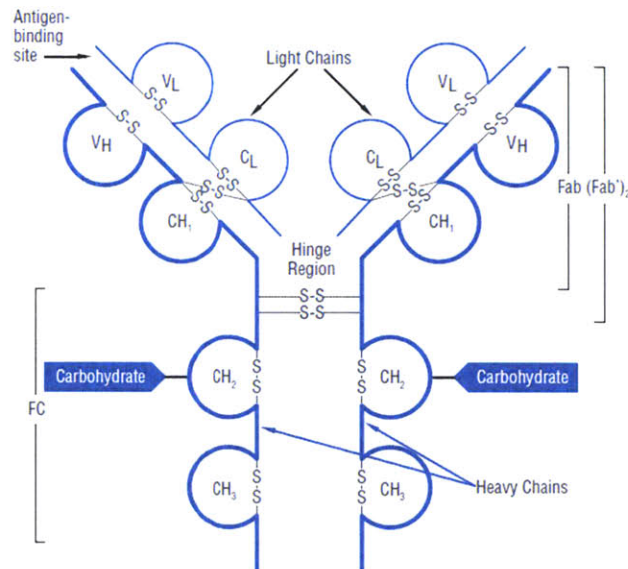


Figure 3: Immunoglobulin (antibody) structure.
Taken from Pierce.

Monoclonal antibody therapy is the use of monoclonal antibodies to specifically bind to target molecules within a patient's body. Unlike vaccines, in which a small amount of antigen is designed to elicit an immune response, monoclonal antibodies are intended to target specific molecules within the pathway of a disease, bind to these targets thereby disrupting the disease pathway. While monoclonal antibodies have shown potential in a clinical environment, ultimately monoclonal antibodies are only produced when necessary because their production is time consuming and expensive. Even so, their unique specificity has instigated investigation of their usage in several previously untreatable diseases. Over twenty monoclonal antibodies, including unmodified antibodies and antibodies armed with toxins or radionuclides, have been approved to prevent allograft rejection or to treat autoimmune diseases and cancer. Hundreds of monoclonal antibodies are also in clinical studies (Waldmann, 2003).

Murine antibodies have a short half-life *in vivo* and only limited penetration into tumor sites (Stern & Hermann, 2005). Murine antibodies have been generally replaced by humanized and chimeric antibodies in modern therapeutic antibody applications (Nelson *et al.*, 2000). The intention of these two types of antibodies is to achieve a more "human-like" antibody, so that immunogenicity might be reduced while a specific immunologic effect is simultaneously increased (Riechmann *et al.*, 1988).

2.3 Hepatitis B monoclonal antibody

Our initial objective was to evaluate delivery of a monoclonal antibody with indication for the treatment of rheumatoid arthritis, a disease afflicting 1.5 million adults in the United States (Myasoedova *et al.*, 2010). This was later revised to assessment of the ability of the jet injector to deliver chemokine receptor type 5 (CXCR5) monoclonal antibody. Anti-CXCR5 antibody binds to CXCR5 (Allen *et al.*, 2007; and Zlotnik *et al.*, 2006), a G-protein coupled

receptor, and is of potential use in the treatment or prevention of CXCR5 related diseases or disorders such as rheumatoid arthritis (Lee *et al.*, 2011) and in the migration of B cells into lymphoid microenvironments (Cyster, 1999). Timely availability of CXCR5 could not be guaranteed and as such the monoclonal antibody chosen for this study was a murine antibody against Hepatitis B surface antigen (HBsAg).

Hepatitis B is an infectious illness caused by the Hepatitis B virus (HBV). It has inflicted 2 billion people, about one third of the world's population (Hepatitis B Foundation, 2009). Approximately 90 percent of healthy adults who are infected will recover and develop protective antibodies against future Hepatitis B infections. However, about 5 percent will be unable to recover completely and will develop chronic infections. For this reason, the World Health Organization recommends prevention using the Hepatitis B vaccine, which has a 95 percent success rate (WHO, 2006).

HBsAg, a viral envelope protein and major component of the Hepatitis B vaccine, is produced by yeast cells into which the HBsAg sequence has been inserted (Miyanojara *et al.*, 1983). The Hepatitis B vaccine, like all vaccines, works by injecting antigen into the body and inducing an immunogenic response (creation of antibodies). The presence of HBsAg in human sera indicates current Hepatitis B infection, and people who develop antibodies against HBsAg are usually considered non-infectious.

We chose to use this particular antibody for several reasons. For HBsAg and its monoclonal antibody, antigen/antibody recognition is structural and not sequence based. As we will illustrate in Section 2.5, one of our primary goals is to evaluate whether monoclonal antibody will shear during ejection. With antibody/antigen structural recognition, we can use the antigen HBsAg to evaluate antibody binding effectiveness after ejection. A reduction in

effectiveness might indicate significant shearing. This antibody was also chosen for its availability. As it is used as a Hepatitis B diagnostic to determine the presence of HBsAg, it is a relatively common antibody. While antibodies are not typically used as vaccines, prophylactic use of human monoclonal antibodies to HBV has been shown to reduce viral load in individuals exposed to the virus (Dagan *et al.*, 2003). In summary, monoclonal antibody to HBsAg is an appealing choice of antibody due to its structural recognition of HBsAg, potential clinical use, and ready availability.

2.4 Applications of jet injection

While intravenous (IV) injection is the current gold standard for delivery of monoclonal antibody and other voluminous, highly viscous formulations, IV therapy does have several key issues as mentioned in the Introduction (Bohony, 1993). These include but are not limited to infusion phlebitis (inflammation of the vein), infection, bruising, extravasation, and infiltration. By reducing the size of the puncture in the body, the needle-free jet injector can reduce the chances of opportunistic infection as well as the potential for contamination of the formulation. With further evaluation of the injection process in a clinical setting, the jet injector technology might be able to simplify a physician's task of safely and efficiently transferring a large quantity of drug into a patient's body.

2.5 Major concerns

One major concern for this application of the jet injector is the viscosity of antibody formulations on the order of 10 g/L to 200 g/L (Marques *et al.*, 2009). Previously, the jet injector had not been tested with highly viscous compounds. It is possible that the force required at viscosities comparable to that of monoclonal antibody concentrates exceeds the voltage

limitations of the jet injection system. In addition, viscous compounds are characterized by their resistance to change in inertia. At the end of an ejection of highly viscous fluid, it is possible that this quality of the fluid will cause it to resist deceleration and continue to exit the orifice. In this instance, the ability of the jet injector to deliver monoclonal antibodies would be impeded by the lack of accuracy and/or precision in each ejection.

The second major concern for this application is the integrity of monoclonal antibody concentrates after ejection. At injection speeds required to pierce the epidermis, the flows within the ampoule and through the exiting orifice are turbulent. This results in a velocity profile as in Figure 4, where the no-slip condition at the walls of the ampoule cause a high shear force at those locations. Equation 1 shows the shear stress on the antibody (τ_{Ab}) when the antibody is located near the walls. It is a function of the viscosity of the fluid (μ) and slope of the fluid velocity ($\frac{dv_x}{dy}$), in addition to the radius (R) of the monoclonal antibody.

$$\tau_{Ab} = \mu \left. \frac{dv_x}{dy} \right|_{y=R} \quad (1)$$

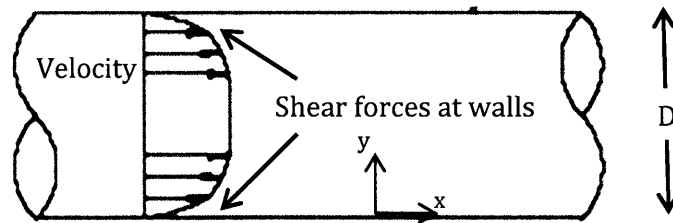


Figure 4: Velocity profile of fluid within ampoule.

At the orifice, there is an even higher shear force due to the fluid being pressurized and directed from the larger cross-sectional area ampoule to the smaller cross-sectional area of the orifice. Because of the large size of individual monoclonal antibodies and these high forces, it is likely that at some threshold concentration and ejection speed the antibody will be sheared. Our

goal is to determine whether concentrations required for effective delivery in a medical setting would cross this threshold and limit the possibility of using the jet injector in this application.

3. Experimental Methods

3.1 Pre-calibration

The current jet injector requires an initial calibration, which defines a relationship between input voltage and the steady state jet velocity. While there are several analytical methods that relate pressure imposed on the fluid with the resulting jet speed, in this case the use of the disposable Injex™ piston makes static and sliding friction at the piston-ampoule interface a significant component of the overall load imposed upon the Lorentz-force motor (Taberner *et al.*, 2010). The relationship between input voltage and jet velocity can be attained by conducting a series of increasing voltage step-response experiments. In each experiment, the steady-state coil current can be measured and the steady state force calculated. Steady state speed is necessary in order to ensure that applied force is balanced exactly by the friction of the piston-ampoule interface and fluid impedance created by the orifice. At steady state, inertia is negligible. The relationship between input voltage and steady state is best approximated with a polynomial fit.

Figure 5 shows the results of our calibration for three different coils. Coils 1 and 2 had a resistance of 11.3 Ω , and Coil 3 was a shorter coil with a resistance of 9.3 Ω . These three coils were used during the repeatability experiments. While they show slightly different velocity-voltage relationships, they are similar in terms of accuracy and precision during delivery of pre-set volumes.

