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QUANTIFICATION OF INJECTABILITY OF A NEW SELF-ADMINISTERED INTRAMUSCULAR INJECTION

Roja Anandakumar
University of Rhode Island

QUANTIFICATION OF INJECTABILITY OF A NEW SELF-ADMINISTERED INTRAMUSCULAR INJECTION

BY

ROJA ANANDAKUMAR

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ROJA ANANDAKUMAR

Approved:

Thesis Committee

Major Professor C T Rhee

Sara Rosenbaum

Daniel Rubin

Harold D Bibb

DEAN OF THE GRADUATE SCHOOL

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ABSTRACT

Liquid Avonex® (Interferon beta-1a) manufactured by Biogen, Inc. is indicated for the treatment of relapsing forms of multiple sclerosis (MS) to slow the accumulation of physical disability and decrease the frequency of clinical exacerbations. The recommended dosage of Avonex® for the treatment of relapsing forms of MS is 30 μg injected intramuscularly once a week. Several complications have been reported following intramuscular (IM) injections, of which pain at the injection site is the most common. Though factors such as site of injection, injection volume, concentration and nature of the drug may affect the reception of pain, one factor which has not apparently been given any attention in the published literature is the ease of injectability of the syringe, which is the amount of force required to expel the drug from the syringe. This factor can be expected to have a substantial effect on the discomfort experienced by patients and on the completeness of the injection (i.e. the percentage of dose delivered). An evaluation of the published literature by the author of this thesis has failed to reveal any publications dealing with the role of injectability as a measure for easing the pain on injection. There is a need for some objective, quantitative method for measuring the ease of injectability such as may be provided by use of the Instron Series 5500.

The purpose of this study was to perform compressive studies on the intramuscular injection, Liquid Avonex® staked and luer-slip pre-filled syringes using the Instron Series 5500. The Instrument generates an expulsion force profile from which the force required for the initial movement of the plunger into the

syringe (breakout force) was calculated. An ideal syringe with good ease of injectability will have minimum breakout force and a smooth expulsion profile. Thus by studying the various factors affecting the expulsion force profile, the ease of injectability of syringes was quantified. The various factors studied were age and viscosity of silicone oil, storage temperature, addition of surfactant and the speed of expulsion. The viscosity of the silicone oil and the method of siliconization were found to be critical factors defining the expulsion force profile. The 1000 cSt silicone oil was found to give better ease of injectability than the 12,500 cSt silicone oil. Increase of storage temperature, addition of surfactant and increase of expulsion speed did not have any substantial effect on the expulsion force profile of the syringe.

Pain on IM injection is also related to the mechanical properties of the needle insertion, which include the maximum force required to penetrate the muscle and the penetration profile of the needle into the muscle. Needle penetration studies were conducted to examine the injection profiles of staked and non-staked needle sharps using a model intramuscular injection pad. The results showed that non-staked needles need lesser penetration force and show linear penetration profile in comparison with staked needles and hence cause less pain on injection.

In conclusion, the results reported in this study clearly indicate that compressive studies using the Instron Series 5500 are an effective and convenient way to quantify the ease of injectability of syringes.

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Finally I would like to dedicate this work to my dearest mother, who means everything to me. Whatever little success I have achieved in my life is because of her. I feel proud to be the daughter of such a wonderful and loving mother.

PREFACE

This thesis has been prepared in the format of the manuscript plan in accordance to section 11-3 of the Graduate Manual at the University of Rhode Island.

The thesis has been divided into three sections. Section I contains a brief introduction on intramuscular (IM) injections, the complications following IM injections especially the pain on injection, importance of injectability studies to ease the pain on injection and use of the Instron Series 5500 to evaluate the ease of injectability of syringes. This is followed by the objectives of the study. Section II forms the central part of the thesis and is composed of a manuscript written in the format prescribed by the scientific journal, *Drug Development and Industrial Pharmacy* to which the manuscript will be submitted for publication. It also includes a summary of the entire thesis and some suggestions for future work. Section III contains appendices that include graphs and tables useful for clear understanding of the results described in the manuscript. A report "*Statement for the use of 1000 cSt silicone oil instead of 12,500 cSt silicone oil*" by Vetter Pharma, a contract manufacturer of Biogen, Inc. has been included in appendix C. This document provided the basis for testing the effect of viscosity of silicone oil on the expulsion force profile of Liquid Avonex® pre-filled syringes. The bibliography for the entire thesis follows the Section III.

It was not possible to include all the graphs related to the study in the manuscript due to adherence to the journal requirements. Hence some representative graphs have been included in the manuscript. For ease of understanding, some graphs have been included in both the manuscript as well as in the appendix.

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SECTION I

INTRODUCTION

1. Routes of Drug Administration

Drug substances are normally administered as part of a formulation in association with one or more pharmaceutical adjuvants (excipients) that serve various pharmaceutical functions. The selective use of these pharmaceutical excipients results in dosage forms (1). Drug dosage forms are designed based on the nature of the disease, the intended route of administration, the size of the dose, the anatomic and physiologic characteristics of the administration site and the interaction of the drug and dosage form at the administration site (2). Amongst the various routes for administration of drugs, the oral route is the most preferred route by the patients. The oral route is a natural, uncomplicated, convenient and safe means for administering drugs. The major disadvantage of this route is the potential problem of poor bioavailability (the rate and extent of active drug that reaches the systemic circulation) due to incomplete absorption. Drugs when taken orally and swallowed are normally absorbed from the small intestine into the hepatic portal vein and are transported to the liver. During this first pass, a portion of the drug substance may be metabolized by the liver enzymes before the drug enters the hepatic vein which drains into the heart and thus reaches systemic circulation. This is known as first-pass effect (1). Slow drug response and degradation of certain drugs by the stomach acid or gastrointestinal enzymes are other disadvantages of the oral route. Generally drugs with high molecular weights are not absorbed well when given orally. Barriers to oral delivery of macromolecules include low pH of the stomach, enzymatic

degradation and limiting diffusivity through the gastrointestinal epithelium (3). An example is Insulin, which is administered parenterally due to the destruction of the hormonal protein substance by the proteolytic enzymes of the gastrointestinal tract (1).

The term parenteral means next to or beside the enteral. Enteral refers to the alimentary tract, hence parenteral means sites that are outside of or beside the alimentary tract. The three primary routes of parenteral administration are subcutaneous (SC or SQ), intramuscular (IM), and intravenous (IV) although there are other less common routes such as intradermal, intracardiac, intraarticular, intrathecal. The nature of the drug determines the particular route of administration to be employed (1). Drugs destroyed or inactivated in the gastrointestinal tract or too poorly absorbed to provide satisfactory response are administered parenterally. The parenteral route is also preferred when a rapid and predictable drug response is desired. However it is the most hazardous way to administer a drug. When done incorrectly, the patient's nerves, bones, tissues or blood vessels could be damaged (4).

Intravenous administration provides the most rapid onset of action of any parenteral route because there is no barrier to absorption. IV injections are usually given into the veins of the forearm. Drugs that are too irritating for IM or SC administration are given by this route.

The SC route of administration of drugs involves their injection through the layers of the skin into the loose subcutaneous tissues of the upper arm, the anterior surface of the thigh, or the lower portion of the abdomen. Absorption after SC administration is generally more rapid and predictable than after oral administration. Heparin and Insulin are the most important drugs routinely administered by this route. Drugs that are administered by this route must be potent in small concentrations since only a small volume can be injected.

2. Intramuscular Injections

IM injections are made into the striated muscle fibres that are under the subcutaneous layer of skin. The principle sites of injection are the gluteal (buttocks), deltoid (upper arm) and vastus lateralis (thigh) muscles. The onset of action is typically longer than with IV administration, but shorter than with SC administration. IM injections generally result in lower but more sustained blood concentrations than after IV administration. This is because IM injections require an absorption step which delays the time taken to achieve peak concentrations. When the formulation is injected, a "depot" forms inside the muscle tissue, which acts as a repository for the drug. The absorption rate from this depot depends on various physiological as well as formulation factors. Drugs which are irritating to subcutaneous tissue are often administered intramuscularly. Also, larger volumes (2-5 ml) may be injected intramuscularly than subcutaneously (2 ml) (1). Patients generally experience more pain via IM administration compared to IV and SC administration.

3. IM Injection Sites

The effect of a medication can be enhanced or diminished depending on the site of injection. Selection of IM injection site is influenced by age of the patient, the nature and amount of medication to be injected and the general consideration of the patient. Damaged or scarred tissue, poor muscle mass or tone and accessibility or mobility factors may contraindicate a particular site (5). There are five injection sites that are commonly used for the administration of IM injections.

3.1 The deltoid site

Drugs are absorbed rapidly from this site. However, the small area of the site limits the number and volume of injections which can be given. The site is close to both the radial nerve and the deep brachial artery. Hence there is potential for patient injury. It is used commonly for tetanus toxoid boosters (4, 5).

3.2 The dorsogluteal site

The presence of major nerves such as sciatic nerve and blood vessels such as superior gluteal artery, the relatively slow uptake of medication from this site compared with others and the thick layer of adipose tissue commonly associated with it, makes this site problematic. However, the large area of the site makes it suitable for multiple injections over a period of time (4, 5).

3.3 The rectus femoris site

Uptake of drugs from this site is slower than that of the deltoid, but faster than from the gluteal. This site is used when other sites are contraindicated or when patients have to self-administer the medication. The main disadvantage is that injections into this area may cause considerable discomfort (5).

3.4 The vastrus lateralis site

This site is free of blood vessels, is well developed in adults and is easily accessible. Since the muscle tissue is well developed, there is less likelihood of injury. But the area has a number of small nerve endings, which causes pain after the injection (4, 5).

3.5 The ventrogluteal site

This site has gained attraction recently and is considered by some to be the site of choice for IM injections. It consists of thick gluteal muscle, is free of penetrating nerves and blood vessels, and has a narrower layer of fat as compared to the dorsogluteal site (6). It is suitable for immobilized patients whose dorsogluteal muscles may be atrophying (5).

4. Complications Following IM Injections

The complications which have followed IM injections are contractures, palsy, local irritation, pain, infection, arterial punctures, abscess and cyst formation, necrosis, slough of skin, scar formation, intravascular injections, periostitis and

peripheral nerve injuries with consequent neurologic deficits (7). There are several factors that contribute to these complications.

4.1 The injection site

Complications following IM injections have been reported to occur with all injection sites except the ventrogluteal site. The less frequent use of this site may be the cause of the apparent lack of complications for this site (5). Presence of important nerves and vascular structures, thickness of the SQ fat are factors to be considered during the selection of an injection site.

4.2 The injectate

The concentration and type of the injectate plays an important role in muscle tissue response. Drugs like cephalothin sodium, tetracycline and digoxin cause injection site complications with a relatively high frequency. Some antibiotics like procaine penicillin and oxytetracycline cause least extensive reactions at the injection site (7). The ability of the muscle to accommodate the injectate is another consideration for muscle complications (8).

4.3 Frequency of injection

Multiple injections into the same site increase the risk of complications. Any drug when repeatedly injected causes fibrosis of the muscle and subsequent joint contracture. Hence it is advisable to change the injection site in case of chronic treatments.

4.4 Patient

The SQ tissue of the gluteal muscle varies from 9 cm in obese to 1 cm in old and emaciated individuals. This causes variability in the depth of musculature. Hence erroneous deposition of the drug into the SQ tissue is quite common. Disease conditions such as diabetes, ketoacidosis, malnutrition or hypotension have been related to SQ localized mucormycosis infection (8).

4.5 Needle

The length of the needle is decided based on the site of the injection and the age of the patient (9). A 1½-inch needle is used for a normal adult; one-inch needles are used for thin adults and ⅝-inch for young children and infants. Longer (2 - 2½-inch) needles are used for obese patients (4). Occurrence of pain is found to be significantly related to the size of the needle and the velocity of insertion (10).

4.6 Method of injection

There are two methods for administering IM injections - the Standard method and the Z-track method. The standard method involves spreading the skin above the injection site between the fingers of the practitioner's non-dominant hand. This method is believed to increase the risk of medication leaking into the needle track. The Z-track technique involves pulling the skin either downward or laterally to about 1-1.5 inches before injection using the non dominant hand (5). The skin and the subcutaneous tissue are displaced before the injection and the valve action of the skin and superficial tissue is used to prevent leakage. The displaced tissue returns to

its normal position and overlaps the needle track once the injection is completed (9). This procedure is shown to decrease leakage of medication into the SQ tissue, thus causing less pain and discomfort for the patient (11).

4.7 Air Bubble Technique

A topic that engenders heated debate among clinicians is the use of an air bubble in the syringe. In the days of glass syringes, an air bubble of approximately 0.2 ml was necessary to account for dead space in the needle hub and to ensure accuracy when drawing up the medication. However, modern disposable syringes are calibrated to deliver an accurate dose of medication, taking into account the volume of medication in the hub (9). An air bubble in the syringe is known to affect the dosage of the medication by a factor of 5% to 100% (12, 13, and 14). In a pre-filled syringe, the dosage is accurately provided by the manufacturer and an air bubble is not needed. Thus drawing up an air bubble is an outdated, unnecessary and potentially dangerous procedure for the patients and should be eliminated from IM injection procedure (9, 11).

Administering an IM injection is a common, yet complex technique that requires skill and knowledge on part of the person administering the injection. There are numerous reports in the literature of patient complications due to improper administration of IM injections (9). Inserting and removing the needle too slowly may distort the epidermis thereby increasing tissue trauma and pain. However, the medication must be injected slowly over several seconds. Injecting the medication

rapidly in the hope of minimizing the patient's reaction, results in the drug being injected throughout the procedure from the time the skin is punctured and continuing into the deep tissues. This can cause deposition of drug in the SQ fat (7).

5. Pain on Injection

Pain at the injection site is the most commonly reported complication following IM injection. Quantification of pain on injection has been done with the use of pain questionnaires. Two commonly used pain questionnaires are the McGill Present Pain Intesity (PPI) and the Brief Pain Inventory (BPI). The PPI measures the pain on a scale of 0 (no pain) to 5 (excruciating pain) and the BPI measures pain on a scale of 0 (no pain) to 10 (pain as bad as you can imagine). It has been found that both the instruments have sufficient discriminative validity to distinguish between different levels of injection-site pain (15). Another commonly used scale to study the pain intensity is the visual analogue scale (VAS) (16, 17, 18). The VAS is a simple scale consisting of a 10 cm line anchored at one end by a label such as "No Pain" and at the other end "Worst Possible Pain". The patient marks a spot on the line for the pain intensity and it is measured.

Though factors such as site of injection, injection volume, concentration and nature of the drug may affect the reception of pain, one factor, which has not apparently been given any attention in the published literature, is the ease of injectability of the syringe, which is the amount of force required to expel the drug from the syringe. This factor can be expected to have a significant effect on the

discomfort experienced by patients and on the completeness of the injection (i.e. the percentage of dose delivered). An evaluation of the published literature by the author of this paper has failed to reveal any publications dealing with the role of injectability as a measure for easing the pain on injection. There is a need for some objective, quantitative method for measuring the ease of injectability such as may be provided by the Instron Series 5500.

6. Instron Series 5500

The Instron Corporation manufactures instruments to test torsion, compression, flexure, peel, sheer, friction, hardness, fatigue, fracture, structure and several other mechanical properties. The Instron Series 5500 allows test samples to be subjected to forces either compressive (crushing), tensile (pulling), deflection (bending), or torsion (twisting) and measures the effect of forces on the physical properties of the material like the strength, brittleness and ductility. This equipment is composed of a load frame which is designed for tension, compression and reverse stress testing. Load frames are engineered for precision, speed, accuracy, safety and reliability. The load frame specifically designed for performing compressive studies on pre-filled syringes is the Model 5542. The testing capacity for Model 5542 is defined as 500 N/112 lb with a speed of 40 in/min. The control electronics of the Model 5542 feature 200 Hz data collection which enables advanced real-time control. They can control the frame using any combination of load, strain or speed rates. Merlin is the software that controls the Instron 5500 testing systems for test setup, test control, data collection, result generation and report preparation. The

software features a graphical user interface fully implemented in windows®. The flexibility of the software allows changes to be made to testing requirements. Merlin's built-in test control and calculation tools allows user to create customized tests without modifying the software macros (19).

The Instrument generates an expulsion force profile from which the force required for the initial movement of the plunger into the syringe (breakout force) can be calculated. An ideal syringe with good ease of injectability will have minimum breakout force and a smooth expulsion profile. Thus by studying the various factors affecting the expulsion force profile, the ease of injectability of syringes can be improved.

7. Avonex® and MS

The intramuscular injection, the ease of injectability of which has been evaluated in this study, is Liquid Avonex® (Interferon beta-1a). Interferon beta-1a is a 166 amino acid glycoprotein with a predicted molecular weight of approximately 22,500 daltons. Avonex® is manufactured by recombinant DNA technology (Biogen, Inc.) and is indicated for the treatment of relapsing forms of multiple sclerosis (MS) to slow the accumulation of physical disability and decrease the frequency of clinical exacerbations. MS is an inflammatory, autoimmune, demyelinating disease of the central nervous system. It involves decreased nerve function associated with formation of scars on the surface of nerve cells. It generally strikes the early adult age, but persists for a period of 30 years. The symptoms

include numbness, impaired vision, loss of balance, weakness, bladder dysfunction and other physiological changes (20). The damage to myelin in MS is believed to be due to an abnormal response of the body's immune system. Avonex® is not a cure for MS; it only helps in slowing the progression of the disease. The current commercial formulation of Avonex® is in the form of a lyophilized powder in a single-use vial and is reconstituted with 1.1 ml of diluent (Sterile Water for Injection, USP).

The recommended dosage for the treatment of relapsing forms of MS is 30 mcg injected intramuscularly once a week. It is intended for use under the guidance of a physician. However, many patients self-inject Avonex® after receiving proper training in intramuscular injections. For the treatment of a chronic disease such as MS, it is highly desirable to have a mode of injection with reliability and little discomfort. In order to avoid the lengthy process of preparing the injection, a second-generation formulation of Avonex® is being developed by Biogen, Inc. to be supplied in the form of a liquid in a pre-filled syringe. Further research is being done for incorporation of the Liquid Avonex® pre-filled syringe into an autoinjector.

An autoinjector is a spring-driven injection device, which penetrates the skin and injects the drug automatically. It is a hand-held device that completely hides the needle through the injection process, making administration more manageable. It allows patients to self-administer injections with great ease. Compressive studies using Instron Series 5500 can play a role in defining the device variables for the

autoinjector. The spring tension of the autoinjector is set based on the breakout force of the syringes.