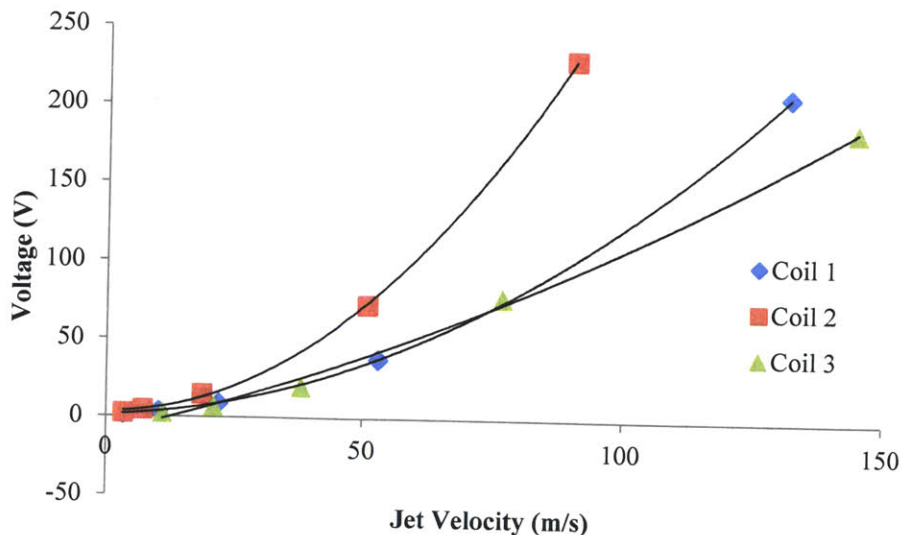


Figure 5: Water calibrations of jet injector using different copper coils.

After calibrating, the user must input four parameters: the initial jet velocity (V_{jet}), the time at the initial jet velocity (T_{jet}), the follow-through velocity (V_{ft}), and the desired volume of fluid to be delivered. These factors determine the depth of injection along with the volume and time taken during each sample, and are then used to define the injection profile of customized waveforms.

Each ampoule holds 300 μL of fluid and each piston has 30 mm of travel. To ensure that the piston never runs up against the ampoule, we never inject more than 250 μL before reloading. The voltage, current, velocity, and displacement (as determined by monitoring the piston of the voice coil using a 10 $\text{k}\Omega$ linear potentiometer with a bandwidth exceeding 1 kHz), and time are monitored in real time using the control software. These values are graphically presented along with the calculated delivery volume. A sample waveform for a water ejection can be seen in Figure 6.

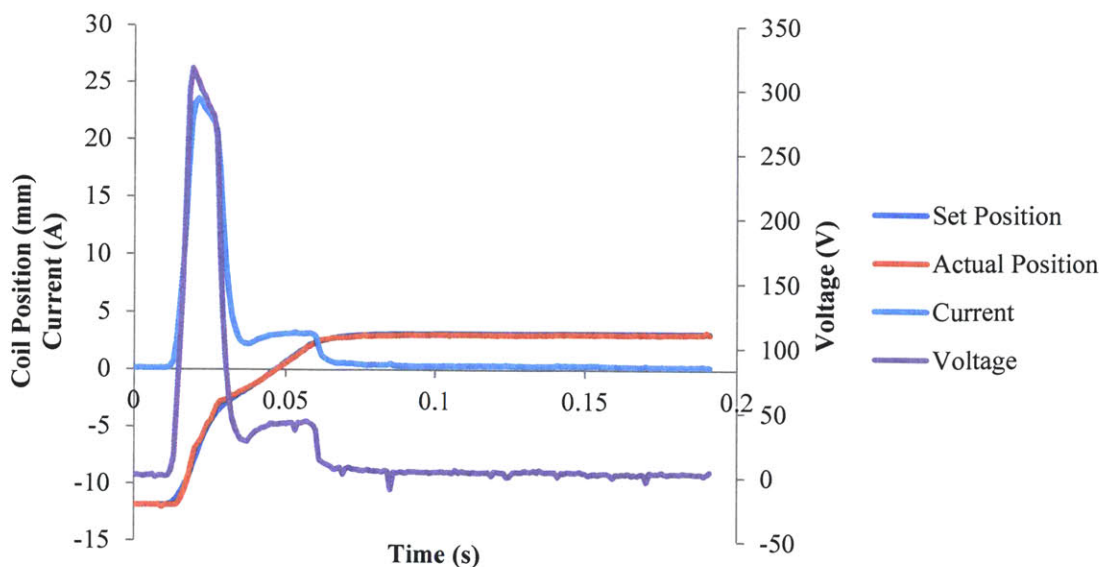


Figure 6: Coil position, current, and voltage during ejection of 150 μL of water at 200 m/s.

3.2 Ejection studies to evaluate repeatability of delivery volumes

3.2.1 Repeatability of increasing concentrations of glycerol

Our intention was to characterize the ability of the jet injector to handle highly viscous compounds, simulating the behavior of concentrated monoclonal antibody. To do this, we decided to find a glycerol concentration that would have similar viscosity to that of such a monoclonal antibody formulation, on the order of 1×10^{-3} to 100×10^{-3} Pa•s. Pure glycerol, in comparison, is about 1.2 Pa•s (Lide, 1994). We characterized the behavior of the jet injector by conducting repeatability studies, in which we repeatedly ejected fluid at constant volumes and jet velocities. We assessed this behavior by evaluating the accuracy and precision of delivered fluid volumes.

To investigate whether or not high-viscosity fluids would limit the jet injector's ability to deliver accurate volumes, we chose to use glycerol for its high viscosity and solubility in water. We first used solutions of glycerol in increasing concentrations to establish a concentration that could be repeatably delivered by the current jet injector. In order to do this, we chose to restrict

the delivery parameters to a V_{jet} of 100 m/s, T_{jet} of 10 ms, V_{ft} of 50 m/s, and volume of 100 μL . This limit was determined based on when the voltage supplied by the amplifier reached close to 300 V, the limit of the amplifier. At such high voltages, we would expect to experience a high shear force at the piston-ampoule interface accompanied by minimal travel of the piston itself. We used 0, 1, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 percent concentrations of glycerol in distilled water for this task. A sample size of ten was used for each respective concentration, in order to establish the precision of the system.

We then selected concentrations of glycerol with a similar viscosity to either the buffer or antibody-buffer formulation and proceeded to do repeatability studies. The viscosities we used were 0, 1, and 10 percent for similarity to buffer, and 30, 50, and 70 percent concentrations for similarity to the monoclonal antibody-buffer formulation. In these repeatability studies, the desired V_{jet} and volume were varied such that at a given V_{jet} , the repeatability of ejected volumes was quantified by delivery of 50, 100, 150, and 200 μL of a known concentration of glycerol, each into a set of pre-weighed vials containing cotton wool. The samples were injected into 1.5 mL Eppendorf tubes containing a small quantity of cotton to encourage absorption and prevent splattering. The difference in weights pre- and post-ejection would reveal the ejected mass. Using the density at that volume, we could calculate injection volume and compare it to the pre-set volume.

The procedure for measuring viscosity is delineated in Section 3.3. These sets of data, one relating glycerol percentage to repeatability and the other relating viscosity to glycerol percentage, allow us to roughly extrapolate the behavior of monoclonal antibody concentrations at similar viscosities. This estimate serves to make an initial review of the possibility of using a jet injector to eject monoclonal antibodies.

3.2.2 Repeatability of MAb buffer formulation

The composition of the buffer we used was 25 mM histidine, 50 mM arginine, 5 percent sucrose, and 0.2 percent polysorbate 20. This polysorbate buffer formulation was used for the stabilization effects of the polysorbate, which have been shown during shaking stress experiments of a polysorbate-antibody solution. A polysorbate 20 level of 0.005 percent was found sufficient to stabilize both at low and high antibody concentration against antibody aggregation and precipitation (Mahler *et al.*, 2009). Viscosity data supplied by colleagues using a comparable buffer solution indicated that formulations of this buffer with monoclonal antibody could have temperature-dependent viscosities ranging from approximately 2.6×10^{-3} to 69.4×10^{-3} Pa•s for a 5° to 25°C temperature range.

The viscosities of the polysorbate buffer at 5°C and 25°C fit into the range of viscosities measured during the viscosity characterizations of different glycerol concentrations. Using the same repeatability test as was used on the varying glycerol concentrations, we can then compare the repeatability of the buffer to the repeatability of a similar glycerol solution. This provides an estimate as to the ability of the jet injector to handle the polysorbate buffer, we can determine if the composition of the buffer itself might be a deterrent in the performance of our jet injection system.

3.2.3 Repeatability of buffer formulation containing MAb

In a clinical situation, a dose of monoclonal antibody could potentially be 200 mg and at a much higher concentration of 175 g/L. We were unable to acquire such high concentration Hepatitis B antibody. In order to get an initial estimate of the repeatability of delivery using a monoclonal antibody, we used a 1 g/L solution of Hepatitis B MAb, diluted in polysorbate

buffer. Using the jet injector, we ejected replicates of 50 μL at 50, 100, 150, and 200 m/s in order to evaluate repeatability of ejected volume.

3.3 Viscosity Measurements

The viscosities of these several of these formulations were measured and recorded using a shear rheometer (TA Instruments AR-G2), so that we could relate our measured viscosities for glycerol concentrations and buffer formulation with known values for buffer formulation containing MAb. The viscosity of the buffer formulation containing MAb was not measured using this method, primarily due to the cost of antibody and large quantity of solution required. Our colleagues have indicated that an 87 g/L solution of MAb in buffer would have a viscosity of approximately 2.6×10^{-3} Pa•s. As we are using 1 g/L concentrations of MAb, we are working with a solution with viscosity on the order of water (1.0×10^{-3} Pa•s).

We utilized a steady-shear experiment using a cone and plate method (Figure 7). Approximately 2 mL of fluid was placed on the flat plate. A shallow cone (about 1 degree) above the flat plate was entered into direct contact with the fluid, and the plate was spun. The forces on the shallow cone were measured as a function of shear rate, which was varied from 0.1 to 100 s^{-1} during a 10 second sample period. As glycerol is a Newtonian fluid, the identified viscosity for each glycerol concentration was a ratio of resultant shear stress to the preset shear rate of the fluid.