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OBJECTIVES

The objectives of this study have been listed below:

1. To review the published literature for data pertinent to the formulation of intramuscular injection with particular reference to factors which may affect ease of injectability.
2. Perform compressive studies on Liquid Avonex® pre-filled luer-slip and staked needle syringes using the Instron Series 5500.
3. Generate the expulsion force profile and calculate the maximum force at breakout, 10 mm and 15 mm extension.
4. Study the effect of various factors on the breakout force and the expulsion force profile of the pre-filled syringes.
5. Perform needle penetration studies to compare the injection profiles of staked and non-staked needle sharps using a model intramuscular injection pad.
6. To evaluate the potential of Instron type equipment for the quantification of ease of injectability

SECTION II

MANUSCRIPT

QUANTIFICATION OF INJECTABILITY OF A NEW SELF-ADMINISTERED INTRAMUSCULAR INJECTION

Abstract

This paper reports compressive studies of the intramuscular injection, Liquid Avonex® staked and luer-slip pre-filled syringes. The ease of injectability of the syringes has been quantified. Compressive studies have been performed using the Instron Series 5500. The effects of various factors such as age and viscosity of silicone oil, storage temperature, addition of surfactant and effect of expulsion speed on the expulsion force profiles were determined. The viscosity of the silicone oil and the method of siliconization were found to be critical factors defining the expulsion force profile. The 1000 cSt silicone oil was found to give good ease of injectability. Also needle penetration studies were conducted to examine the injection profiles of staked and non staked needle sharps using a model intramuscular injection pad. The results showed that non-staked needles showed a better penetration profile and minimized the pain on injection.

Keywords: Injectability, Instron, expulsion force profile, siliconization, luer-slip, staked needles.

1. INTRODUCTION

Avonex® (Interferon beta-1a) manufactured by Biogen, Inc. is indicated for the treatment of relapsing forms of Multiple Sclerosis and is used at a

dose of 30 mcg injected intramuscularly once a week. Avonex® is a recombinant, fully glycosylated Interferon beta-1-a that is produced by Chinese hamster cells and is identical to native human Interferon-beta (1). The current commercial formulation of Avonex® is in the form of a lyophilized powder in a single-use vial and is reconstituted with 1.1 ml of diluent (Sterile Water for Injection, USP). A second-generation liquid formulation of Avonex® is being developed by Biogen, Inc. to be given in the form of a pre-filled syringe. In addition to pre-filled syringes, autoinjectors are in development for the delivery of Avonex®.

Administration of intramuscular injections can cause discomfort, and in some instances moderately severe pain to the patients. Though factors such as injection volume, site of injection, nature of the drug and muscle tone may play roles in defining the chances of such problems, one factor which has not apparently been given any attention in the published literature is the ease of injectability of the syringe, which is the amount of force required to expel the drug from the syringe. This parameter may well be expected to have a substantial effect on patient discomfort and even possibly the completeness of the injection (i.e. the percentage of dose delivered). Thus ease of injectability may modify the safety and efficacy of a parenteral dosage form. The increase in licit self-administration of parenterals makes an investigation of this topic especially timely.

In the present study, compressive studies have been performed on Liquid Avonex® pre-filled luer-slip and staked needle syringes using the Instron

Series 5500. The Instron allows test materials to be subjected to forces either compressive (crushing), tensile (pulling), deflection (bending), or torsion (twisting) and measures the effect of forces on the physical properties of the material like the strength, brittleness and ductility. The Instrument generates an expulsion force profile from which the force required for the initial movement of the plunger into the syringe (breakout force) is calculated. The breakout force of a pre-filled syringe is regarded an important factor for the development of the device variables for the auto-injector. The spring tension for an auto-injector is set based on the breakout force and the force at the end of the stroke of a pre-filled syringe. The effects of various factors on the expulsion force profile of Liquid Avonex® were determined. From the nature of the expulsion force profile, the ease of injectability of the pre-filled syringes was evaluated.

2. MATERIALS

Interferon beta-1 a (Liquid Avonex®)(Biogen, Inc., Cambridge, MA), Tween 20 (Polyoxyethylene Sorbitan Monolaurate)(Sigma Aldrich Co. St. Louis, MO), 0.22 μm STERIVEX-GV filter unit (Millipore Corporation, Bedford, MA), SCF 1 ml Long Luer CCT Syringe (Becton Dickinson™, Zp9843100, France, S.A.), Needle 23G 1¼ Precision Glides® (Becton Dickinson™, Franklin Lakes, NJ), Intramuscular injection pad (IMIP), Model # 00250 (Limbs and Things Ltd., Bristol, BS2 ORA, UK).

3. METHODS

3.1 Instron Parameters/ Measurements

The Instron model 5542 was used for the study, since it is a single column tester specifically designed for compressive testing on pre-filled syringes. Merlin software controls the Instron series for test-setup, test control, data collection, result generation and report preparation. The three main parameters to run a manual method using the Instron 5500 series are the Motion (speed) and Data (time and load). The Data (time) refers to the time interval for data collection and the Data (load) refers to the load needed for the data collection. Other parameters are the Extension and the Load. The load refers to the actual force that is applied by the instrument. The extension is set based on the distance traveled by the plunger into the syringe. Liquid Avonex® pre-filled syringes were subjected to different levels of speed, time and load and based on the nature of the expulsion force profile, i.e. the amount of noise produced and the number of data points collected, a suitable combination of the three parameters was achieved. The parameters set for the syringe expulsion studies were: Speed: 25 mm/min, Data (time): 150 msec, Data (load): 0.15 N, Extension: 20 mm, Load: 30N.

3.2 Force data

A typical load vs. extension curve (expulsion force profile) of Liquid Avonex® pre-filled syringe is shown in figure 1. The curve characteristically demonstrates a peak, followed by a plateau. The rise in the curve at the end of the expulsion force profile occurs when the entire drug is expelled from the syringe. The

breakout force (load at which the first peak occurs) is the force required for the initial movement of the plunger into the syringe. The Fmax refers to the maximum force at any particular extension. The Fmax at 10 mm gives an indication of the amount of force required to expel the liquid when it is half way through the syringe. The Fmax at 20 mm refers to the state when the liquid nears the needle portion of the syringe.

3.3 Intramuscular Injection Pad (IMIP) Parameters

The injection penetration profiles of staked and non-staked needle sharps were studied by penetration into a model intramuscular injection pad (IMIP). Parameters were developed for the needle penetration studies similar to the syringe expulsion studies. The motion (speed) to inject both luer-slip and staked needles was 50 mm/min. Penetration data was captured at a load of 0.15 N and a time of 150 msec. A Precision Glides® 23 G needle was attached to the luer-slip syringes. The depth of penetration of the Precision Glides® 23 G needle and the staked needle into the IMIP was 32 mm. Hence the extension was set to –35 mm. Thirty staked and luer-slip syringes were penetrated into the IMIP.

3.4 Size Exclusion Chromatography (SEC)

Distributions of intact protein, soluble aggregate and/or fragments of interferon beta-1a were determined using SEC. A high-performance liquid chromatography (HPLC) system (Waters Alliance 2690) was used with a Superdex® G-75 column at a flow rate of 0.8 ml/min using 4.8A buffer as the mobile phase. 200 µl of protein sample was injected into the column and detected by absorbance at 215

nm. Standards of known protein concentration were run as controls for each set of samples. Samples were run six times and the average value was used to determine the fraction of aggregated protein in the sample. The fractions of aggregate, intact protein and protein fragments were determined by assessing the relative peak areas of each species. The SEC yielded complete protein recovery (no protein loss) when compared with appropriate controls.

4. RESULTS AND DISCUSSION

4.1 Examination of Breakout force and Expulsion force profile for variations within batches and between batches

Staked needle syringes

Five batches of concentration 60 μg/ml and three batches of concentration 120 μg/ml were tested. Thirty samples were tested from each batch. To study the within batch variability, the thirty samples were obtained as ten beginning, ten middle and ten end samples.

Luer-slip needle syringes

Five batches of concentration 60 μg/ml were tested. Thirty samples were tested from three batches and five samples were tested from two batches.

Substantial intra-batch (i.e. within the beginning, middle and end syringes) and inter-batch variability were seen in the expulsion force profiles of the syringes tested. Some examples are shown in figure 2 and 3. It was believed that the

variability could be due to the effect of age of silicone oil or uneven amounts of silicone oil. The batches tested were of ages from 8 months to 35 months. It was assumed that the silicone oil might have lost its lubricant action with age and hence caused the inter-batch variations. The breakout force for all the batches tested along with their ages are tabulated in table 1 and graphed in figure 4. Though there were substantial differences in the breakout force from batch-batch, no consistent increase in the breakout force with increase in age of the batch was seen. Thus the data was inconclusive in determining the effect of age of silicone oil on the breakout force of a syringe.

It is noteworthy that the batches tested did not show a smooth expulsion force profile as shown in figure 1. From figure 5, it can be seen that the Fmax at 15 mm extension was greater than the Fmax at 10 mm extension for all the batches tested, indicating that more force was required to expel the drug as it neared the needle end of the syringe.

4.2 Effect of viscosity of silicone oil

Silicone oils used as lubricants have a degree of polymerization (n = 400 to 1200) such that their kinematic viscosities are nominally between 1000 cSt and 30,000 cSt (2). Vetter Pharma is the contract manufacturer for Biogen Inc., for pre-filled syringe filling. Two types of silicone oils (the 1000 cSt and 12,500 cSt silicone oils) have been used by Vetter Pharma to siliconize the syringes. An in-house testing by Vetter Pharma was performed to analyze the possibilities of the current

siliconization process with regard to the oil quantity and plunger forces. The tests showed that the low viscosity 1000 cSt oil was more evenly distributed within the syringes in comparison to 12,500 cSt silicone oil. Batches produced with 1000 cSt oil delivered acceptable and substantially lower plunger forces than the 12,500 cSt oil batches. Moreover the expulsion force profiles of the 12,500 cSt oil batches were not smooth. They showed an increase of forces towards the right side of the force diagram similar to that seen in the batches tested previously (figures 2, 3). This was explained by less silicone oil at the needle end of the syringe compared to the upper end, due to a striping method used by Vetter Pharma to siliconize the syringes.

The syringes used to study the intra-batch and inter-batch variability were siliconized by Vetter siliconization method. Testing was also performed on development syringes which were siliconized using the BD (Becton Dickinson) siliconization method. Characteristic differences between the two types of syringes are given in Table 2. A comparison of the expulsion force profile of development syringes and Vetter processed syringes is shown in figure 6. The BD siliconized syringes had break out force ranging from 3-5 N in comparison to 5-10 N in case of Vetter siliconized syringes. Increase of force towards the right side of the force diagram as seen with the Vetter processed syringes were not seen with the BD siliconized syringes. The obtained data thus correlated with the findings by Vetter.

4.3 Effect of Temperature

Avonex® (Interferon beta-1a) should be stored at 2-8°C. However, if refrigeration is not available, Avonex® can be stored at 25°C for up to 30 days (3). The effect of accelerated storage temperature on the expulsion force profiles was studied. Two batches were tested at temperatures, 2-8°C and 30°C (60% RH) for 6 hrs. It was seen that the breakout force of the syringes did not show any substantial change with the increase in temperature (figure 7).

4.4 Effect of surfactant

Proteins drugs like Liquid Avonex® are susceptible to aggregation and precipitation when subjected to high forces. It is generally accepted that the pathway leading to aggregate formation is related to the conformational changes a protein molecule undergoes when exposed to an interface (4). Protective excipients such as non-ionic surfactants are usually added to solutions of therapeutic proteins in order to maintain adequate shelf life and protect patients against possible adverse effects of protein aggregation and degradation (5). The protective excipient present in Liquid Avonex® is the non-ionic surfactant, tween 20. The mechanism by which a surfactant acts to protect the protein is not clear, although several possibilities exist. One such mechanism is competition with the protein for adsorption on various interfaces, such as the air/solution interface or vial/solution interface thus protecting against denaturation and aggregation at these interfaces (5).

It was believed that the presence of surfactant would reduce the breakout force of the pre-filled syringes. Four formulations were prepared: 4.8A Buffer (73.06 mN/m), 4.8A Buffer with 0.005% Tween (49.81 mN/m), IFN-beta-1a with 4.8A Buffer (52.59 mN/m), IFN-beta-1a with 4.8A Buffer and 0.005% Tween 20 (43.03 mN/m). Kruss Tensiometer was used to determine their surface tension. The four formulations were filled in to luer-slip syringes, siliconized by BD siliconization method and were subjected to compressive testing by Instron. Unlike the luer-slip batches previously tested, the expulsion force profiles of these syringes were smooth (no increase of forces towards the right side of the force diagram, Figure 8). This was believed to be due to the BD siliconization process, which used 1000 cSt silicone oil for the barrel and plunger. The breakout force had an average of 4.01 ± 0.38 N for the four formulations. Hence it can be confirmed that 1000 cSt silicone oil was more evenly distributed and gave better ease of injectability than the 12,500 cSt silicone oil. There were no substantial differences in the breakout force for the four formulations (Table 3). Thus presence of surfactant did not affect the expulsion force profile of the pre-filled syringes.

4.5 Effect of expulsion speed

The characterization of the structure of a protein drug is complex because of the presence of primary, secondary, tertiary, and quaternary structures (6). The complex hierarchy of the protein may be disrupted by multiple possible destabilizing mechanisms (7). When a protein drug is given as an intramuscular injection using an autoinjector, there are possibilities that the force of expulsion

might have a significant effect on the integrity of the protein. The effect of high speed on the expulsion force profile of Liquid Avonex® was studied. Liquid Avonex® formulations were expelled at a speed of 500 mm/min (maximum speed limit of Instron). The extension was set to 20 mm, load to 0.15 N and time to 150 msec. The structural integrity of the protein was studied by subjecting the expelled liquid to size exclusion chromatography (SEC). The samples were divided into two groups: Syringe samples that were expelled through syringes and Non syringe samples (control samples) that were formulated and directly subjected to size exclusion chromatographic analysis. The syringe samples were in turn expelled in two ways: expulsion through Instron and manual expulsion using high force. The percent total aggregate of the test samples were found to be within the specifications when compared to the control (Table 4). The integrity of the protein was thus found to be unaffected by the speed of expulsion.

4.6 Needle Penetration Studies

With regard to the needle, pain associated with IM injections can be considered to be related to three factors: needle penetration of the skin, penetration of the needle through the skin and muscle, and pulling/tearing of the skin and/or muscle tissues as the needle moves non-linearly with some curvature in its penetration path (8). Little attention had been given in the literature regarding the movement patterns of an injection and how movement patterns might be associated with pain (9). It is believed that an optimal injection should have relatively linear motion of the needle during penetration (8). Factors affecting the linear motion are

the size, shape and sharpness of the needle. Previous studies have shown that the geometry of a needle is an important factor affecting the ease of tissue penetration (10, 11). Sharpness of a needle has been evaluated in terms of the maximum force required to penetrate synthetic membranes. However these studies did not examine the load-displacement curve of the needle penetration (12).

An Investigation Report conducted on a Clinical Trial summarized approximately thirty to forty complaints regarding duller sharps and increased pain upon injection from six clinical sites that used Liquid Avonex® staked needles. It was believed that during the processing, siliconizing and shielding of staked needles, the tips and shafts become dull and in some case defective. To further confirm the assumption, the penetration force profile of luer-slip and staked needles were studied by penetrating the needles in to an IMIP. The IMIP is a multi-layered soft tissue block used for practicing tissue injection techniques.

The penetration studies indicated that a slightly greater Fmax was required to penetrate the IMIP for staked needle (2.3 ± 0.3 N) in comparison to the luerslip (1.7 ± 0.2 N). The penetration profile for the staked needle appeared skewed compared to the luerslip (figure 9). The data suggested that a greater resistance occurred with the staked needles during penetration into the IMIP, which resulted in non-linear motion of the needles. This is believed to be due to the dull sharps of the staked needles. Thus staked needle syringes would cause a considerable amount of pain when injected intramuscularly.

5. CONCLUSION

In this study Liquid Avonex® luer-slip and staked needle syringes were subjected to compressive testing by Instron and the effects of various factors on the expulsion force profile were studied. The expulsion force profiles were used to evaluate the ease of injectability of the syringe. The viscosity of silicone oil used for siliconizing the syringes was found to be a critical factor affecting the ease of injectability. 1000 cSt silcone oil was found to give better injectability than 12500 cSt silicone oil. Storage temperature, presence of surfactant and expulsion speed did not have any major effect on the expulsion force profile. A comparative study of luer-slip and staked needles was done and it was seen that luer-slip needles had a better penetration profile.

This study demonstrates the utility of injectability measurements for the development of one intramuscular protein injection, Liquid Avonex®. However, the results reported in this paper clearly indicate that use of the Instron is very probably an effective and convenient way to quantify ease of injectability for many parenterals. It is believed that although the data reported in this paper was derived from investigations of one particular injection, there is no reason to believe that this approach would not be applicable to many other parenteral products.