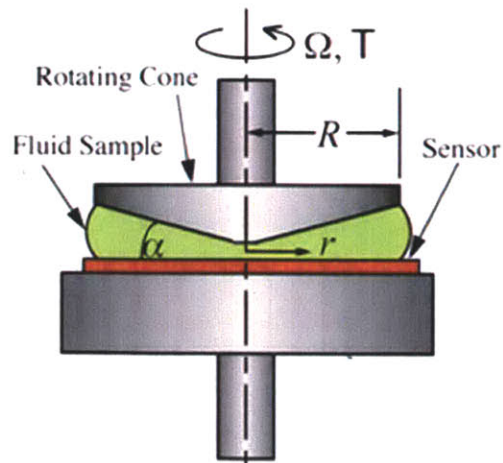


Figure 7: Cone and plate rheometer.
Taken from Pressure Profile Systems.

3.4 Evaluation of MAb Integrity Following Ejection

To avoid loss due to backsplash, MAb was ejected into tubes containing cotton wool as was done for volume repeatability studies with some modifications. Because post ejection evaluation of MAb integrity required recovery of the ejected samples, the volume and packing of the wool into tubes was adjusted by trial and error using ejection of water until a density was achieved that prevented any sample from pooling in the bottom of the tube but also precluded backsplash. Rather than using 1.5 mL Eppendorf tubes, 0.5 mL tubes were used as these could then be seated in 2.0 mL tubes for sample recovery. In each case, sample was ejected into the 0.5 mL tube, the end of the tube clipped, the tube seated in a 2.0 mL tube, and the 2.0 mL tube centrifuged at 13,000 rpm for 30 seconds. Volume recovered was determined by accessing the difference in weight between the combination of tubes and cotton wool prior to and after ejection. On average ($N=8$), ejection of 50 μL of water resulted in recovery of $34 \pm 2 \mu\text{L}$ or 71% of the ejectate. A schematic of the procedure is shown in Figure 8. This procedure was then

implemented for recovery of ejected MAb. Recovered samples were stored at 4°C until ready for evaluation.

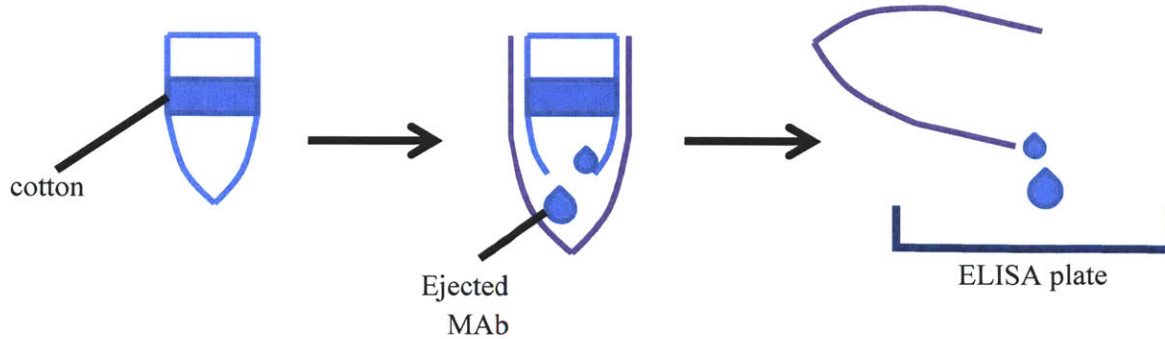


Figure 8: Procedure to evaluate the integrity of monoclonal antibody after ejection.

3.4.1 MAb integrity as determined by ELISA

The ELISA (enzyme-linked immunosorbent assay) is a very versatile, highly sensitive, quantitative technique that permits detection of specific antibodies, soluble antigens, or cell-surface antigens. All ELISAs require that a solid-phase reagent (antigen or antibody) interact with a secondary or tertiary reagent (antigen or antibody) either of which is covalently coupled to an enzyme. Unbound reagents are removed by washing and unbound sites blocked using a range of proteins from non-fat milk to purified proteins to reduce non-specific binding of proteins. A substrate (chromogenic or fluorogenic) is then added which is hydrolyzed by the bound enzyme resulting in a colored or fluorescent product. The relative amount of this product is proportional to the amount of antigen or antibody being analyzed (Abmayr *et al.*, 1999). In our case, we wanted to evaluate the ability of the ejectate, a Hepatitis B MAb (C86332 MAb from Meridian Life Science, Inc.), to bind with the 24 kDa recombinant HBsAg (R86783 from Meridian Life Sciences, Inc.).

Prior to evaluation of ejectates using the ELISA, we needed to determine the optimal concentration of antigen and antibody required to generate the best signal to noise ratio, provide a robust and reproducible assay, and provide a measure of the antigen over a biologically relevant range (dynamic range) (Pierce). A criss-cross ELISA with different antigen and primary antibody concentrations was conducted initially in order to determine optimal signal for both primary antibody and antigen. Optimal signal was defined as a high signal below saturation and without loss of sensitivity. The antigen concentrations we used were 5000, 2500, 1250, 625, 312.5, 156.3, 78.13, 39.06, 19.53, 9.77, 4.88, and 0 $\mu\text{g/L}$. The monoclonal antibody concentrations used were 3550, 1775, 887.5, 443.75, 221.88, 110.94, 55.47, and 27.73 $\mu\text{g/L}$.

The criss-cross ELISA used allowed us to define an optimal HBsAg (initial antigen) concentration and an optimal C86322M (primary antibody) concentration. As seen in Figure 9, the antigen concentration we chose was 300 $\mu\text{g/L}$ and the monoclonal antibody concentration we chose was 150 $\mu\text{g/L}$. The box shows comparable antigen-antibody concentrations to the one we chose. The absorbance for this point was thus approximately 1, which is just below saturation. This point was chosen to be just below saturation, to emphasize maximum sensitivity if any of the concentrates were demonstrating a lower absorbance after ejection. Our primary objective was to ensure that if the monoclonal antibody had been sheared that we might be able detect this from the inability of the secondary antibody to effectively bind.

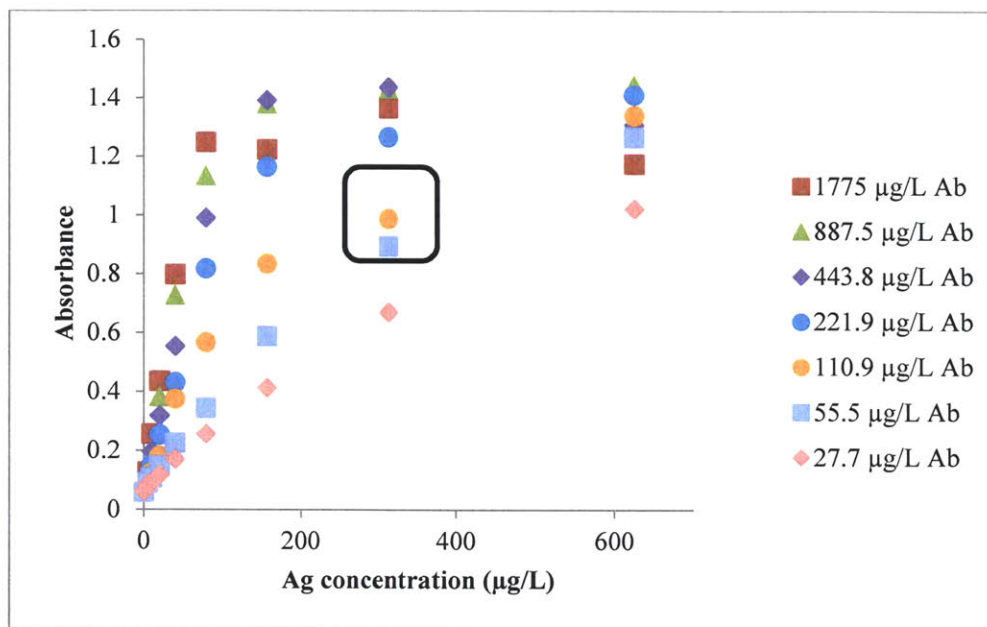


Figure 9: Results from criss-cross ELISA.

With the results optimized from this ELISA, we proceeded to do another ELISA that would compare ejected monoclonal antibody formulation with pre-ejected monoclonal antibody formulation. We set up a plate using the optimized antigen and antibody concentrations from above along with our ejectates and pre-ejectate in triplicate. The jet injector speeds used to potentially shear our antibody were 50, 100, 150, and 200 m/s. In addition, we included controls of no antigen with our Ab formulations, a non-specific antigen (Collagen-III Ag) with our Ab formulations, and HBsAg with the Collagen-III Ab6310. Each of our controls was intended to yield no binding. Resulting differences in absorbance of the ejectates and pre-ejectate detected using the photo-spectrometer would indicate shearing of the monoclonal antibody.

3.4.2 MAb integrity as determined by gel electrophoresis

3.4.2.1 SDS polyacrylamide gel electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), is a technique widely used to separate proteins according to their electrophoretic mobility. This mobility can be

a function of polypeptide chain length or molecular weight. In SDS-PAGE, samples are mixed with SDS, an anionic detergent that denatures secondary protein structure and tertiary structure with the exception of disulfide bonds. The latter are broken by addition of β -mercaptoethanol. Together with SDS and the β -mercaptoethanol, the sample is heated to approximately 95°C allowing the SDS to bind to most proteins in a constant weight ratio. Given the charge due to the bound SDS dominates the intrinsic charge on the protein, the resulting SDS-protein complexes have identical charge densities and as such migrate in the gel based on size (Hames, 1981). This procedure permits both size and composition of the sample to be determined.

Reducing SDS-PAGE was used to evaluate Hepatitis B MAb structure before and after ejection at variable velocity using the needle-free jet injector. More specifically, given that we are using a purified MAb, we were attempting to determine if ejection resulted in degradation. This degradation might be inferred by accumulation of products other than the expected MAb subunits. A constant concentration of each sample mixed with two volumes of Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue) containing 5% β -mercaptoethanol was loaded into the appropriate wells of a 4 to 20 percent gradient polyacrylamide gel. Gels were subjected to electrophoresis for 40 minutes at 150 V, after which they were washed three times with distilled water (5 minutes/wash), stained with Coomassie Brilliant Blue R-250 for 60 minutes, de-stained in water for 30 minutes, and photographed using a Canon EOS-1Ds camera. A schematic of the gel setup for SDS-PAGE is shown in Figure 10.

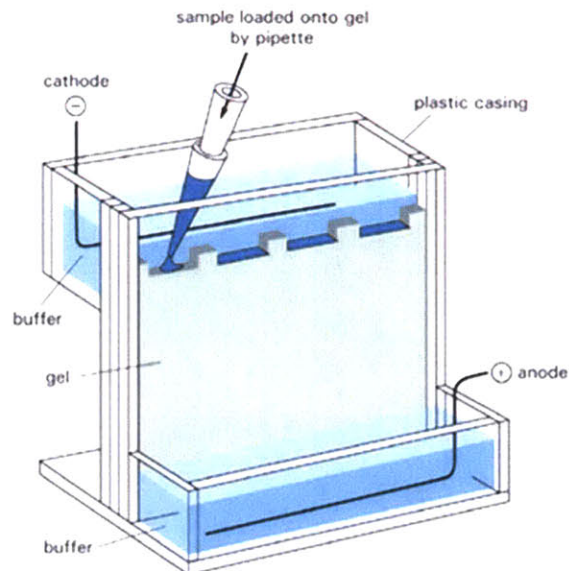


Figure 10: Setup for SDS-PAGE gel cassettes.

When voltage is applied, the negatively charged molecules migrate through the gel to the positively charged anode. Schematic taken from Wagner, 2011.

At least one well of each gel was loaded with Kaleidoscope™ pre-stained standards made up in Laemmli buffer containing 5% β -mercaptoethanol and heated to 95°C for 5 minutes prior to loading. These Kaleidoscope™ samples contain proteins with known molecular weights, ranging from 5,886 Daltons for aprotinin to 192,988 Daltons for myosin, and allow us to estimate the molecular weight of each component of our antibody concentrates following electrophoresis.

Hepatitis B MAb samples were also subjected to SDS-PAGE under non-reducing conditions. The difference between reducing and non-reducing SDS-PAGE concerns the treatment of the samples prior to loading; the procedure for gel electrophoresis is the same for reducing SDS-PAGE. In non-reducing SDS-PAGE, sample is mixed with Laemmli buffer that does not contain β -mercaptoethanol and the samples are not boiled prior to their being loaded onto the gel. However, the presence of SDS denatures the protein giving it a net negative charge and constant charge-to-mass ratio.

3.2.2.2 Native gel electrophoresis

The difference between SDS-PAGE and native or non-denaturing polyacrylamide gel electrophoresis involves differences in both sample preparation and running conditions. Samples for native gel electrophoresis are diluted in buffer lacking both SDS and β -mercaptoethanol and are not boiled prior to loading. In addition, SDS is not included in the electrophoresis buffer. The net result is that samples are separated on the basis of both charge and size (*i.e.* hydrodynamic size) with preservation of subunit interactions, native protein conformations, and activity. Changes in stability resulting in degradation or aggregation for example will alter the charge or conformation of the test molecule and manifest as a change in migration on the gel compared to an intact sample molecule.

3.4.2.3 IEF gel electrophoresis

IEF (isoelectric focusing) gel electrophoresis uses ampholytes (*i.e.* molecules with both positively- and negatively-charged moieties) to establish a pH gradient. Under an electric field, proteins will migrate through the gel until they reach their isoelectric point (pI) or the point at which they have no net charge. If the charge on the protein changes as in degradative processes such as deamidation, this change should be reflected as a change in the pI.

4. Results and Discussion

4.1 Ejection studies to evaluate the repeatability of delivery volumes

4.1.1 Repeatability of increasing concentrations of glycerol

These solutions were ejected from the jet injector at 100 m/s for a volume of 100 μL (shown in Figure 11). Additionally, the limit of ejection for this experiment occurred at 80 percent glycerol. We were unable to eject the 90 percent or 100 percent solution without risking excessive current and possibly short-circuiting the system.

From these data, we know that the jet injector's limiting viscosity occurs between that of 80 and 90 percent, or between 49.7×10^{-3} and 169.7×10^{-3} Pa•s. We calculated injection volumes by taking the difference between post-ejection vials containing fluid and dry pre-weighed vials. This procedure is detailed in Section 3.2.1. After ejecting using the jet injector and calculating injection volumes using this method, we found that ejection volumes were constant as the glycerol concentration (and viscosity) increased. The instrument's precision was fairly constant with increasing glycerol concentration. On average, the jet injector was able to deliver 100.3 ± 2.6 μL .

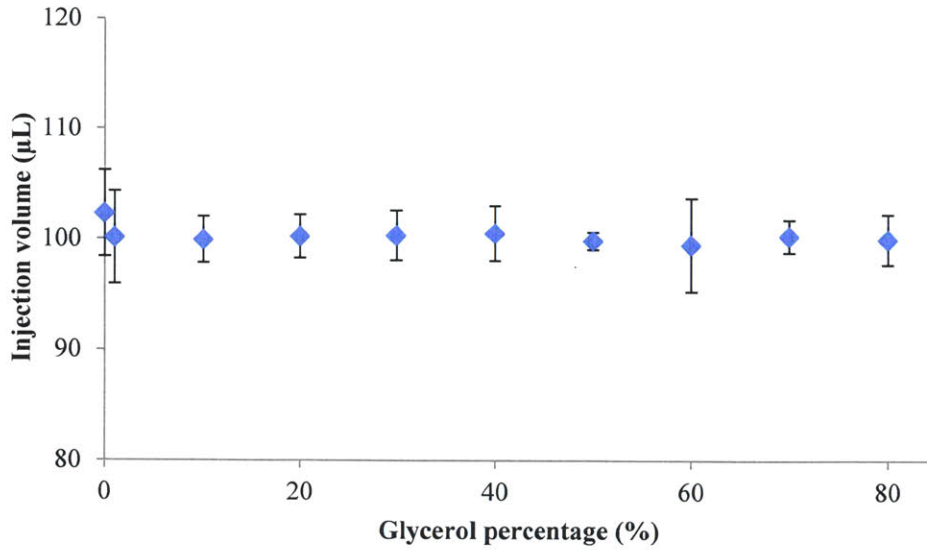
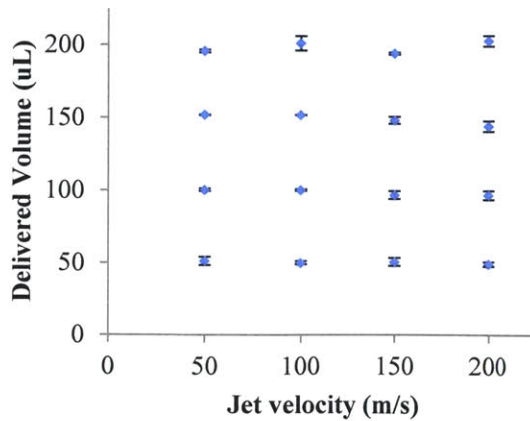
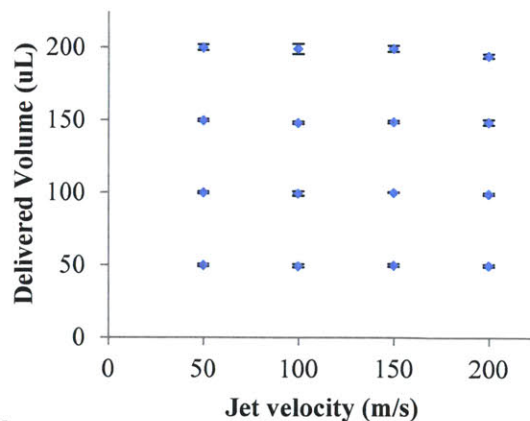


Figure 11: Injection volumes with increasing glycerol percentage.

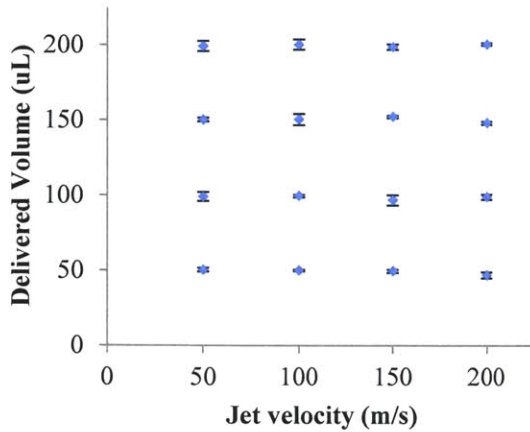
We also chose to investigate the repeatability of delivery for higher viscosities at different delivery volumes and speeds. Figure 12a-f show repeatability of delivery for 0, 1, 10, 30, 50, and 70 percent solutions. As shown in the figure, the spread at each delivery volume and jet injection speed increases as the glycerol concentration increases. However, even at 50 percent glycerol with a viscosity of 6.6×10^{-3} Pa•s, the average error is $2.9 \mu\text{L}$, compared to $2.0 \mu\text{L}$ for pure distilled water.