6. ACKNOWLEDGMENTS

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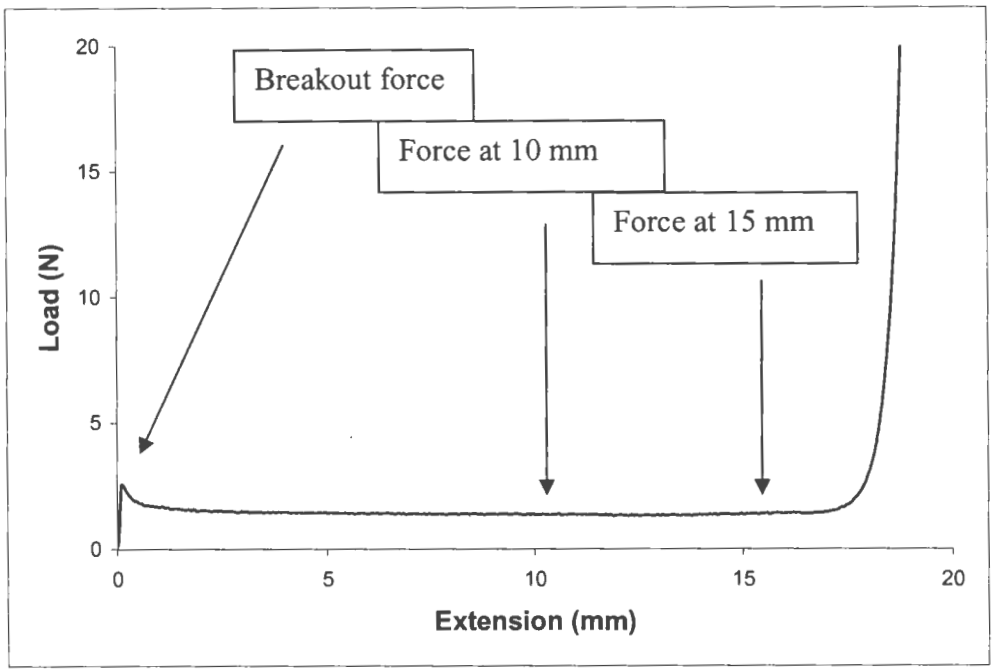


Figure 1. Expulsion force profile of Liquid Avonex® pre-filled syringe

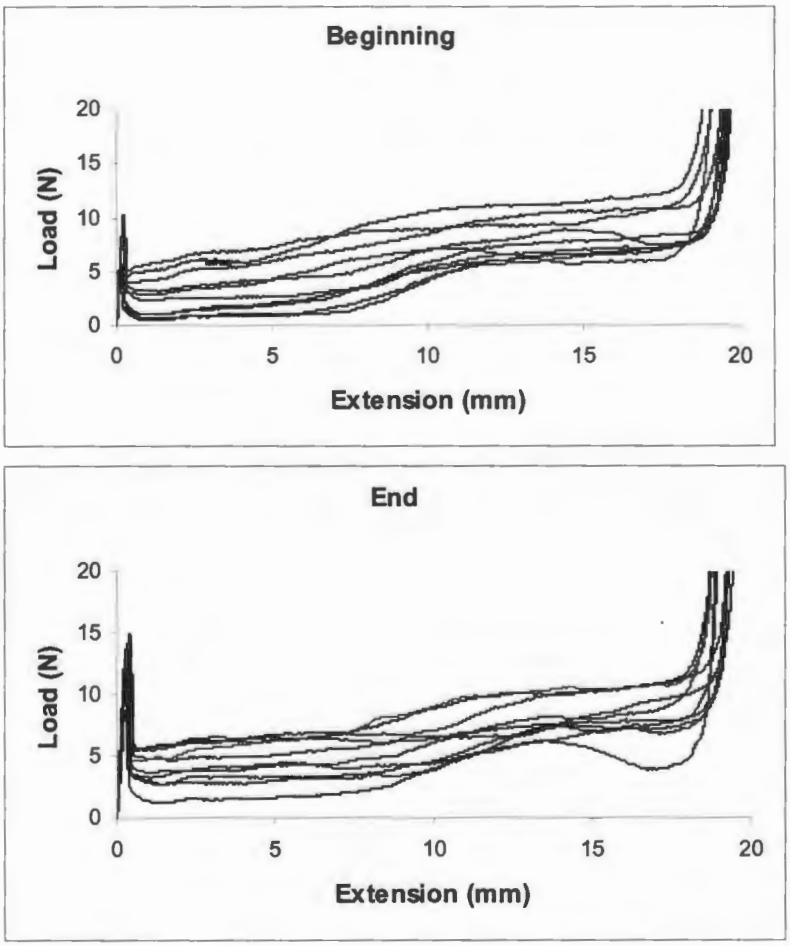


Figure 2. Expulsion force profile of Liquid Avonex® Staked needle syringes, Batch No. 022002, 60 μg/ml, n=10

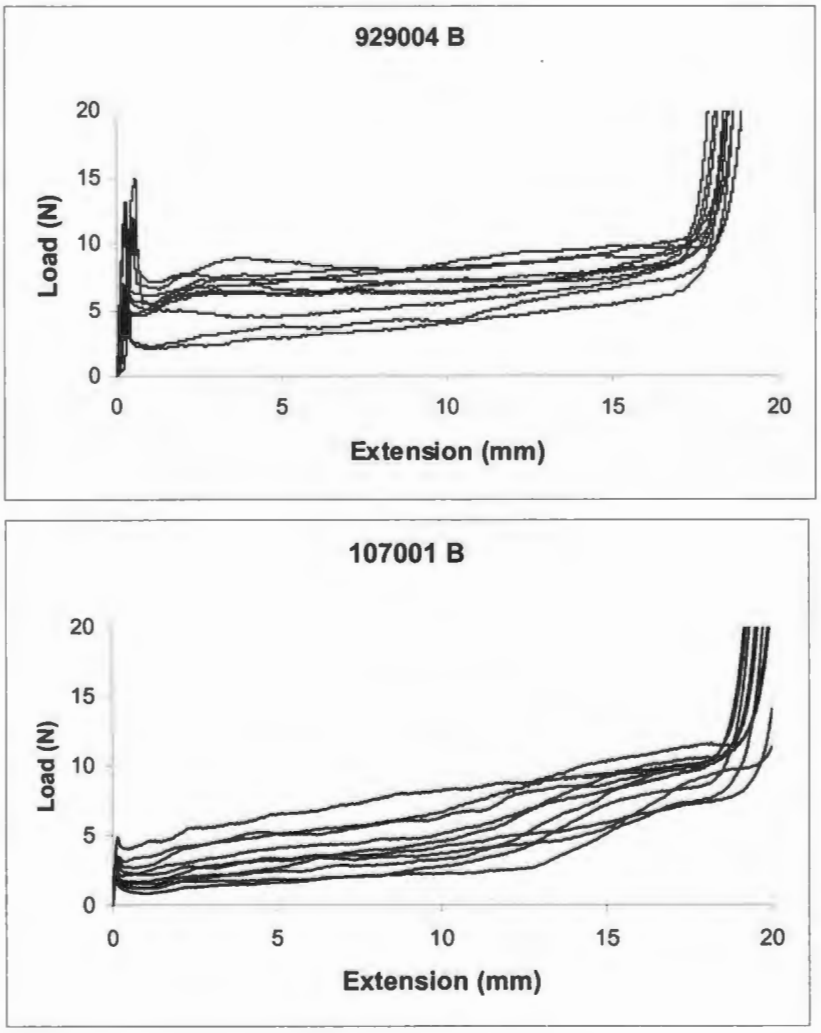


Figure 3. Expulsion force profile of Liquid Avonex® Luer-slip syringes, Batch No. 929004 and 107001, 60 μg/ml, n=10

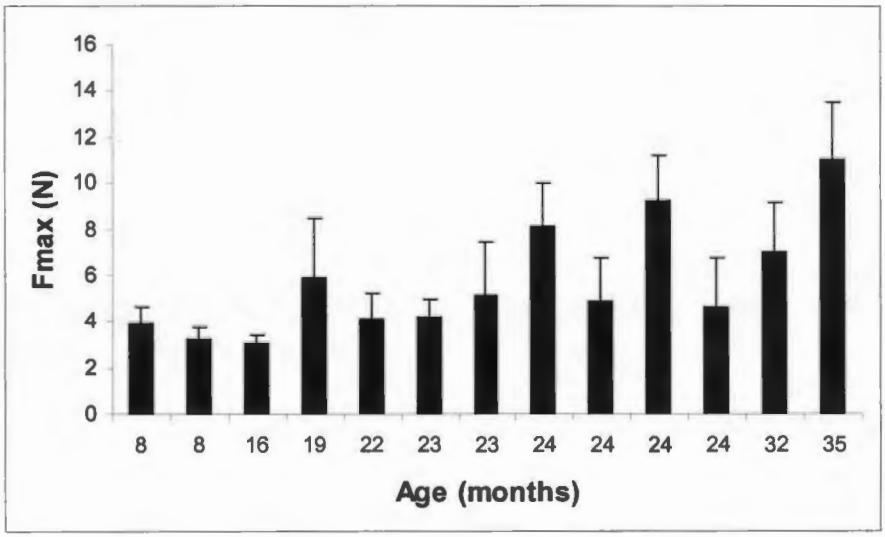


Figure 4. Age vs. breakout force for all the batches tested

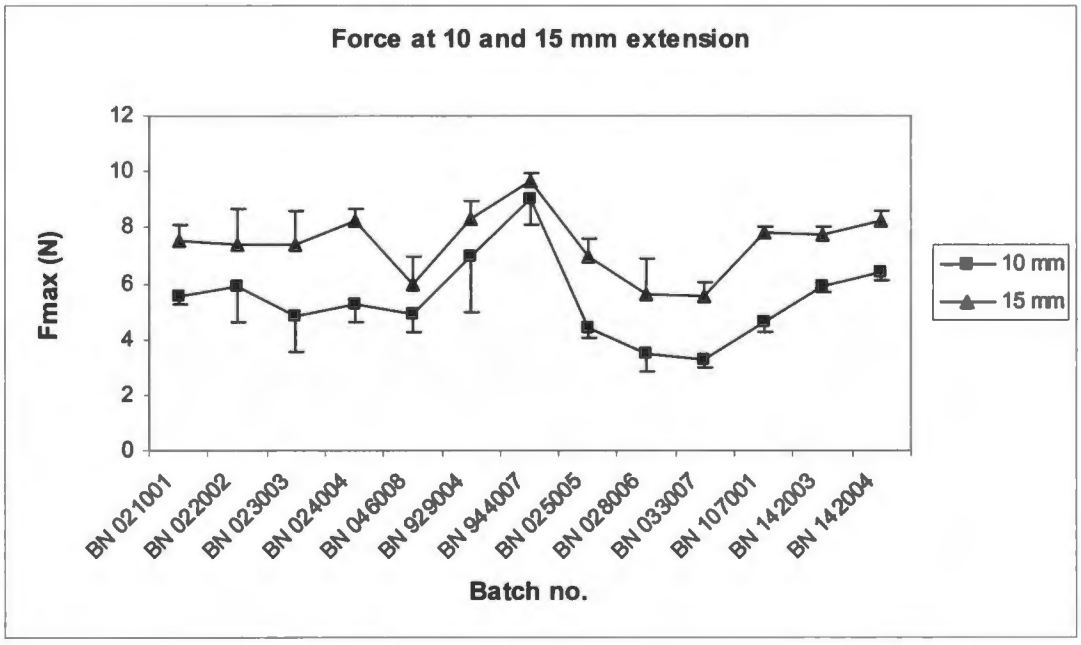


Figure 5. Force at 10 and 15 mm extension for all the batches tested

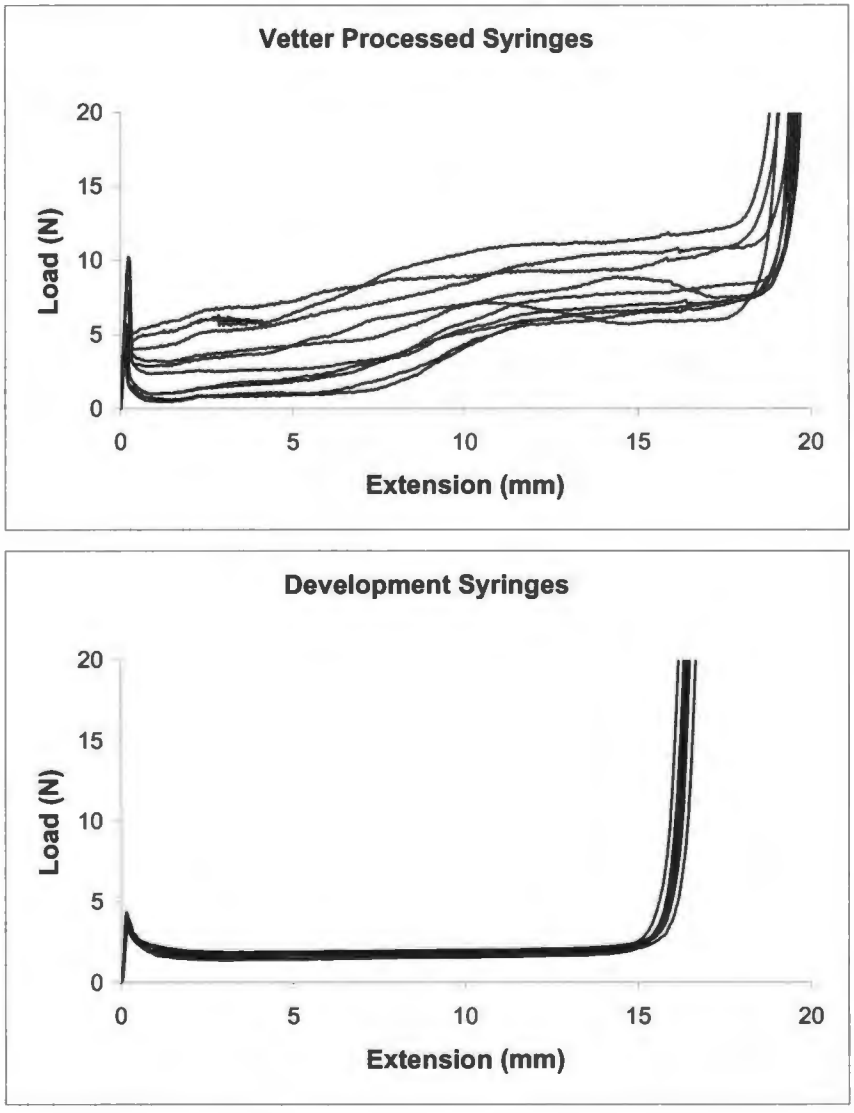


Figure 6. Comparison of expulsion force profile of Vetter processed syringes and Development syringes, n=10

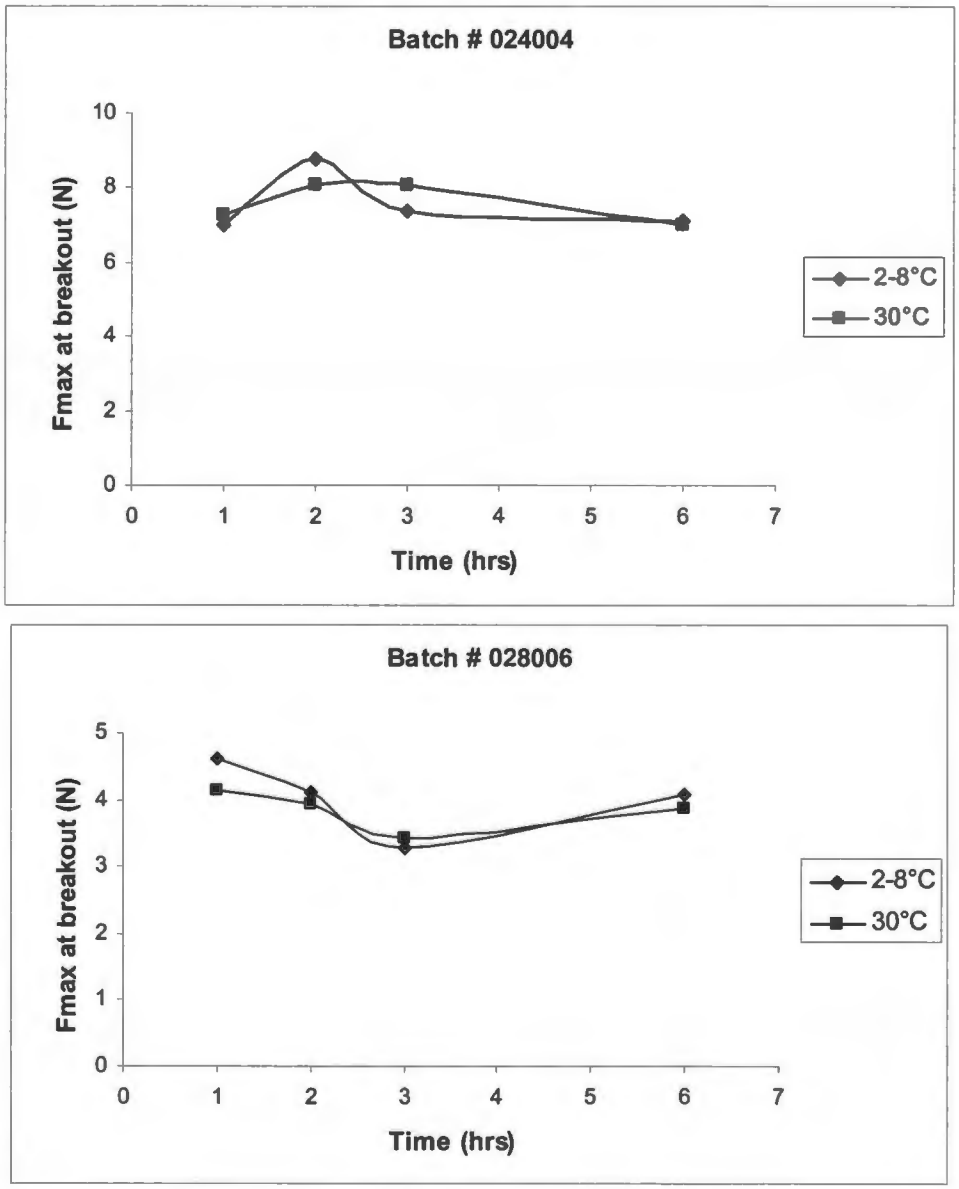


Figure 7. Comparison of the Fmax at breakout of Liquid Avonex® staked needle syringes, batch # 024004 and 028006 stored at two temperatures, 2-8°C and 30°C, n=5