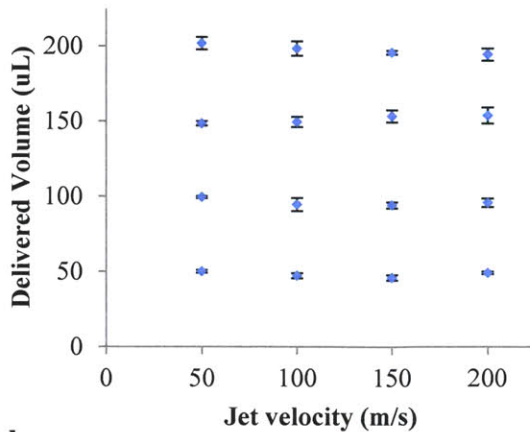
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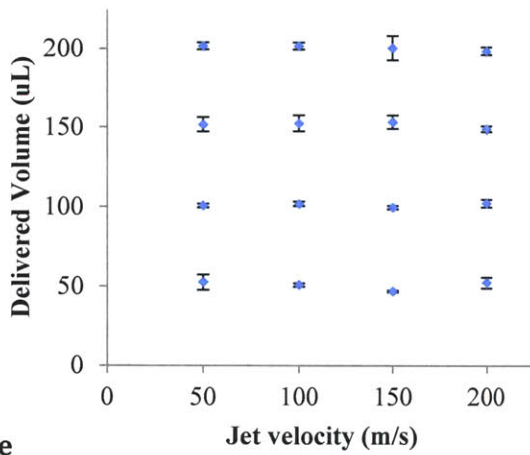
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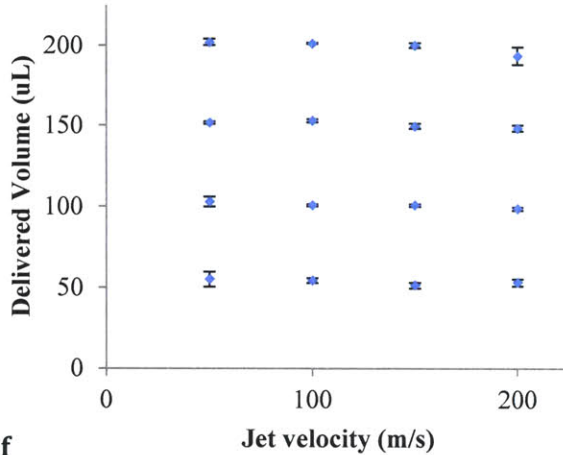
c



d



e



f

Figure 12a-f: Repeatability of delivery for 0, 1, 10, 30, 50, and 70 percent glycerol solutions. Error bars show standard deviations above and below the mean.

While the repeatability studies for glycerol show a high degree of precision even at high viscosities (70 percent glycerol), there are several notes to be made about the process. At higher viscosities (above $6.6 \times 10^{-3} \text{ Pa}\cdot\text{s}$ corresponding to a glycerol concentration of 50 percent), there is a tendency for the ampoule to collect air bubbles near the piston during reloading. Slow manual reloading was required to minimize the presence of these pockets of air. Since the set velocity of auto-reloading was comparably high, it could not be used. However, with the adjustment of a slower auto-loading velocity, reloading of the piston becomes both more efficient and more accurate.

Another note to make about the jet injection process is the effect of the quality of ampoules on the behavior of the jet injector. The Injex™ Ampoule is a disposable and commercially-available drug ampoule specifically chosen for its availability and relatively low cost. Although this ampoule had been designed for single-use, we had been routinely achieving 50-100 injection cycles before the ampoule or piston exhibited evident wear and required replacement (Taberner *et al.*, 2010). With higher speeds and higher viscosities, this life cycle is considerably shorter, sometimes even noticeably affecting accurate delivery in the first several ejections. Rapid and successive ejections at 200 m/s are limited by the useful lifetime of the ampoule. Since the ampoule is currently disposable, it may be beneficial to look into designing and manufacturing ampoules made of more robust materials which could be intended for re-use.

4.1.2 Repeatability of MAb buffer formulation

Repeatability studies for the MAb buffer at both 5 and 25°C (Figure 13) showed a comparable response with water. Initial jet velocities vary from 50 to 200 m/s. Standard deviations, shown by error bars, are comparable to water and low-concentration glycerol. There was not a significant difference in the behavior of the buffer at 5°C and the buffer at 25°C, which could be expected due to the low viscosity (similar to water) in both instances.

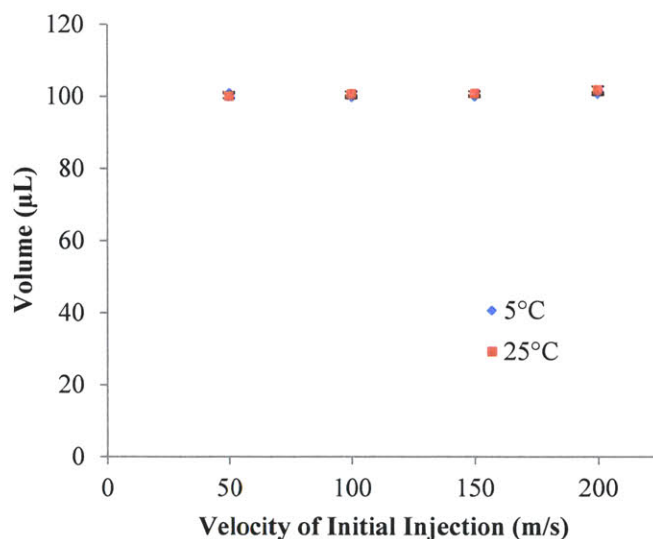


Figure 13: Repeatability of 100 µL MAb buffer ejections. Error bars, while negligible, show standard deviations above and below the mean.

While the behavior of the MAb buffer during ejection was both accurate and precise, the polysorbate within the buffer itself is an emulsifier, with some characteristics similar to soap. When reloaded or ejected at high velocity, the buffer had a tendency to develop small bubbles in the form of foam. With a slower reloading speed and diligent cleaning of the edges of the orifice, this problem can be resolved. This phenomenon of the MAb buffer required careful monitoring during auto-reloading and may have contributed to a small amount of error in the repeatability studies. However, the benefits of the presence of polysorbate in the formulation outweigh the error contributions. As remarked upon in Section 3.2.2, inclusion of polysorbate in the buffer has been shown to stabilize the antibody from aggregation and precipitation.

4.1.3 Repeatability of buffer formulation containing MAb

Repeatability studies of monoclonal antibody in buffer showed that the jet injector remained fairly accurate when delivering volumes of 50 µL. At higher speeds and particularly at 200 m/s, there is some inaccuracy in this method of measurement due to splash-back, despite the

presence of the cotton. As in the repeatability studies for the buffer alone, the tendency for the polysorbate buffer to form froth may have also contributed to some error.

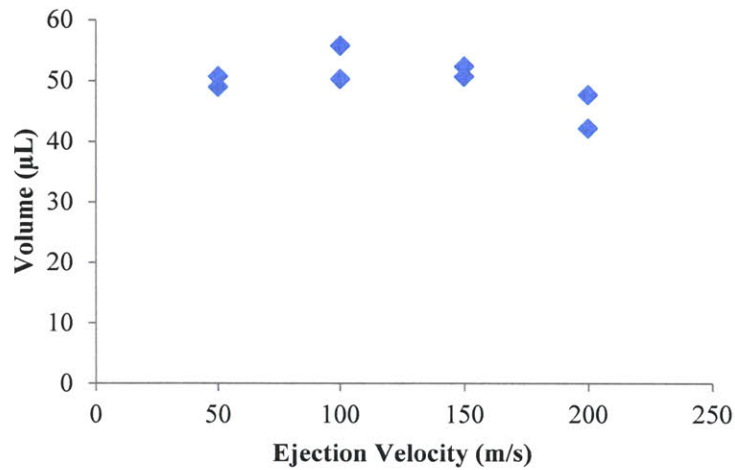


Figure 14: Repeatability of Hepatitis B MAb delivery.

4.2 Viscosity measurements

4.2.1 Viscosity of increasing concentrations of glycerol

Using the rheometer, we developed relationships between the shear rate and shear stress of glycerol at different glycerol concentrations, and were able to compare these viscosities with those of our buffer formulation containing MAb. The results are shown in Figure 6. For our glycerol concentrations, viscosities range from 1.73×10^{-3} Pa•s for a 1 percent solution up to 763×10^{-3} Pa•s for pure glycerol. This appears to be slightly low compared to tabulated values, which show a viscosity of 1.2 Pa•s for pure glycerol as mentioned previously in Section 3.2.1.

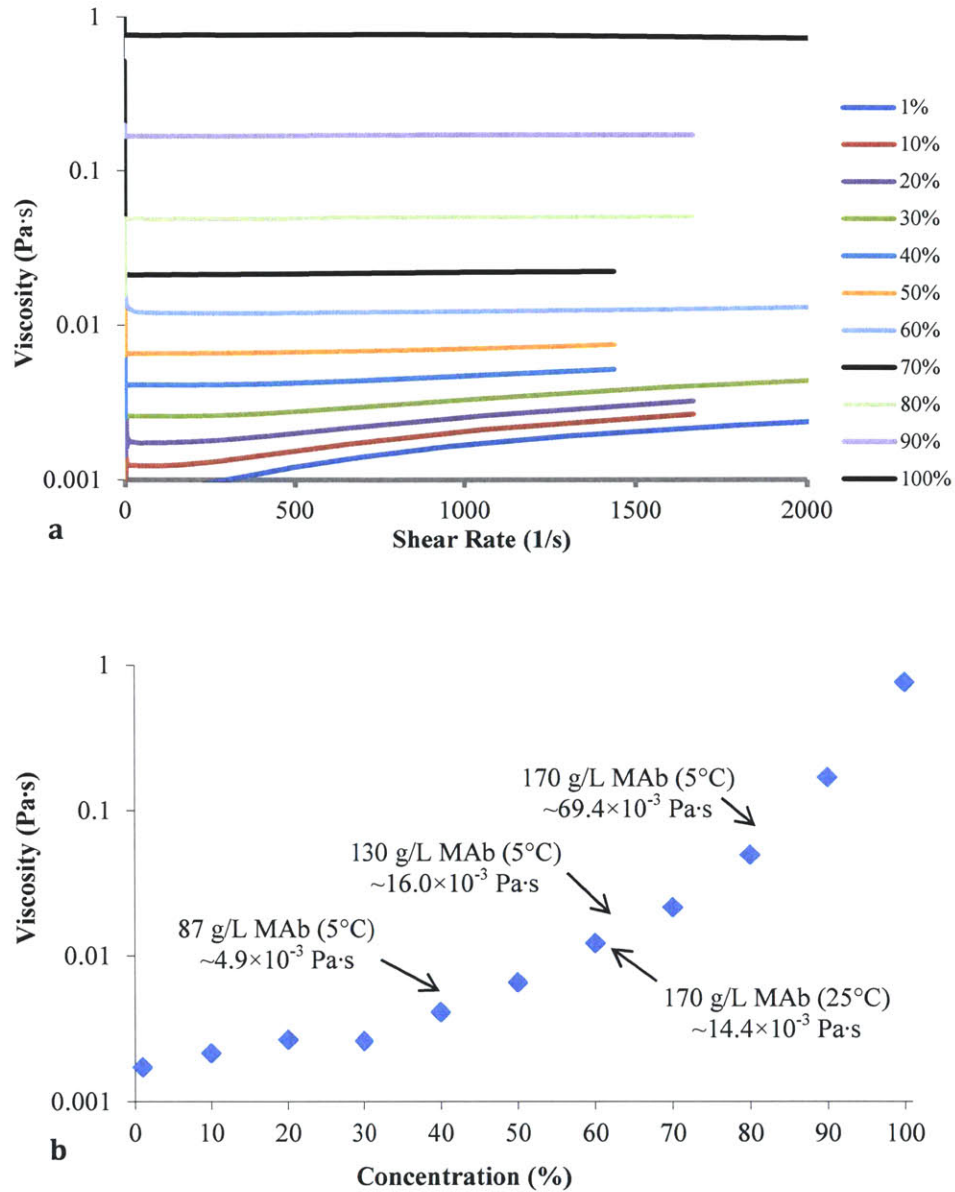


Figure 15: (a) Viscosities at increasing glycerol concentrations and shear rates. (b) Viscosities as a function of concentration.

4.2.2 Viscosity of buffer formulation at variable temperature

The rheometer was used to find a viscosity for the buffer alone (25 mM L-histidine, 50 mM L-arginine, 5% sucrose, and 0.2% polysorbate 20), at two different temperatures, 5°C and 25°C (Figure 16). These viscosities were 1.44×10^{-3} Pa·s and 0.93×10^{-3} Pa·s, respectively. These viscosities for the buffer show that the presence of monoclonal antibody contributes significantly

to the viscosity of the final solution. This antibody-buffer solution is approximately 69.4×10^{-3} Pa·s for 25°C and 14.4×10^{-3} Pa·s for 5°C, as stated previously. When these viscosities are compared to the viscosities for each of the glycerol concentrations, the polysorbate buffer falls in between 0 and 1 percent glycerol solutions, while the antibody-buffer solution falls between a 60 and 70 percent glycerol solution.

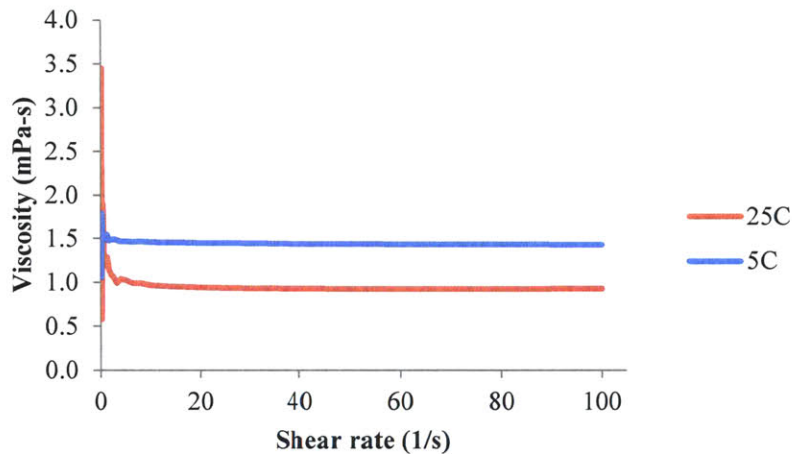


Figure 16: Viscosity of buffer formulation for MAb.

4.3 Evaluation of MAb integrity following ejection

4.3.1 MAb integrity as determined by ELISA

The ELISA was run to compare each of these ejectates with the untouched antibody (pre-ejectate). As shown in Figure 12, there was not a statistically significant difference between any of the ejectates and the pre-ejectate. As the Hepatitis B antigen-antibody relationship is a conformational one (Section 2.3), this would seem to indicate that shearing during ejection was negligible. If the antibody had been sheared, its ability to bind to antigen had not been noticeably compromised and the method of ELISA thus could not definitively indicate shearing.

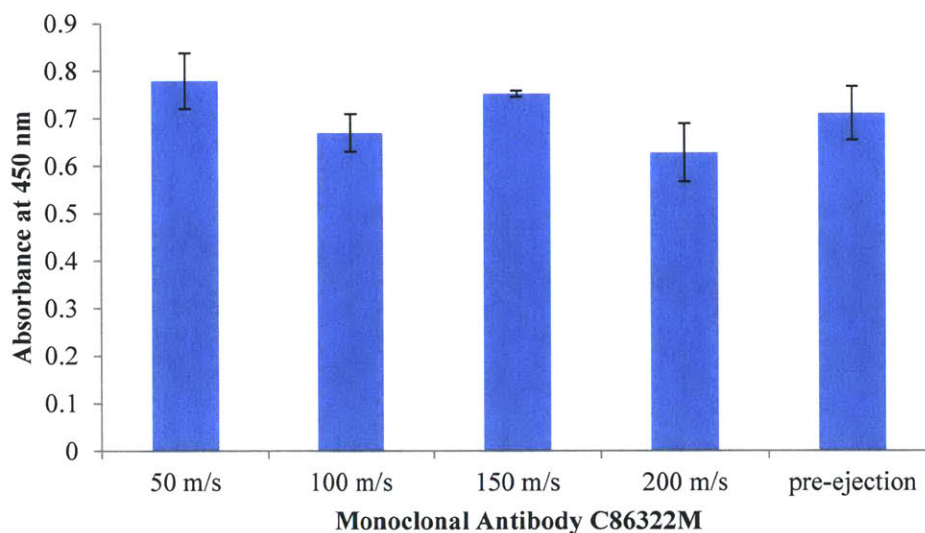


Figure 17: Comparison of MAb after 50, 100, 150, and 200 m/s ejection with pre-ejectate. Error bars show standard deviations above and below the mean.

4.3.2 MAb integrity as determined by gel electrophoresis

Several methods of gel electrophoresis were used to evaluate the integrity of the MAb following ejection using the needle-free jet injector, each designed to tell us something about the gross structure of the molecule.

4.3.2.1 SDS polyacrylamide gel electrophoresis

Initially, a constant amount (*i.e.* 5 μ g) of each ejected sample was reduced and loaded into individual wells for separation by SDS-PAGE. SDS-PAGE is often used to determine the purity and/or heterogeneity of MAb's. Reduction of the disulphide linkages of IgG molecules should yield two distinct bands corresponding to the heavy (~50 to 53 kDa) and light (~23 to 25 kDa) chains comprising the MAb (Pierce). The presence of additional bands could reflect structural heterogeneity due to contamination with host immunoglobulins since MAb is derived from ascites, differences in glycosylation prior to purification, or proteolysis prior to or after purification (Grebenu *et al.*, 1991); possibly due to handling and/or ejection. Reduction and electrophoresis of the Hepatitis B MAb following ejection at variable velocity using the needle-

free jet injection yielded two bands with relative molecular weights of approximately 53 and 25 kDa as expected for heavy and light chains comprising IgG molecules (Figure 18). A comparable set of bands was observed in the pre-ejectate control sample.