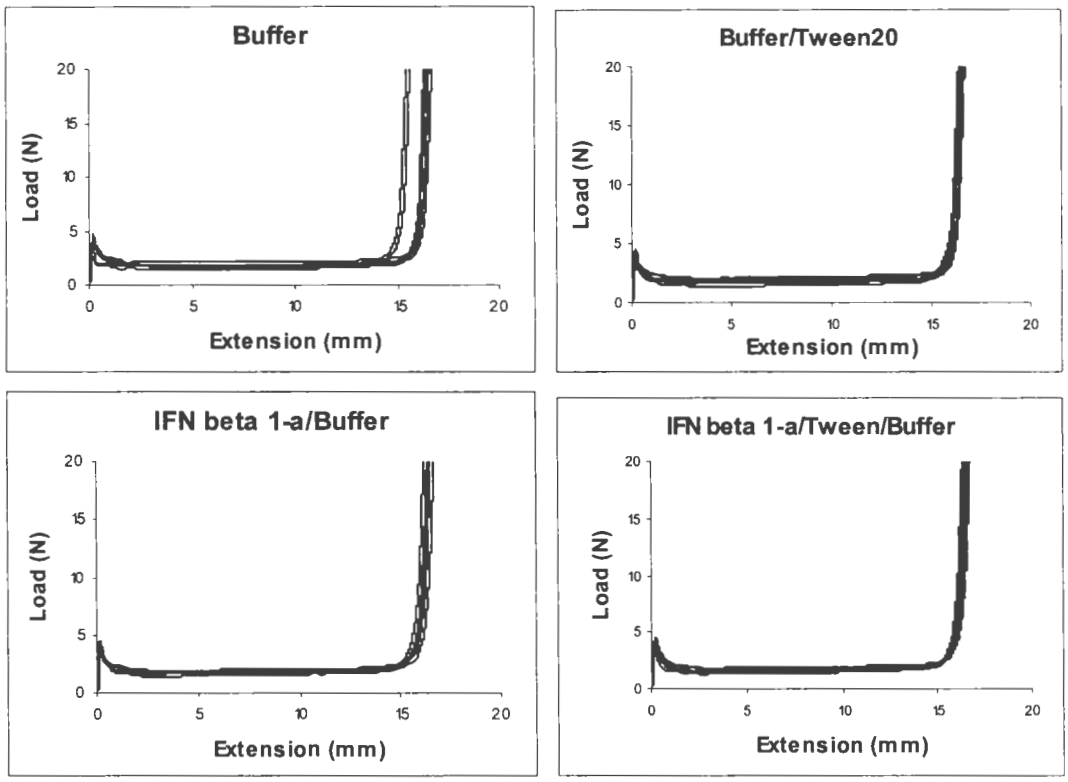


Figure 8. Effect of surfactant on expulsion force profile of Liquid Avonex® luer-slip syringes (siliconized by BD), n=10

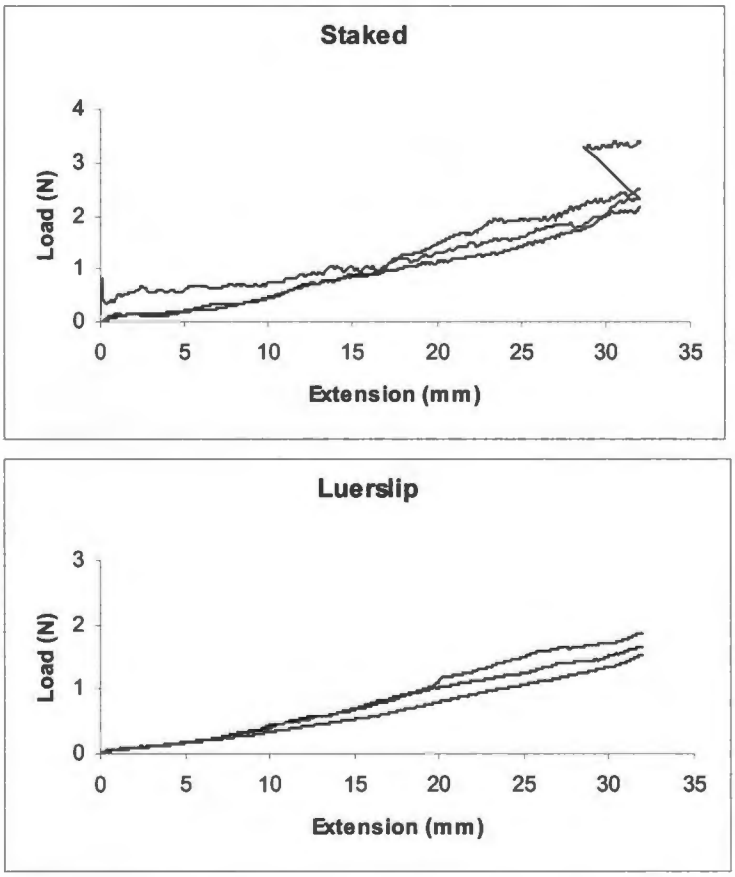


Figure 9. Needle penetration profile of luer-slip and staked needles (n=3: samples having low, medium and high Fmax at 32 mm)

Table 1.

Age of silicone oil of the batches tested and their force at breakout

Age (months)	Batch no.	Conc. (μg/mL)	Average Fmax (N) Beg (10)	Mid (10)	End (10)	AVG	SD
8	142004	60	3.96 (5)	-	-	3.96	0.69
8	142003	60	3.26 (5)	-	-	3.26	0.53
16	107001	60	3.00	3.49	2.72	3.07	0.39
19	046008	60	6.67	5.63	5.46	5.92	2.56
22	033007	120	4.55	4.55	3.28	4.12	1.13
23	028006	120	4.28	4.19	4.00	4.15	0.78
23	025005	120	5.25	4.57	5.52	5.11	2.31
24	024004	60	6.97	9.23	8.3	8.16	1.84
24	023003	60	5.60	3.71	5.41	4.9	1.86
24	022002	60	6.90	8.54	12.42	9.28	1.93
24	021001	60	4.99	3.48	5.34	4.6	2.15
32	944007	60	6.92	7.44	6.6	6.98	2.16
35	929004	60	10.92	12.38	9.8	11.03	2.52

Table 2.

Differences between the Development syringes & Vetter processed syringes

Parameters	Development Syringes	Vetter Processed Syringes
Siliconization	BD Siliconization	Vetter Siliconization
Viscosity of silicone oil used for plunger	1000 cSt	1000 cSt
Viscosity of silicone oil used for barrel	1000 cSt	12500 cSt
Nature	Hypak SCF syringes	Bulk Syringes
Method	Spraying method	Striping method
Sterilization process	Gamma radiation	Autoclaving

Table 3.

Force at breakout for the four formulations

S. No.	Buffer	Buffer/Tween	IFN/Buffer	IFN/Tween /Buffer
1.	4.56	4.44	3.85	3.86
2.	4.36	4.39	4.06	3.41
3.	4.24	4.02	4.09	4.21
4.	4.35	3.73	3.71	4.15
5.	4.26	3.63	4.37	4.50
6.	2.67	3.86	3.89	3.61
7.	3.36	3.61	3.74	3.89
8.	4.25	4.03	3.77	4.06
9.	4.66	4.39	4.03	4.07
10.	4.25	4.39	3.64	4.08
AVG	4.10	4.05	3.92	3.98
SD	0.61	0.33	0.22	0.31

Table 4.

Percent peak area of the samples subjected to SEC

Tween 20	Sample Condition	Percent Peak Area			
		Monomer %	Dimer %	Agg %	Total Agg %
Present	Control (n=3)	99.8	0.1	0	0
	Instron (n=6)	99.5	0	0	0
	Manual (n=6)	99.6	0	0	0
Absent	Control (n=3)	99.6	0.1	0.2	0.3
	Instron (n=6)	99.7	0	0.3	0.2
	Manual (n=6)	99.6	0.1	0.2	0.2

SUMMARY

1. Avonex (Interferon beta-1a) is a protein drug used for the treatment of relapsing forms of Multiple Sclerosis, and is administered intramuscularly once in a week. Intramuscular injections are a common yet complex technique used to deliver the medication deep into the large muscles of the body. Several complications have been reported following IM injections, of which pain at the injection site is the most common.
2. Though factors such as site of injection, volume, concentration and nature of the drug may affect the reception of pain, one factor which has not apparently been given any attention in the published literature is the ease of injectability of the syringe, which is the amount of force required to expel the drug from the syringe. This factor can be expected to have a substantial effect on the discomfort experienced by patients and on the completeness of the injection.
3. A comprehensive review of the published literature failed to reveal any publications dealing with the formulation of intramuscular injections with particular reference to factors which may affect the ease of injectability. Hence there is a need for some objective, quantitative method for measuring the ease of injectability such as may be provided by use of the Instron Series 5500. The Instron Series 5500 has been used for several purposes in several fields; however no studies report the use of Instron to study the ease of injectability of a syringe.
4. The potential of Instron type equipment for the quantification of ease of injectability of syringes has been evaluated. Compressive studies have been

performed on Liquid Avonex® pre-filled luer-slip and staked needle syringes using the Instron series 5500. The ease of injectability of the syringes was quantified by studying the various factors affecting the expulsion force profile (breakout force).

5. Substantial inter-batch and intra-batch variations were seen in the expulsion force profile, which were due to the improper distribution of silicone oil during siliconization.
6. The viscosity of silicone oil and the method of siliconization were found to be critical factors defining the expulsion force profile. The low viscosity 1000 cSt oil was more evenly distributed within the syringes and delivered acceptable and substantially lower breakout forces than the 12,500 cSt oil. The increase of forces towards the end of the syringe as seen with the 12,500 cSt oil was not seen with the 1000 cSt oil. Thus 1000 cSt oil gave better ease of injectability than the 12,500 cSt silicone oil.
7. The presence of surfactant tween 20 in the formulation did not contribute to a reduction in breakout force of the syringes.
8. Increasing the storage temperature from 2-8° C to 30° C did not have any substantial effect on the breakout force of the syringes.
9. Increasing the expulsion speed from 25 mm/min to 500 mm/min (maximum speed limit of Instron) did not increase the percent total aggregate of Interferon beta-1a. Thus the integrity of the protein was unaffected by the speed of expulsion.

10. Needle insertion is a very common invasive procedure, but little study has been done to study how pain is related to the mechanical properties of the needle insertion and how pain from the procedure can be minimized.
11. Needle penetration studies were conducted using the Instron to examine the injection profiles of staked and non-staked needles into a model intramuscular injection pad. The maximum force required to penetrate the muscle and the penetration profile of the needle into the muscle were recorded. The results showed that non-staked needles need lesser penetration force and show linear penetration profile in comparison to staked needles and thus cause less pain on injection.
12. The billion-dollar biotechnology market is driving the demand for improved protein and peptide drug delivery products. A majority of these compounds have high molecular weights and solubility and stability issues that prevent them from being delivered via any other route other than the parenteral route. Hence a clear need remains for injectability studies on parenterals to be able to deliver the drug efficiently.
13. It is believed that although the data reported in this paper was derived from investigations of one particular injection, there is no reason to believe that this approach would not be applicable to many other parenteral products.
14. A variety of devices such as autoinjectors have been developed to ease the self-administration of injections. Injectability studies play a significant role for injections via autoinjectors in which the improper delivery of the drug might not be known to the patients.

SUGGESTIONS FOR FUTURE WORK

Some suggestions for future work which the author of the thesis was not able to perform due to time constrains are as follows:

1. Of the various differences present between the development syringes and the vetter processed syringes, the method used to apply the silicone oil is a notable one. The former were siliconized using spraying method, whereas the latter were siliconized using striping method. Detailed studies of the effect of the method of siliconization on the breakout force of the syringe can be carried out.
2. In-vitro needle penetration studies were carried out using the model intramuscular injection pad and it was found that luer-slip needles needed less force for penetrating the deep muscles and hence would cause less pain on injection. It may well be desirable to perform in-vivo tests (with approval from Institutional Review Board) with luer-slip and staked needles and record the pain produced on a pain measuring scale to confirm the in-vitro study.
3. It may also be desirable to conduct long-term stability studies and evaluate the percentage aggregates of Interferon beta-1a and the ease of injectability of the pre-filled syringes to study the effect of long-term storage.

SECTION III

APPENDIX A

Series 5500 System Overview

The major components of the Instron Series 5500 system are the load frame, control panel and the merlin windows-based software. The control panel provides instant access to test functions such as start and stop, balance load, reset gauge and jog. The Merlin screen consists of several icons to perform several functions. Few examples are the load frame icon which enables to load frame after a frame limit is tripped, extension icon to set the maximum and minimum limits, load cell icon to calibrate the load cell.

The *Test Control* icon is the main control panel for developing a test method. It consists of the following test control features:

- *Motion* – The Motion window is mainly used to set the speed, i.e. the rate at which the crosshead moves during the test.

- *Events*- This window is used to enable the channel events at set values and to select a resulting action to occur when the event is tripped. For example, we might want to run a test to a specific extension (channel) limit of 4 inches, and when the extension reaches 4 inches, we might want to stop (action) the test.

- *Data*- The Data window allows to select automatic or manual data capturing. Automatic data capture sets up two data channels with preset intervals to acquire

data. The first channel is the time with a 1 second interval. The second channel is load with an interval of 0.1% of the full scale of the installed load cell. Manual data capture enables to select three data channels.

Other icons are the *Sample* icon which is used to set parameters like the sample name, number of specimens etc. The *Results* icon allows the user to setup result tables. The *Graph* icon is used to perform the graphing functions.

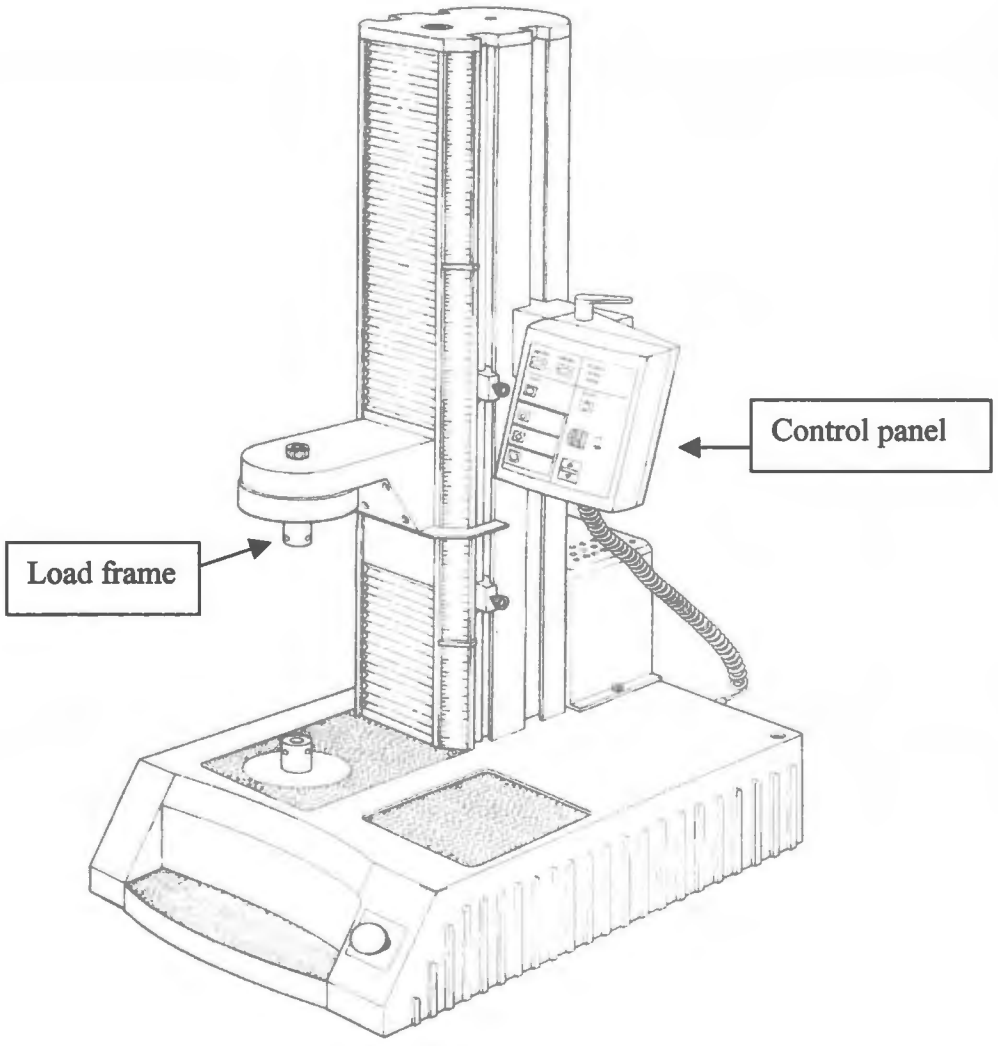


Figure 1. Schematic diagram of the Instron Series 5500

Instron Parameters/ Measurements

The three main parameters to run a manual test method using the Instron 5500 series are the Motion (speed) and Data (time and load).

1. Speed: The speed is the rate at which the crosshead moves during a test. Liquid Avonex® pre-filled syringe samples were dispensed at four different speeds: 5, 10, 25 and 50 mm/min and the expulsion profiles were studied. The Load was set to –30 N and the Extension to –20 mm. The Data parameters (time and load) were set to 50 msec and 0.15 N. The Load, Extension and Data parameters were set according to Biogen, Inc.'s internal document. From the four speeds tested the 25 mm/min speed was found to give the least amount of noise in the expulsion force profile (figure 1). Hence 25 mm/min speed was chosen for further studies with Liquid Avonex®.

2. Data (Time): The Time parameter denotes the time of the test run. The data points will be taken according to the time interval that is set. The basic principle of setting the parameters is that one parameter is set constant and the others are varied. Then based on the nature of the expulsion profile (i.e. the amount of noise produced and number of data points generated), the parameters are chosen. A speed of 25 mm/min with a time of 50 msec gave a good expulsion profile. However with time set to 50 msec, the amount of data points collected was too large that it generated small amounts of noise in the expulsion profile. Hence Liquid Avonex® was subjected to other time points like 25, 50, 75, 100, 150 msec. It was found that the 25 mm/min

speed gave a better expulsion profile with a time interval of 150 msec in comparison to 50 msec.

3. Data (Load): The third parameter to be set was the load for data capture. A load of 0.15 N was initially used for the speed test and the time test. However, Liquid Avonex® was also subjected to 0.15, 0.25, 0.5, 0.75 N load levels. The expulsion profiles were studied and the 0.15 N load was considered suitable to develop the manual test method for the Instron 5500 series.

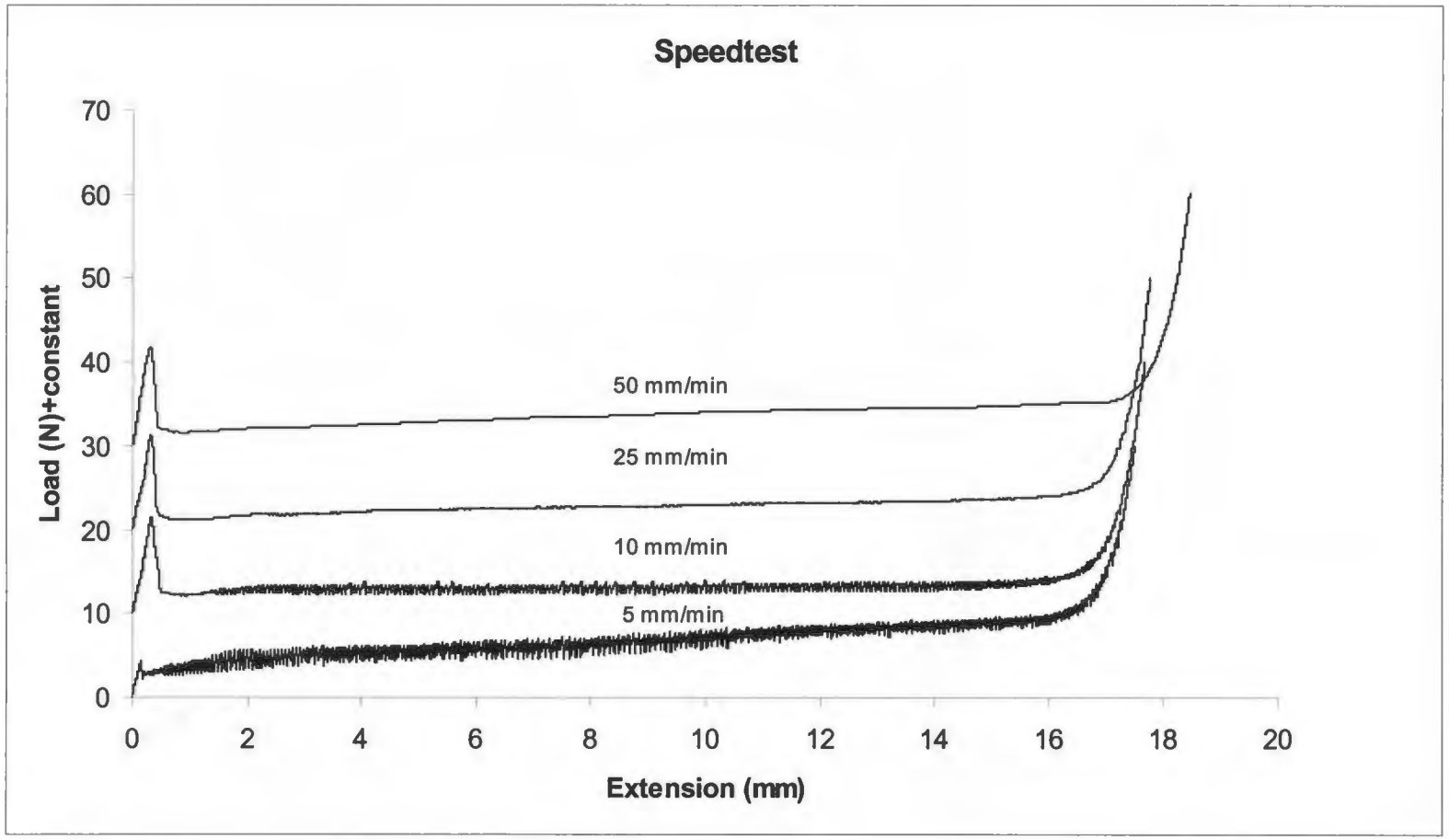


Figure 2. Speedtest with 50 msec time and 0.15 N load

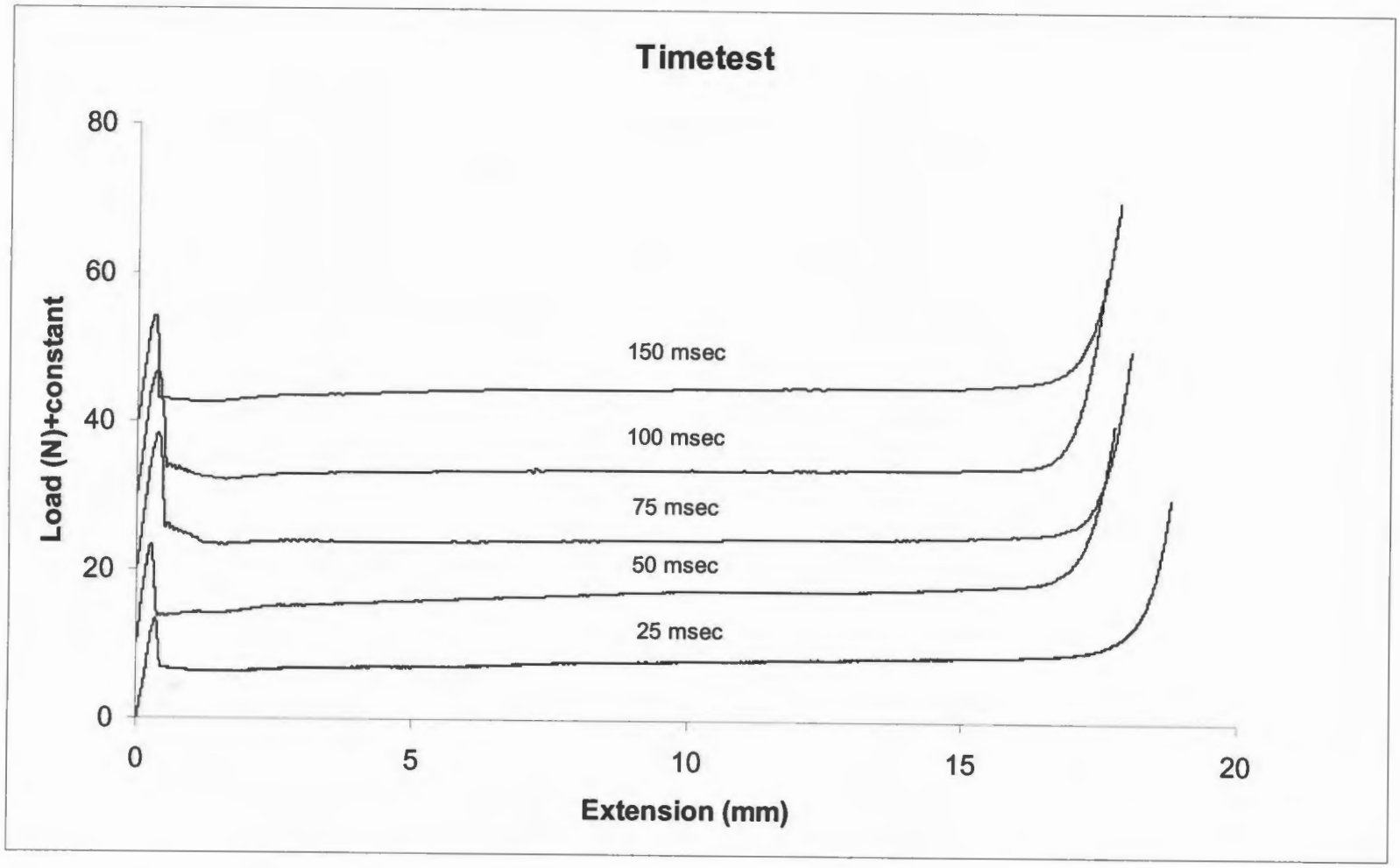


Figure 3. Timetest with 25 mm/min speed and 0.15 N load.

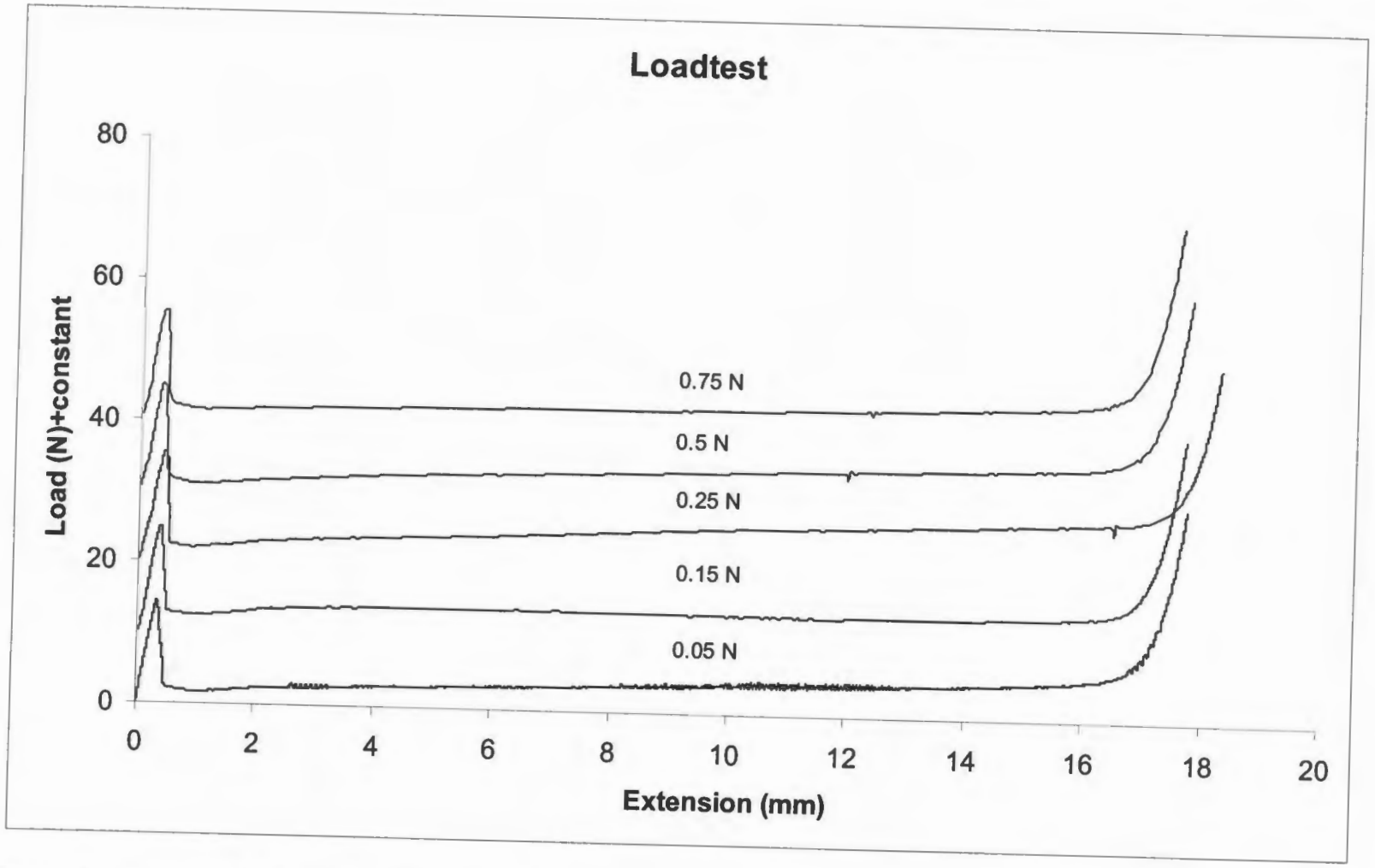


Figure 4. Loadtest with 25 mm/min speed and 150 msec.

APPENDIX B

Appendix B contains

1. Expulsion force profiles of Liquid Avonex® staked (Figure 1-8) and luer-slip (Figure 9-13) syringes tested to study the within batch and batch-batch variability.
2. Force at breakout for the syringes tested to study the within batch and batch-batch variability (Table 1 and 2).
3. Force at breakout for the syringes tested to study the effect of storage temperature (Table 3).
4. Expulsion force profile of development syringes (Figure 14).