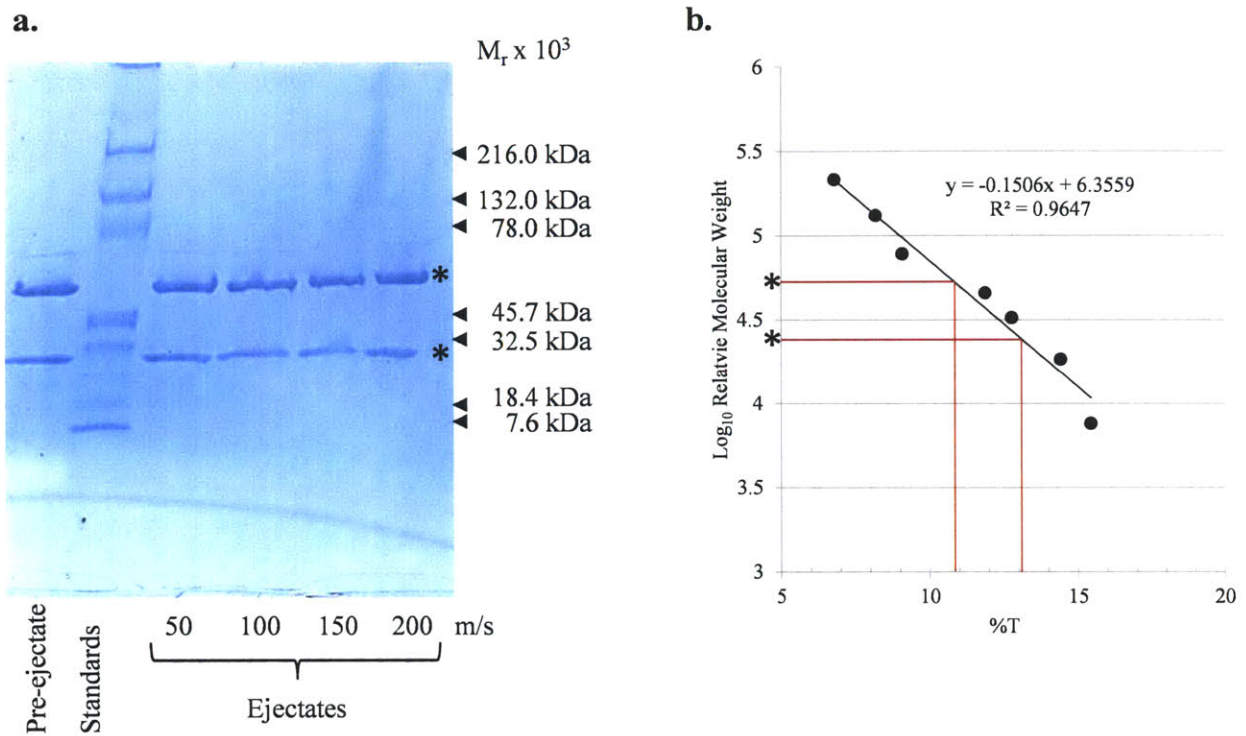


Figure 18: (a) SDS-PAGE of Hepatitis B MAb under reducing conditions. One part MAb (constant concentration of 5 μg assuming 1 g/L sample) was mixed with 2 parts Laemmli buffer (62.5 mM Trish-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue) containing 5% β -mercaptoethanol, heated to 95°C for 5 min, and loaded into appropriate wells of a 4-20% polyacrylamide gel. Relative molecular weights of calibration proteins are indicated on the right. Pre-ejectate served as a control. (b) Plot of relationship between \log_{10} molecular weight of known calibration proteins and polyacrylamide concentration (%T). Relative molecular weights of unknowns, marked by *, are determined by interpolation. Line of best fit is described by the equation, where x represents %T and y represents the \log_{10} relative molecular weight.

Replicates of the samples separated by SDS-PAGE under reducing conditions were evaluated under non-reducing conditions (*i.e.* no β -mercaptoethanol and no heating to 95°C). In each case, 5 μL (5 μg) of sample was mixed with 10 μL of Laemmli buffer minus β -mercaptoethanol and samples loaded directly into the wells of a 4 to 20 percent polyacrylamide gradient gel. Electrophoresis was as for reducing SDS-PAGE. Figure 19 shows that a major band with mobility comparable to the 132 kDa β -galactosidase calibration standard is detected in the

pre-ejectate and ejectates (Figure 18). Given that the average mass of IgG₁ is approximately 150 kDa, this variation from the expected mobility may be due to a change in conformation resulting from the presence of the disulfide linkages.

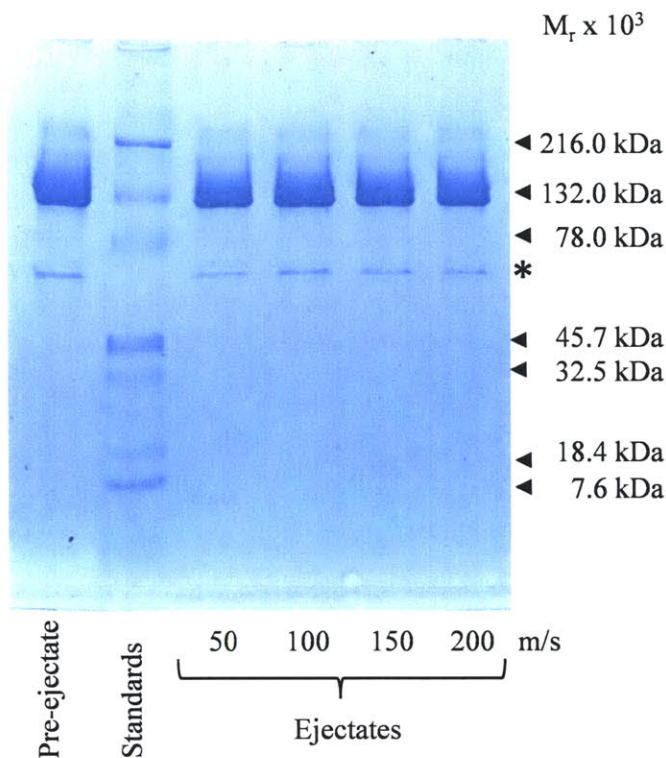


Figure 19: Non-reducing SDS-PAGE of Hepatitis B MAb. Pre-ejectate and samples ejected using variable V_{jet} with constant T_{jet} and V_{ft} . * marks the migration of the heavy chain.

Marginal bands of higher relative molecular mass, possibly suggestive of aggregation, are detected, albeit faintly, in all wells containing Hepatitis B MAb. Their presence in the pre-ejectate together with the observation that their intensity does not appear to change as V_{jet} increases suggests that they may be an artefact of production and/or handling prior to or during this study. However, it should be noted that if oligomers are present at less than two percent, Coomassie Blue staining may not be sensitive enough to detect them. More sensitive dyes such as silver stain (~10 times more sensitive) could be used in future. Finally, a second band having a relative molecular weight comparable to the heavy chain is detected in all wells suggesting

denaturation of the MAb; the concentration of the corresponding light chain being too low for detection by the Coomassie Blue stain. However, once again the presence of this band in all wells does not suggest loss of integrity due to ejection.

4.4.2.2 Native gel electrophoresis

Evaluation of MAb integrity post ejection using native polyacrylamide gels shows the presence of a single predominant band exceeding 216 kDa, in wells loaded with pre-ejectate or post ejectate (Figure 20). However, determination of relative molecular mass in this case is suspect as the conformation of the proteins comprising the calibration standards could affect their migration. What can be stated is that the migration of the Hepatitis B MAb whether ejected or not appears to be comparable suggesting that the net charge, shape, and size of this MAb has not been altered as a result of ejection at increasing V_{jet} . This result is consistent with that obtained for electrophoresis of the Hepatitis B MAb using non-reducing SDS-PAGE.

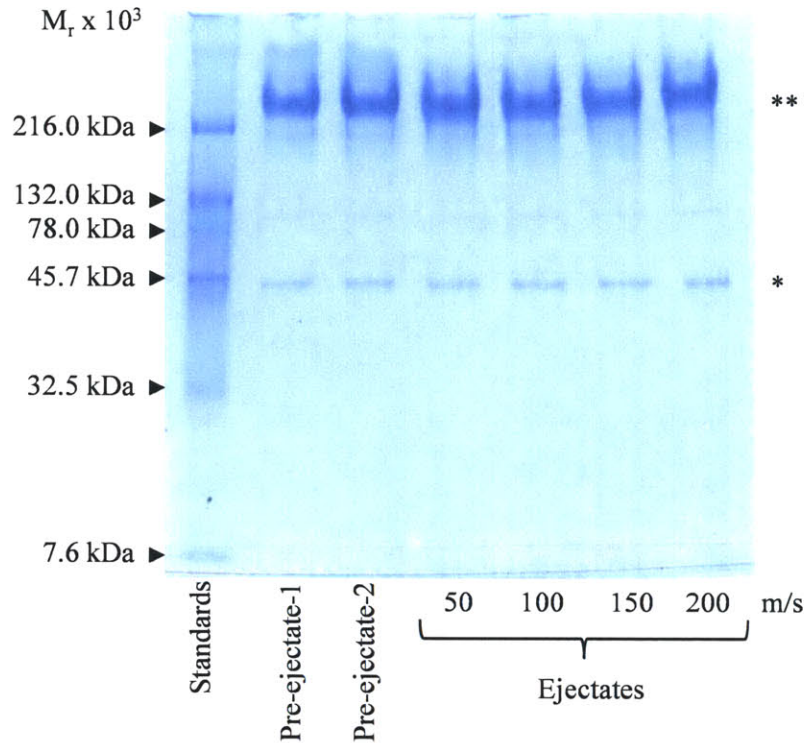


Figure 20: Native polyacrylamide gel electrophoresis of Hepatitis B MAb pre-ejectates and samples ejected from the nozzle of the needle-free jet injector at increasing velocities as marked below the appropriate lanes. ** designates the migration of the native conformation of the Hepatitis B MAb while the single * marks the migration of the heavy chain.

4.4.2.3 IEF gel electrophoresis

Charge heterogeneity of monoclonal antibodies is often a result of deamidation events (Daugherty & Mrsny, 2010). Deamidation involves the removal of an amide functional group thereby damaging the amide-containing side chains of the amino acids asparagine and glutamine (Robinson, 2001). It is considered to be relevant to the degradation of proteins and has been postulated to limit the usefulness of certain recombinant therapeutic proteins (www.ionsource.com). In antibody preparations, differences in charge distribution or content, often markers for deamidation are detected by IEF gel electrophoresis or high performance cation-exchange chromatography (www.dionex.com). We have attempted to assess changes in the charge on the Hepatitis B MAb as a result of treatment using IEF gel electrophoresis.

Individual wells comprising IEF slab gels, pH 3 to 10, were loaded with 5 μ g of Hepatitis MAb ejected using variable jet speeds and electrophoresis and staining carried out as described in Section 3.4.2.3. As shown in Figure 21, four prominent bands and two to three less intense bands that together span a pI range of 6.5 to 7.5 were detected, consistent with the published range for IgG (Hamilton, 2001).