Staked needle syringes, 60 µg/mL
Batch # 021001

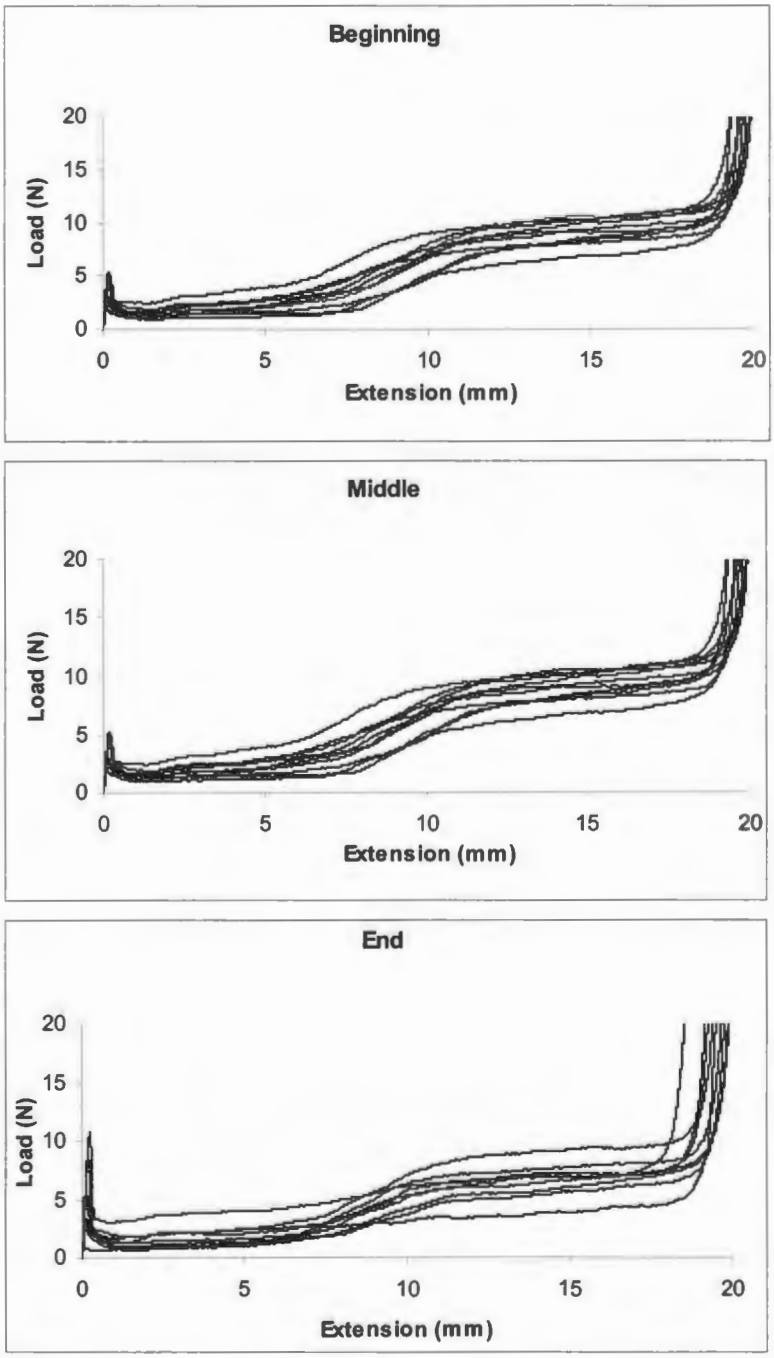


Figure 1. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 021001, n=10

Batch # 022002

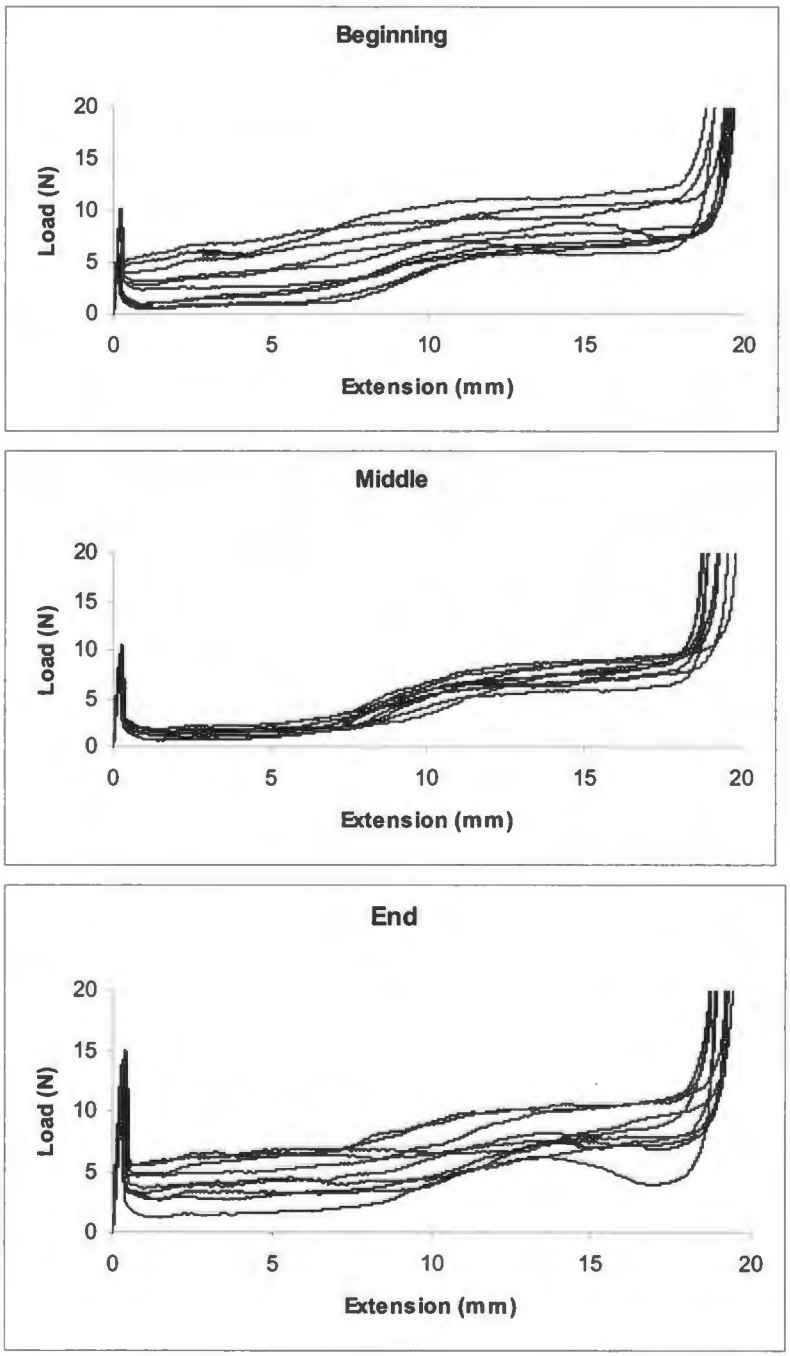


Figure 2. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 022002, n=10

Batch # 023003

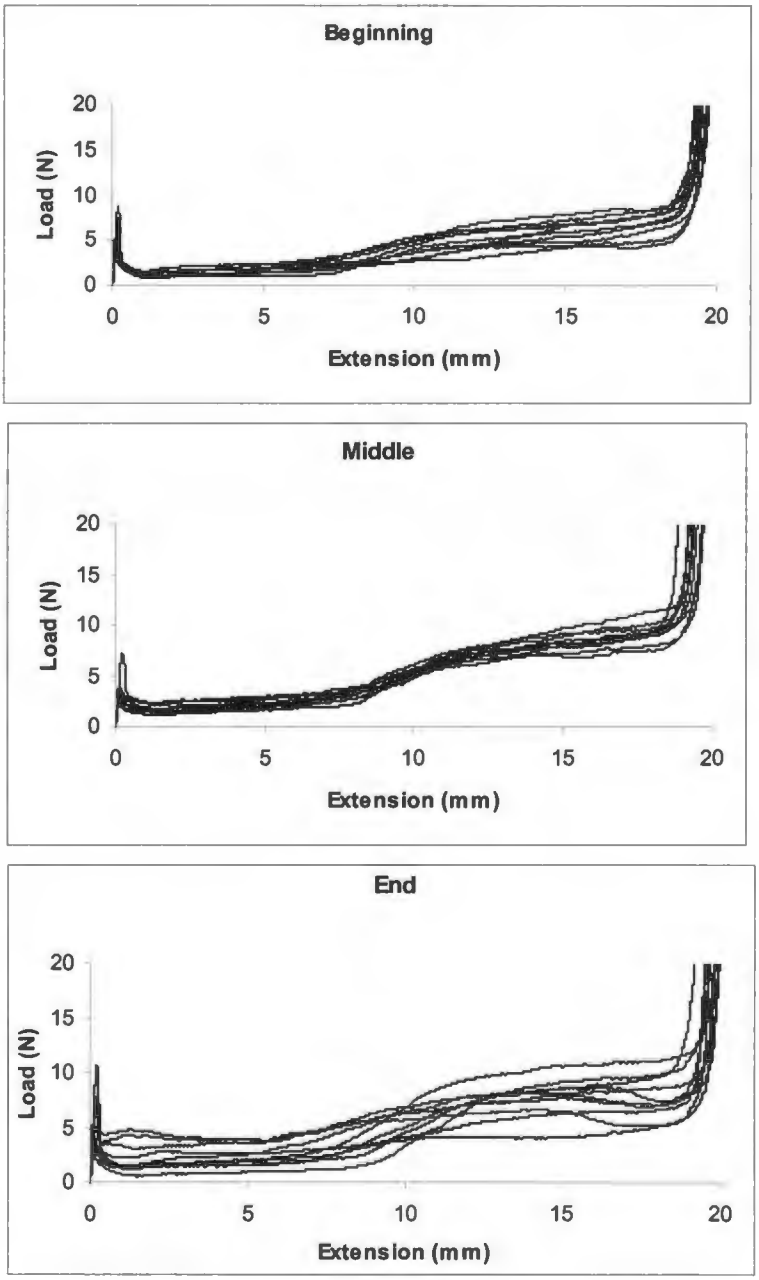


Figure 3. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 023003, n=10

Batch # 024004

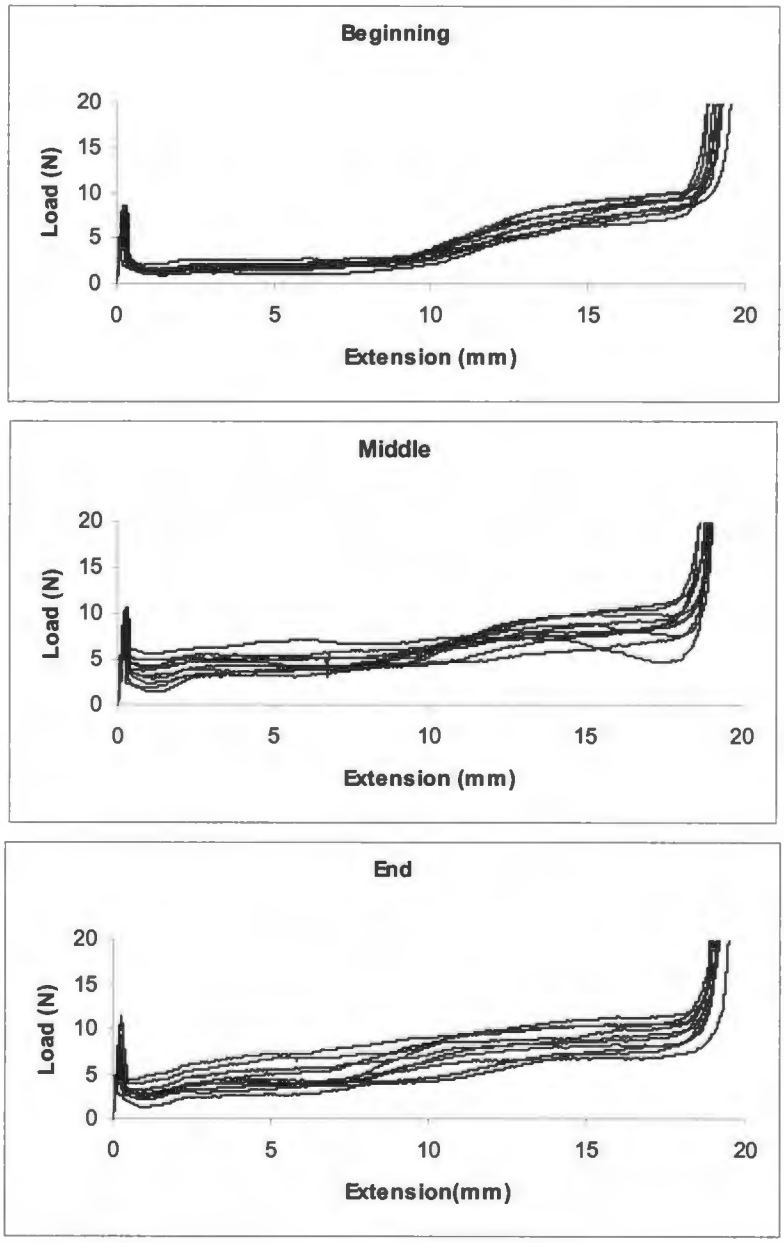


Figure 4. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 024004, n=10

Batch # 046008

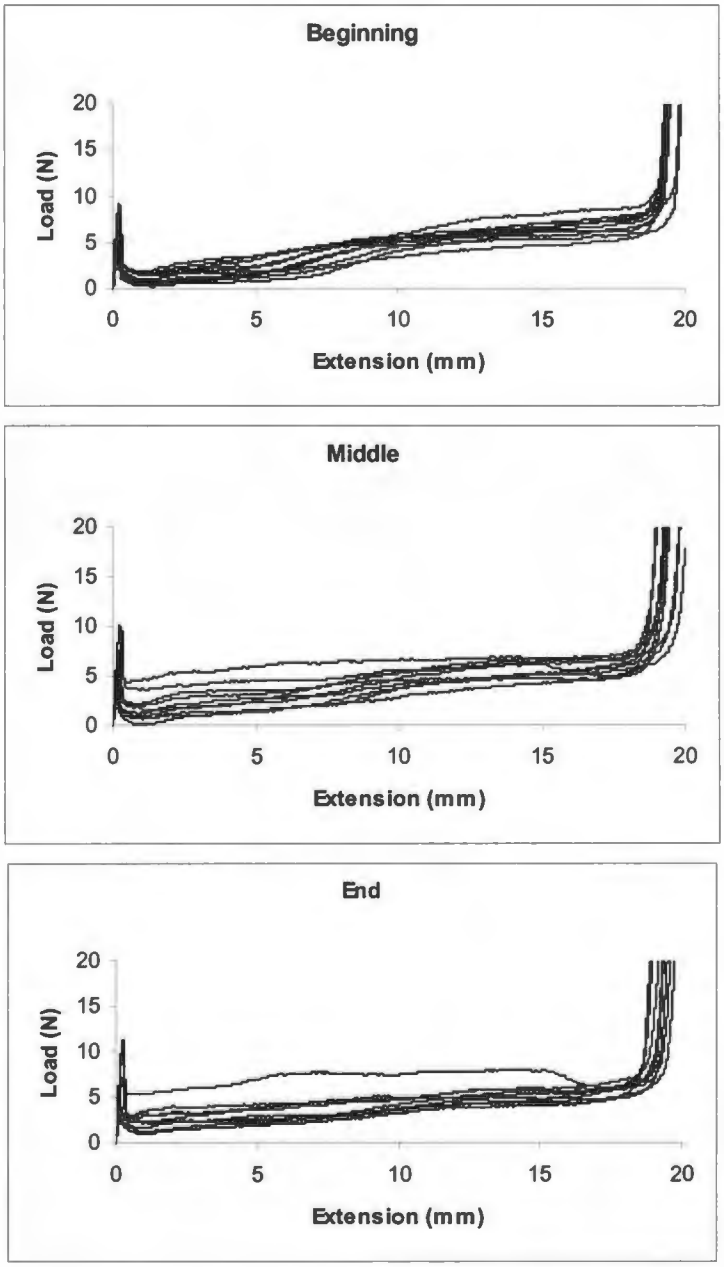


Figure 5. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 046008, n=10

Staked needle syringes, 120 g/ml
Batch # 025005

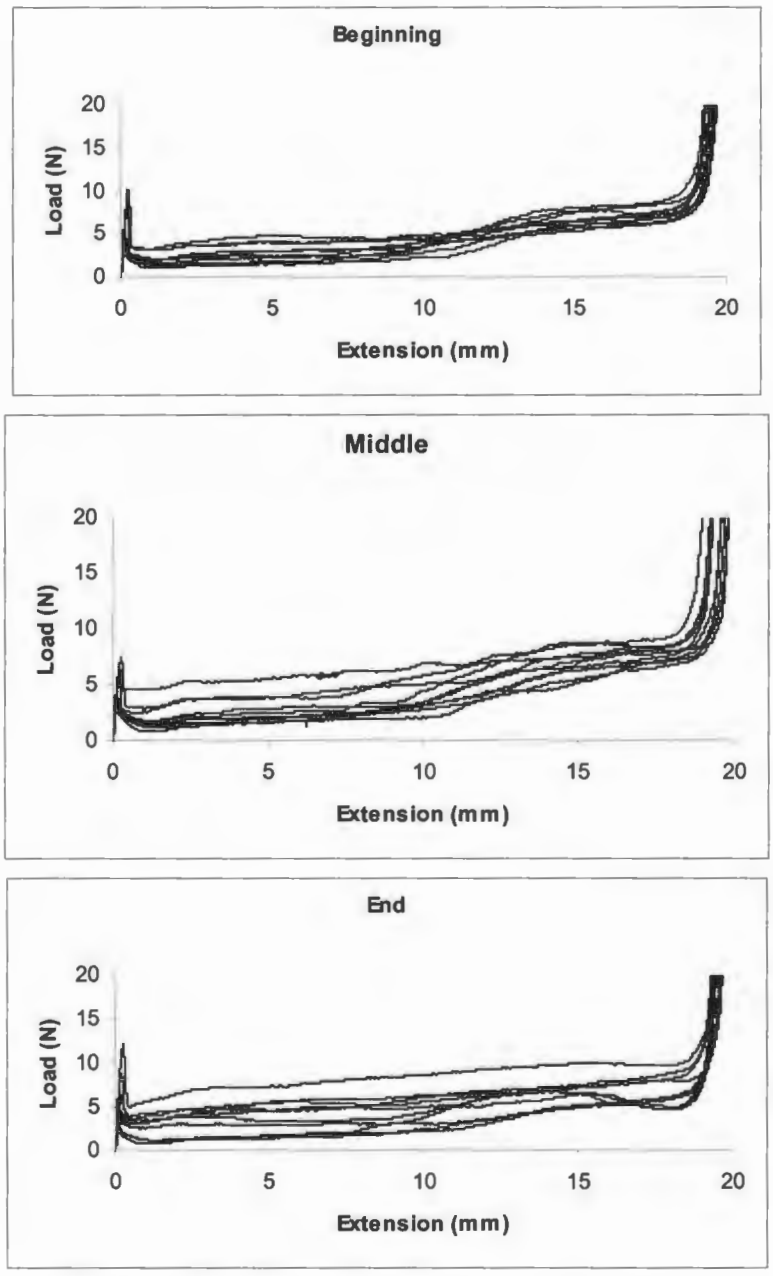


Figure 6. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 025005, n=10

Batch # 028006

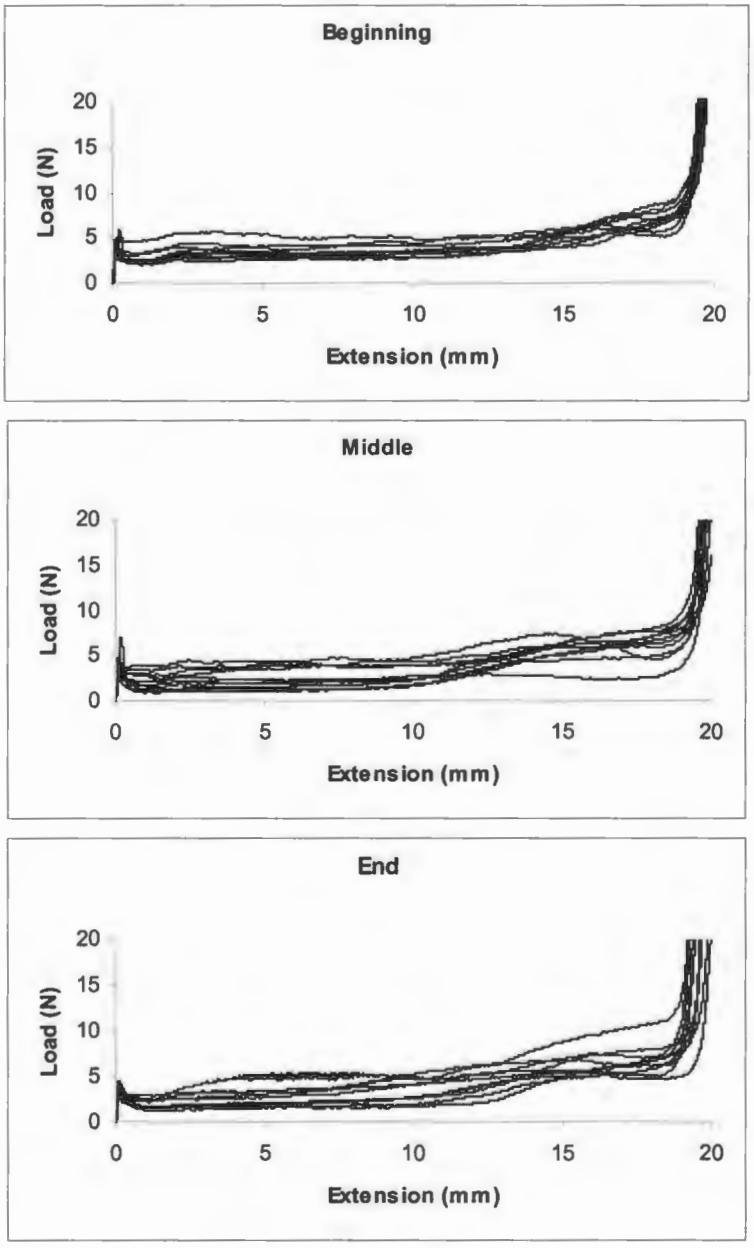


Figure 7. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 028006, n=10

Batch # 033007

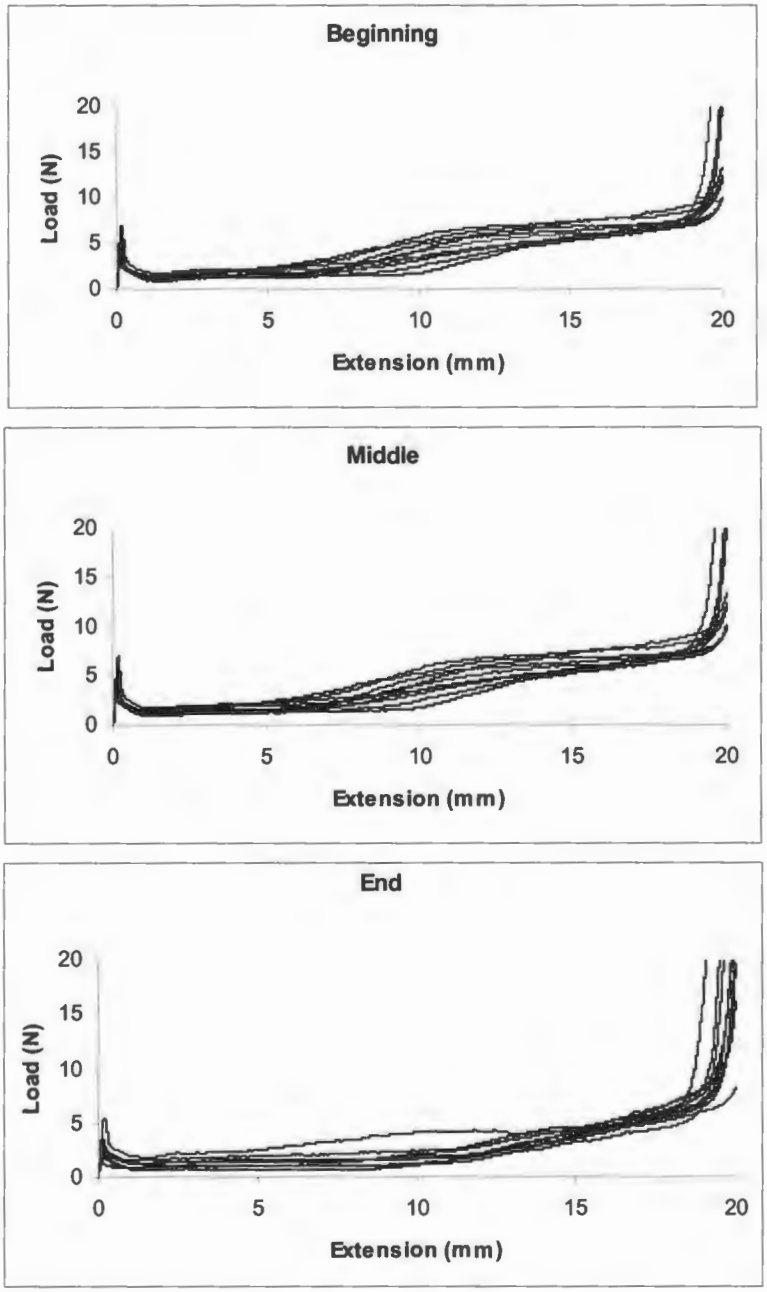


Figure 8. Expulsion force profile of Liquid Avonex® staked needle syringes, Batch # 033007, n=10

Batch No.		Force at breakout										AVG (10)	SD (10)	AVG (30)
		1	2	3	4	5	6	7	8	9	10			
021001	Beg	3.32	3.22	7.52	7.03	2.65	2.96	7.46	4.3	3.39	8.07	4.99	2.23	4.60
	Mid	4.77	2.83	3.56	5.19	2.98	2.91	5.33	2.59	1.94	2.64	3.48	1.20	
	End	3.24	3.43	8.64	3.23	8.48	5.49	5.49	3.05	1.56	10.82	5.34	3.03	
022002	Beg	5.6	7.86	10.21	10.22	7.74	5.72	5.57	4.54	3.56	8.00	6.90	2.27	9.29
	Mid	7.6	5.97	7.64	10.58	8.80	10.31	8.10	9.58	9.14	7.72	8.54	1.41	
	End	15.02	14.19	13.5	11.52	10.33	13.07	8.76	14.10	13.75	10.10	12.42	2.11	
023003	Beg	5.47	7.00	3.28	6.71	4.56	4.9	8.86	4.09	7.38	3.77	5.60	1.81	4.91
	Mid	3.44	3.14	2.75	7.19	2.83	3.47	3.15	4.02	3.19	3.87	3.71	1.29	
	End	4.04	3.14	10.75	3.67	4.19	7.45	5.18	3.03	4.89	7.75	5.41	2.49	
024004	Beg	8.50	8.38	7.80	5.24	7.45	2.99	7.90	7.90	4.86	8.64	6.97	1.92	8.17
	Mid	9.54	9.86	10.58	7.99	8.72	10.61	6.05	10.62	10.17	8.18	9.23	1.49	
	End	9.28	8.46	11.48	10.75	7.51	3.90	7.95	6.54	8.47	8.66	8.30	2.12	
046008	Beg	8.3	3.36	9.32	9.13	6.31	7.18	6.35	7.4	5.17	4.22	6.67	2.00	5.92
	Mid	3.85	3.32	4.95	9.68	7.35	7.01	10.1	3.29	4.11	2.66	5.63	2.72	
	End	3.59	3.88	3.68	11.38	3.20	7.21	9.92	3.86	3.76	4.11	5.46	2.97	
025005	Beg	4.02	3.18	3.45	4.55	4.00	3.34	9.21	7.27	3.46	10.02	5.25	2.59	5.11
	Mid	3.48	3.91	4.01	4.10	3.43	6.43	3.79	7.50	5.65	3.41	4.57	1.44	
	End	3.13	6.34	5.16	3.44	8.82	4.34	3.06	4.19	4.58	12.17	5.52	2.91	
028006	Beg	5.91	4.05	3.56	5.15	3.49	5.05	4.25	3.7	3.77	3.91	4.28	0.81	4.16
	Mid	4.22	4.41	2.56	3.05	6.93	3.73	4.72	3.47	4.17	4.67	4.19	1.19	
	End	3.90	4.48	3.74	3.79	4.17	3.30	4.37	3.94	4.12	4.18	4.00	0.34	
033007	Beg	4.00	3.15	3.65	5.05	4.23	3.65	9.20	7.54	3.50	9.75	5.37	2.49	4.40
	Mid	4.13	5.01	6.31	4.25	4.47	3.53	3.41	4.30	7.01	3.10	4.55	1.	
	End	2.91	2.85	5.55	2.62	3.36	3.33	3.39	3.50	2.15	3.13	3.28	0.90	

Table 1. Force at breakout for Liquid Avonex® pre-filled staked needle syringes.

Luer-slip needle syringes, 60 µg/ml
Batch # 929004

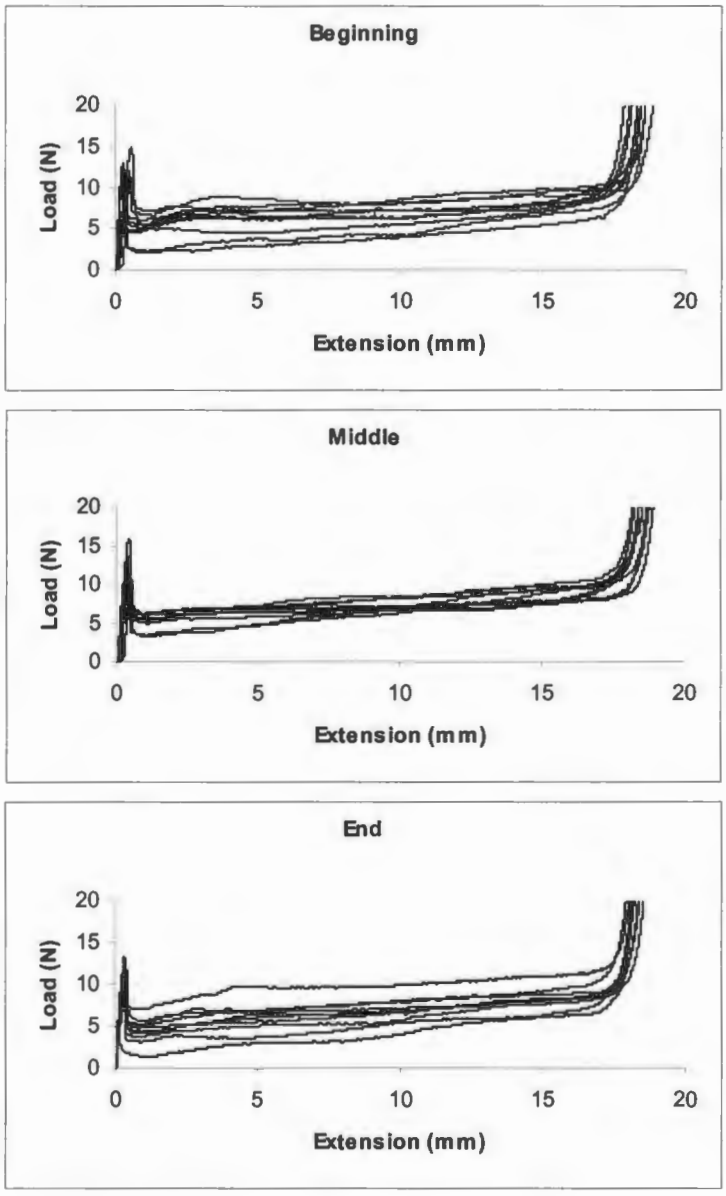


Figure 9. Expulsion force profile of Liquid Avonex® luer-slip syringes, Batch # 929004, n=10

Batch # 944007

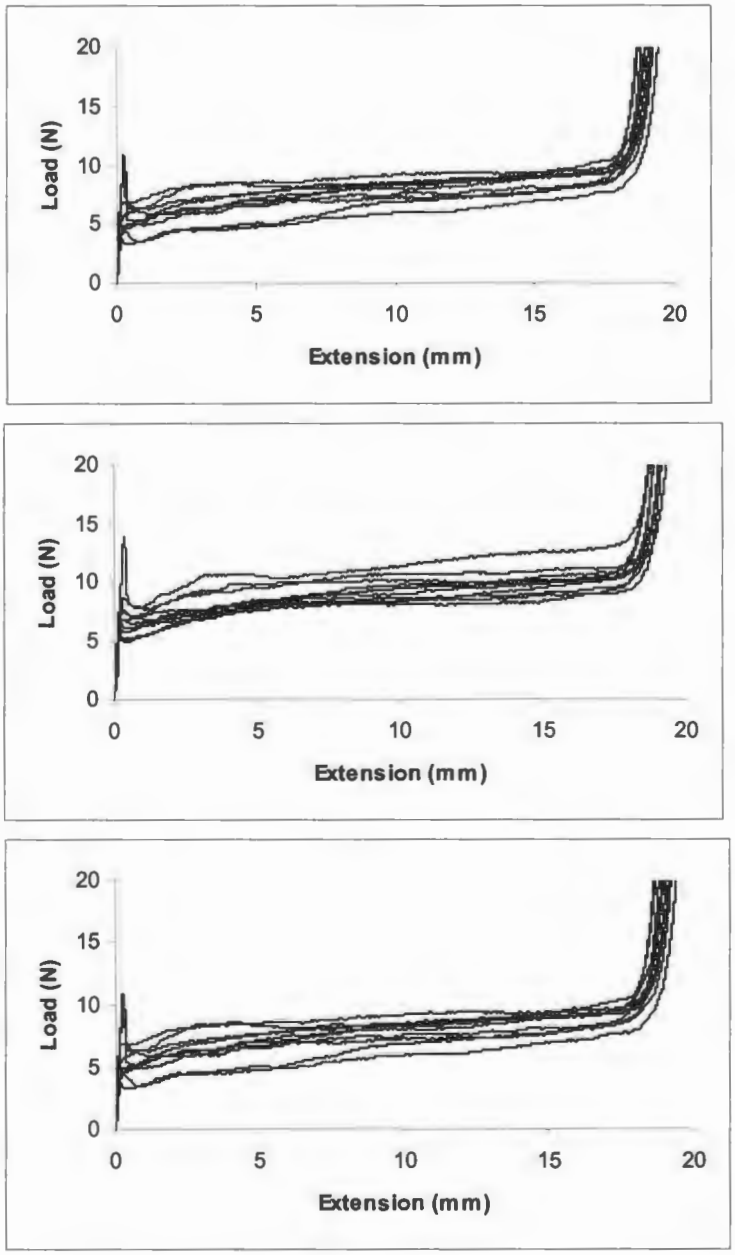


Figure 10. Expulsion force profile of Liquid Avonex® luer-slip syringes, Batch # 944007, n=10

Batch # 107001

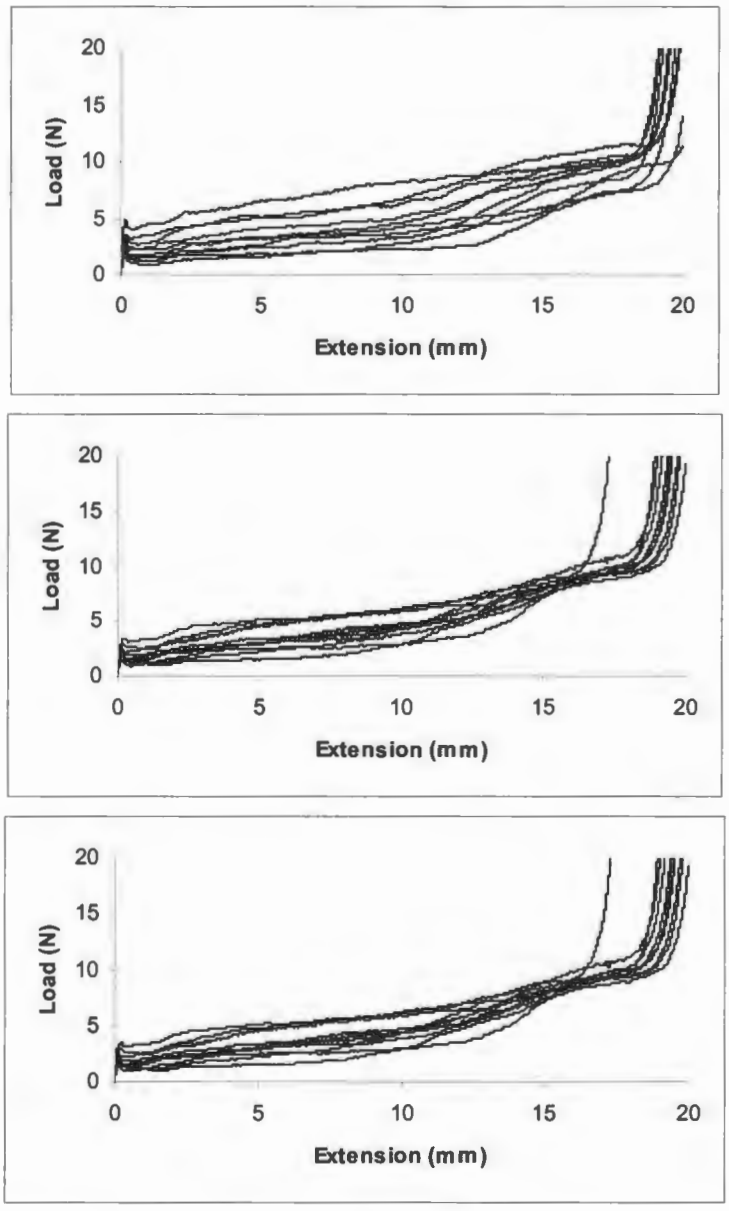


Figure 11. Expulsion force profile of Liquid Avonex® luer-slip syringes, Batch # 107001, n=10

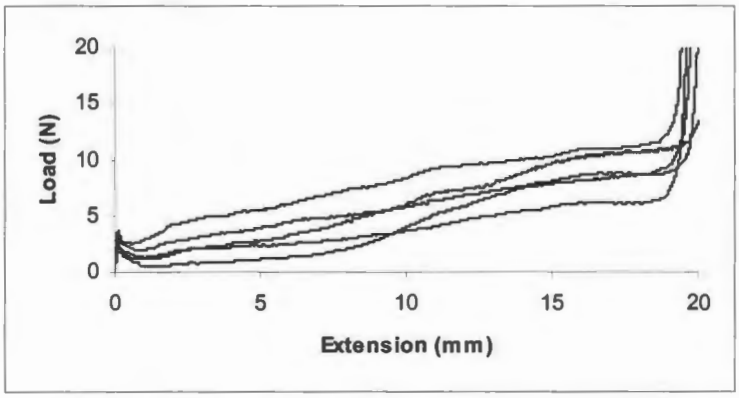


Figure 12. Expulsion force profile of Liquid Avonex® luer-slip syringes, Batch # 142003, n=5

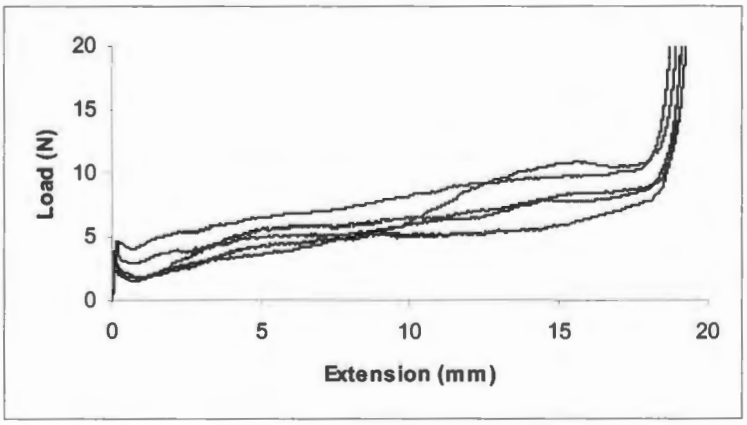


Figure 13. Expulsion force profile of Liquid Avonex® luer-slip syringes, Batch # 142004, n=5

Batch No.		Force at breakout										AVG (10)	SD (10)	AVG (30)
		1	2	3	4	5	6	7	8	9	10			
929004	Beg	11.33	11.92	7.06	14.94	11.79	10.35	13.09	12.86	10.71	5.1	10.92	0.15	11.03
	Mid	9.13	10.49	13.46	13.48	11.64	15.8	12.32	12.04	13.83	11.61	12.38	1.87	
	End	10.63	13.24	8.24	7.43	10.3	10.63	11.91	10.54	4.04	11.03	9.80	2.61	
944007	Beg	7.14	6.29	6.63	7.83	4.13	6.04	8.66	7.16	8.98	6.30	6.92	1.40	6.98
	Mid	6.86	8.65	13.85	6.79	7.55	5.99	4.96	7.37	7.17	5.21	7.44	2.51	
	End	9.88	6.8	10.02	4.43	10.7	5.54	4.75	4.35	4.78	4.72	6.60	2.59	
107001	Beg	3.48	2.65	2.35	4.88	3.07	2.81	3.43	2.96	2.32	2.06	3.00	0.81	3.07
	Mid	2.95	2.6	3.3	4.00	2.94	4.62	3.56	4.09	3.85	2.99	3.49	0.64	
	End	2.41	2.01	2.6	3.02	2.40	2.96	2.83	3.5	2.58	2.95	2.72	0.42	
142003		3.01	3.45	3.74	3.64	2.46	-	-	-	-	-	3.26(5)	0.53(5)	3.26(5)
142004		4.62	4.71	3.87	3.36	3.22	-	-	-	-	-	3.96(5)	0.69(5)	3.96(5)

Table 2. Force at breakout for Liquid Avonex® pre-filled luer-slip syringes.

Batch no.	Temperature	Time (hrs)	Breakout force (N)					AVG
			1	2	3	4	5	
024004	2-8°C	1	8.15	6.89	7.36	6.01	6.58	7.00
		2	10.9	10.08	8.65	8.27	6.03	8.79
		3	9.41	7.48	6.62	6.99	6.29	7.36
		6	8.26	7.4	6.62	6.99	6.29	7.11
	30°C	1	8.91	8.66	7.65	6.01	5.24	7.29
		2	8.85	8.44	8.2	6.93	7.78	8.04
		3	10.96	8.98	8.3	6.33	5.87	8.09
		6	8.57	7.71	7.2	6.21	5.27	6.99
028006	2-8°C	1	6.71	4.22	4.23	3.68	4.22	4.61
		2	6.29	3.82	3.67	3.83	2.92	4.11
		3	3.85	3.47	3.17	3.05	2.84	3.28
		6	6.65	3.53	3.57	3.25	3.38	4.08
	30°C	1	4.7	4.37	3.69	3.36	4.54	4.13
		2	6.24	3.33	3.5	3.48	3.03	3.92
		3	3.86	3.54	3.49	3.24	2.99	3.42
		6	4.59	4.01	3.83	3.13	3.86	3.88

Table 3. Force at breakout for Liquid Avonex® pre-filled staked needle syringes, batch # 024004 and 028006 stored at two temperatures 2-8°C and 30°C.