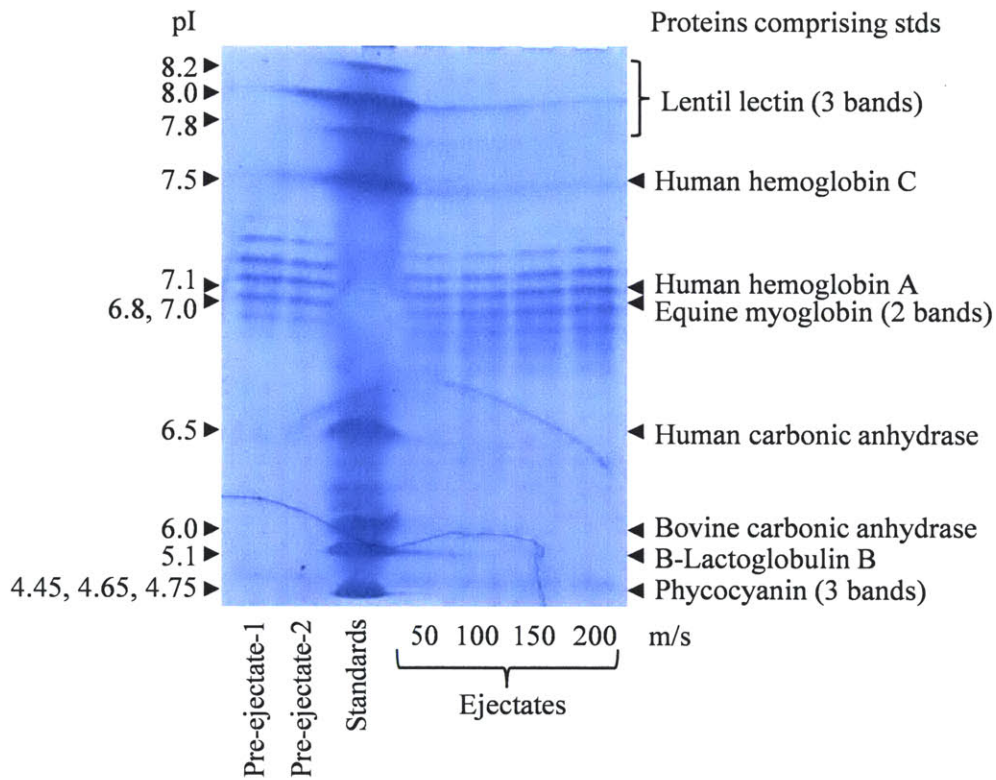


Figure 21: IEF gel electrophoresis of Hepatitis B MAb. MAb buffer containing 1 g/L Hepatitis B MAb was ejected at variable velocity into test tubes and each sample analyzed by IEF gel electrophoresis.

Of the three less intense bands, one appears by Coomassie Blue staining to be of comparable intensity in all samples having a pI of approximately 7.3. The remaining two bands with pI's of less than 6.8 are more intense in the ejected samples, with intensity appearing to increase with increasing velocity of ejection. While this interpretation is qualitative and needs to be quantified by further experimentation, it suggests that ejection may be changing the charge on the MAb which could result in its turnover.

5. Analysis

We initially hypothesized that the factors that might limit the ability of the jet injector to be used for this application were the high viscosity of this formulation and the shearing of the large monoclonal antibody particles during high ejection speeds.

From extensive studies on the ejection of glycerol solutions at varying formulations, we have characterized the jet injector's ability to handle compounds of varying viscosities. We can see that viscosity alone is not a limiting factor for the jet injector until the viscosity exceeds about 49.7×10^{-3} Pa•s (comparable to an 80 percent glycerol concentration). The monoclonal antibody formulation has a known viscosity of approximately 69.4×10^{-3} Pa•s for 175 g/L at 5°C and 14.4×10^{-3} Pa•s at 25°C. For a 130 g/L concentration of antibody, the viscosity is approximately 16.0×10^{-3} Pa•s. With this limitation of viscosity, the jet injector would be unlikely to effectively deliver monoclonal antibody at 175 g/L and 5°C without some redesign. This redesign might present simply as an increased maximum voltage on the amplifier or a more involved redesign of the ampoule to limit friction at the ampoule-piston interface.

The viscosity of 70 percent glycerol was a representative upper limit for that of MAb concentrations ranging from 87 to 130 g/L at 5°C to 25°C. 175 g/L at 5°C, as mentioned previously, exceeds the current capabilities for the jet injector system. Repeatability studies done for up to 21.7×10^{-3} Pa•s (70 percent glycerol solution) showed that even at increased viscosities the feedback-control system within the jet injector contributed to fairly precise delivery volumes. At 21.7×10^{-3} Pa•s, the average standard deviation across all delivery volumes and jet velocities was 2.9 μ L, compared to 2.0 μ L for pure distilled water measured at 1.7×10^{-3} Pa•s. With continued improvements in the software of the jet injection system, we would hope to be able to further optimize precision and accuracy.

The use of multiple coils (Figure 5), while necessary due to the lifetime of each coil, presented some variations. Coil 3, used in conjunction with a more recently modified software, was used for 50 and 70 percent glycerol and buffer formulation with MAb repeatability ejections. Coil 3 had a resistance of 9.3 Ω compared to the original 11.3 Ω for Coil 1 and 2. This lowered resistance allowed the amplifier to provide a lower voltage in order to achieve equivalent jet velocities. The water calibration, which related voltage and velocity, indicated that at lower velocities the jet injector did not have a significant difference in performance from when it contained the Coil 1 and the original software. However, at higher velocities the new coil requires less voltage. Further work would require a firmer grasp of the impact of the coil and software on repeatability and control of injection waveforms.

Furthermore, it will be necessary to empirically measure viscosities of MAb solutions from 1 g/L to the desired 175 g/L, at both 5°C and 25°C. We will also need to conduct repeatability studies using solutions containing monoclonal antibodies in order to better evaluate the jet injector's ability to eject MAb relative to comparably viscous glycerol solutions.

We assessed the monoclonal antibody's ability to handle high ejection speeds using two primary methods: an ELISA and gel electrophoresis. The ELISA we ran showed data (Figure 17) that would indicate that the conformational binding between the antigen HBsAg and MAb was unhindered by ejection. This finding supported our belief that 1 g/L antibody had not aggregated or degraded significantly.

Gel electrophoresis gave further evidence towards this conclusion. With SDS polyacrylamide gel electrophoresis for non-reducing and reducing conditions, and for native gel electrophoresis without the charging effects of SDS, we saw similar migration for each of the ejectates compared to the pre-ejectate. As each of these experiments compared varying

combinations of molecular size and charge for pre- and post-ejectates, we can conclude that the size and charge of the protein remained relatively unchanged as a result of ejection.

Our final gel electrophoresis method of isoelectric focusing revealed two faint bands of pI less than 6.8, increasing in intensity with increasing jet velocity during ejection. These faint bands may indicate changes in amino acid side-chains of the protein, in particular asparagine and glutamine. Changes in these residues are used as indicators for the stability of monoclonal antibody therapeutics. If this alteration in charge due to ejection can be quantified and revealed to be significant, then the usefulness of the bio-therapeutic will be limited. In such an instance, using the jet injector in a monoclonal antibody delivery application may not be feasible.

6. Conclusions and Future Work

We have conducted an initial set of tests to demonstrate the possibility of utilizing a Lorentz-force actuated needle-free jet injector to deliver monoclonal antibodies. From repeatability studies of glycerol and relations of the viscosity of glycerol concentrates with that of monoclonal antibody solutions, we were able to characterize the performance of the jet injector over increasing viscosities that might be relevant to the monoclonal antibody solutions. Additionally, we conducted a first-pass analysis of the shearing of monoclonal antibody at high velocities but relatively low concentrations (1 g/L). At this concentration, we determined using two primary methods (ELISA and gel electrophoresis) that the monoclonal antibody was unlikely to have significantly degraded or aggregated during ejection. However, two bands displayed by ejection during the IEF gel electrophoresis, which increase in intensity with increasing jet velocity, indicate that jet ejection may have resulted in a change in the charge of the molecule.

There are many future steps to be taken before we can recommend delivery of monoclonal antibodies with the jet injector. We will need to attain a better understanding of the characteristics of the jet injection system, particularly where the copper coil and the jet injection software is concerned. We are aware that a shorter coil results in a lower resistance and thus a different relationship between applied voltage and resultant jet velocity, but have not as of yet been able to relate this information by comparing repeatability studies or evaluating system limitations due to high viscosity fluids. The software system we have been using is still a work in progress, and as such we will need to make some improvements to the waveforms of the new software for better accuracy in jet velocity. We may also need to acquire injection ampoules

better suited for multiple high-velocity ejections and less inclined to piston wear and tear at high pressures.

To fully evaluate the applicability of the jet injection system to injection of monoclonal antibodies, further work would need to be done to test high-concentration monoclonal antibodies (on the order of 175 g/L). Here we have demonstrated that viscosity alone should not prevent the jet injector from effectively delivering fluid up to 130 g/L; however, there may be other factors that contribute to the behavior of the system that cannot be accounted for until we eject the actual monoclonal antibody concentrate. Additionally, at higher concentrations of monoclonal antibody there will be a large number of antibody molecules trapped in the region close to the ampoule wall. At this location, the shear forces due to the no-slip condition at the wall will affect a larger number of the molecules.

In the future, we will need to pursue the possibility that jet ejection instigates a change of charge for the molecule. As described previously, changes in charge may correlate with changes in several key side-chains of the molecule. These residues are used as a marker of usefulness in some bio-therapeutics, and thus may indicate protein turnover. We will require replication of these data accompanied with a more quantitative assessment.

While we have begun to account for the ability of the jet injector to handle monoclonal antibodies and the ability of the antibody to handle high-speed jet ejection, we have not yet begun to characterize injection of the monoclonal antibodies. Injection of the monoclonal antibody would require the jet fluid to impact with the surface of the skin before proceeding to deliver to a precise depth. We will need to evaluate and then optimize the process of injection into tissue analogues and then live animals.

Finally, we would require an assessment of the behavior of a wide range of monoclonal antibodies using the jet injector before we can attempt to apply this delivery system to the general application of injection of monoclonal antibodies in a clinical setting.

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