Expulsion force profile of development syringes
Batch # 4978-18

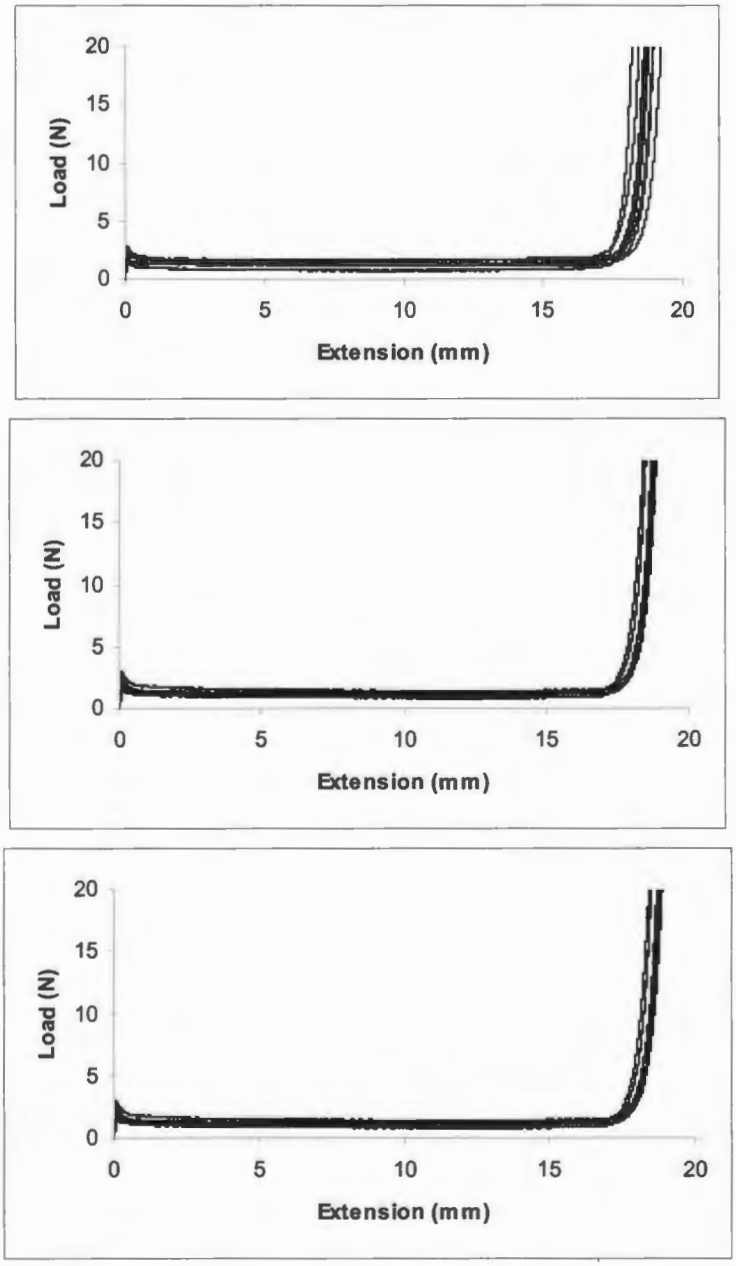


Figure 14. Expulsion force profile of development syringes, Batch # 4978-18, n=10

APPENDIX C-VETTER REPORT

Rationale Version: 1

24.01.2002

Statement for the use of 1000cSt Silicone Oil instead of 12.500cSt Silicone Oil

Content:

Statement for the use of 1000cSt Silicone Oil instead of 12.500cSt Silicone Oil VETTER

1. Introduction

Within the scope of a Vetter internal project regarding the improvement of the existing siliconisation process (wipe down siliconisation), tests with different silicon oil viscosities have been performed. During these tests, Dow Corning DC 360 1000cSt and Dow Corning DC 360 12500cSt silicone oils have been used. Both viscosities comply to the specifications of the European Pharmacopoea and the USP/NF.

In order to compare both silicone oil viscosities the tests have been carried out under identical production conditions (using the same machine and in the same production area) and with the same material (using the same syringe batch). The silicon pumps have been set to the same output in silicone oil.

For the evaluation of the tests the plunger forces have been measured and the amount of silicon oil has been determined.

2. Testresults

2.1 Material and Methods

The plunger forces have been determined according to Vetter work instruction W-A 08 with an electronic force measuring device. The limits for the forces are: $F_{max\ single\ value} \leq 20$ N, $F_{max\ mean\ value} \leq 15$ N for all measured values.

The determination of silicone oil content was done according to Vetter work instruction T-GB11S02 by using FTIR-spectroscopy. The limits for the contents are described in the Vetter form QCCA 162-8, and are max. 2,4 mg / syringe for 1 ml long syringes.

Material: For all tests the same syringe material was used, i. e., for the comparison of the different dosing quantities and types of silicone oil, materials from the same batch have been utilized. The determination of plunger forces has been performed using washed, siliconized, and sterilized stoppers 4106/50 and 4023/50 and syringe barrels of the same batch. The dosing cycles corresponded have been set to deliver 1,0 mg, 0.6 mg, 0.3 mg of silicone oil.

2.2 Reduction of Silicone Oil Amount

The tests were performed to analyze the possibilities of the current siliconisation process with regards to the oil quantity and plunger forces. During the tests, different dosing quantities (cycle times of the oil pumps) and different types of silicone oil (1000 cSt, and 12500 cSt) were used.

The aim of the study was to reduce the amount of silicone oil inside the syringe barrel as far as possible. The amount of oil in the syringe barrels and the plunger forces have been determined.

2.3 Further tests planned

The distribution of the silicone oil over the different sections of the inner surface of the syringe will be determined. The range of the total amount of silicone oil in the syringe with fingerrest and without fingerrest will be determined.

2.4. Results

Silicone oil 1000 cSt:

The plunger forces have been acceptable, even when the lowest dosing quantity was used (about 0.43 mg per syringe).

Silicone oil 12500 cSt:

Only the highest dosing quantity (about 0.89 mg per syringe) ensured plunger forces within the acceptable range.

During all tests, the Flurotec stoppers 4023/50 showed higher plunger forces than stoppers 4106/50.

See Appendix 1, Gliding Forces

For one customer diluent product a change from 12500cSt to 1000cSt silicone oil was successfully implemented. The evaluation of this change was done by measuring the plunger forces on pulling the stoppers back (measurements of 200 different syringes per batch).

Batches produced with 1000cSt oil delivered in every case acceptable plunger forces. The achieved plunger forces have been significantly lower than with 12500cSt oil.

See Appendix 2, Diagram Pulling Forces

3. Conclusion / Discussion

Only by using silicone oil 1000 cSt a reduction of silicone oil quantities can be achieved ensuring acceptable plunger forces.

Plunger forces for pushing the stopper, and even for pulling the stopper have been improved by using 1000cSt instead of 12500cSt silicone oil, without having to increase the silicone oil amount within the syringe. The increase of the forces to the right side of the force diagram (Appendix 1, the right side of the diagram corresponds to the needle-side of the syringe) can be explained by less silicone oil at this location compared to the fingerrest side. This effect is more pronounced with 12500cSt treated syringes. Silicon distribution data (data not shown) confirm this finding in that 1000cSt silicone oil can be more evenly distributed than 12500cSt silicone oil.

4. Approval

25.01.02

Prepared: D. Baumann/ R&D [Date / Signature]

25.01.02

Verified: Dr. P. Gässmann/Production [Date / Signature]

25.01.02

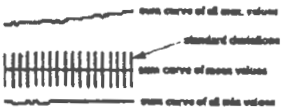
Approved: J. del Boca/Quality Assurance [Date / Signature]

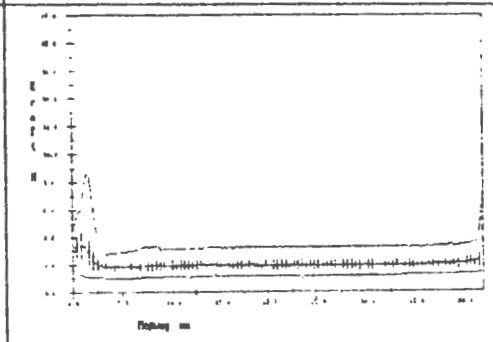
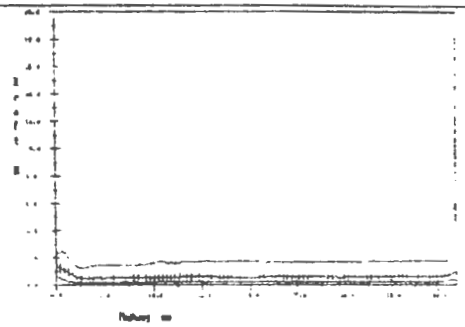
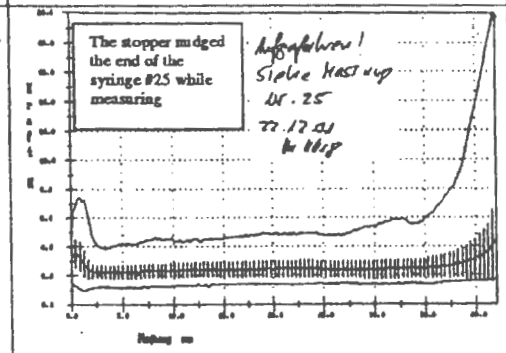
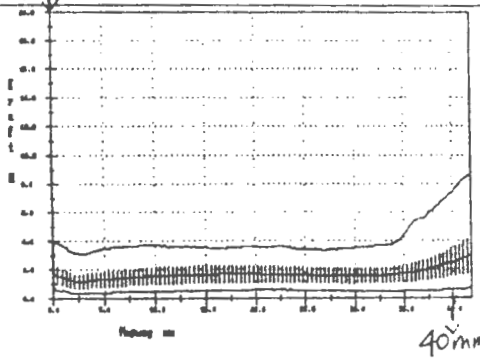
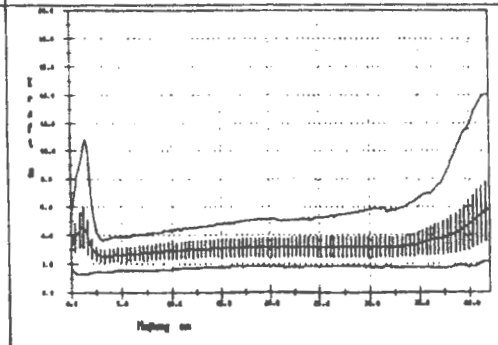
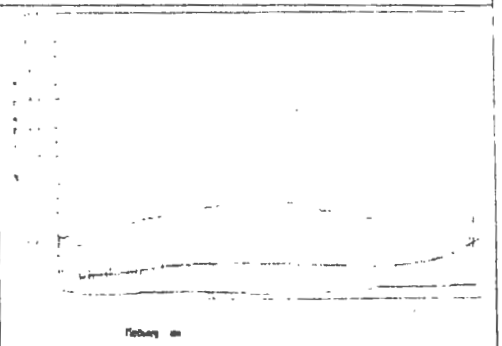
Statement for the use of 1000cSt Silicone Oil instead of 12.500cSt Silicone Oil VETTER

Appendix 1, 1000 cSt Silcone Oil

Each diagram includes 50 force measurements
(measurements of 50 different syringes)
Syringe batch: WE 0003217-01

For explanation the force diagrams:



	Stoppers 4023/50 (Flurotec)	Stoppers 4106/50
1,0mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 1,09	 20N
0,6mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 0,61	 40mm
0,3mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 0,43	

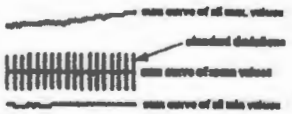
Statement for the use of 1000cSt Silicone Oil instead of 12.500cSt Silicone Oil

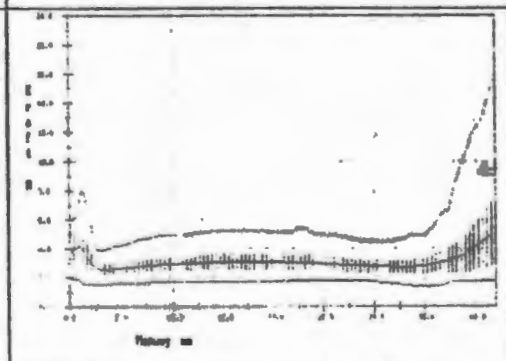
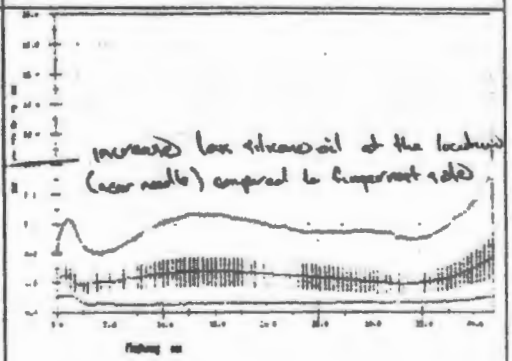
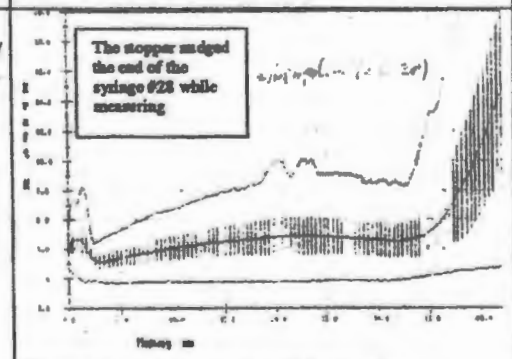
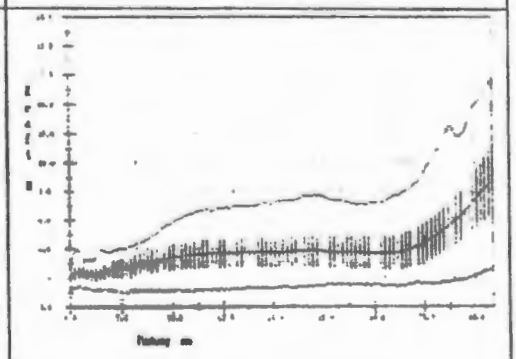
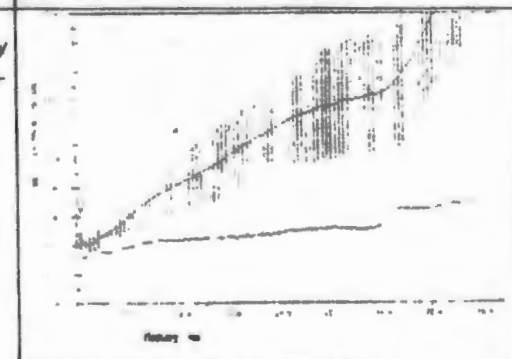
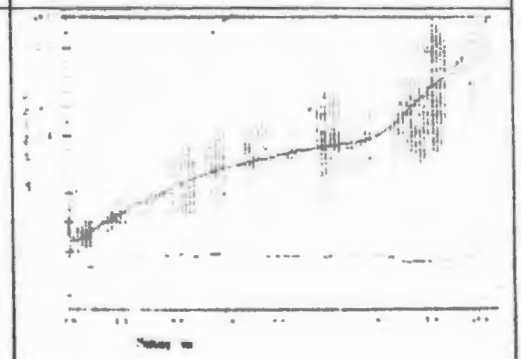
VETTER

Appendix 1, 12.500 cSt Silicone Oil

Each diagram includes 50 force measurements
(measurements of 50 different syringes)
Syringe batch: WE 0003217-01

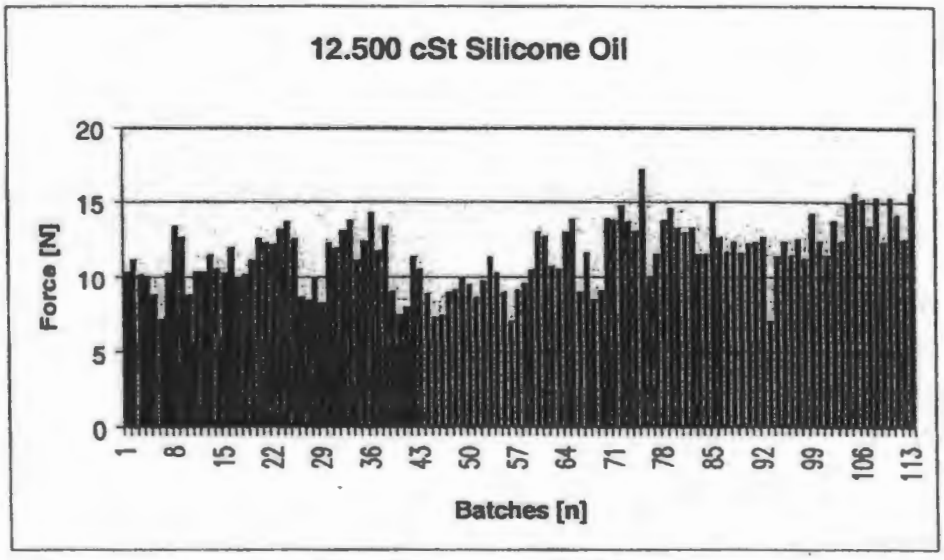
For explanation the force diagrams:



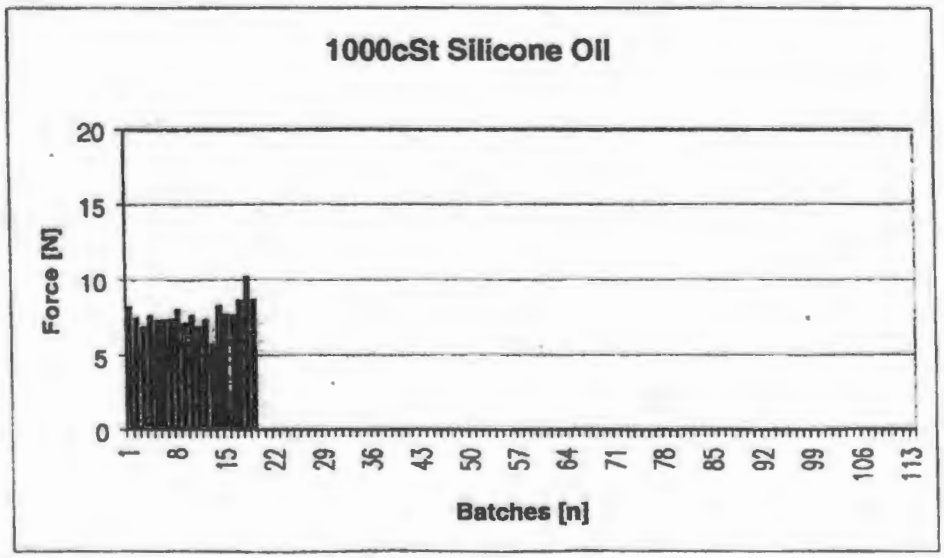
	Stoppers 4023/50 (Flurotec)	Stoppers 4106/50
1,0mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 0,89	
0,6mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 0,45	
0,3mg/ Pump-cycle	 Silicone oil content , meanvalue, mg/syringe: 0,10	

Appendix 2

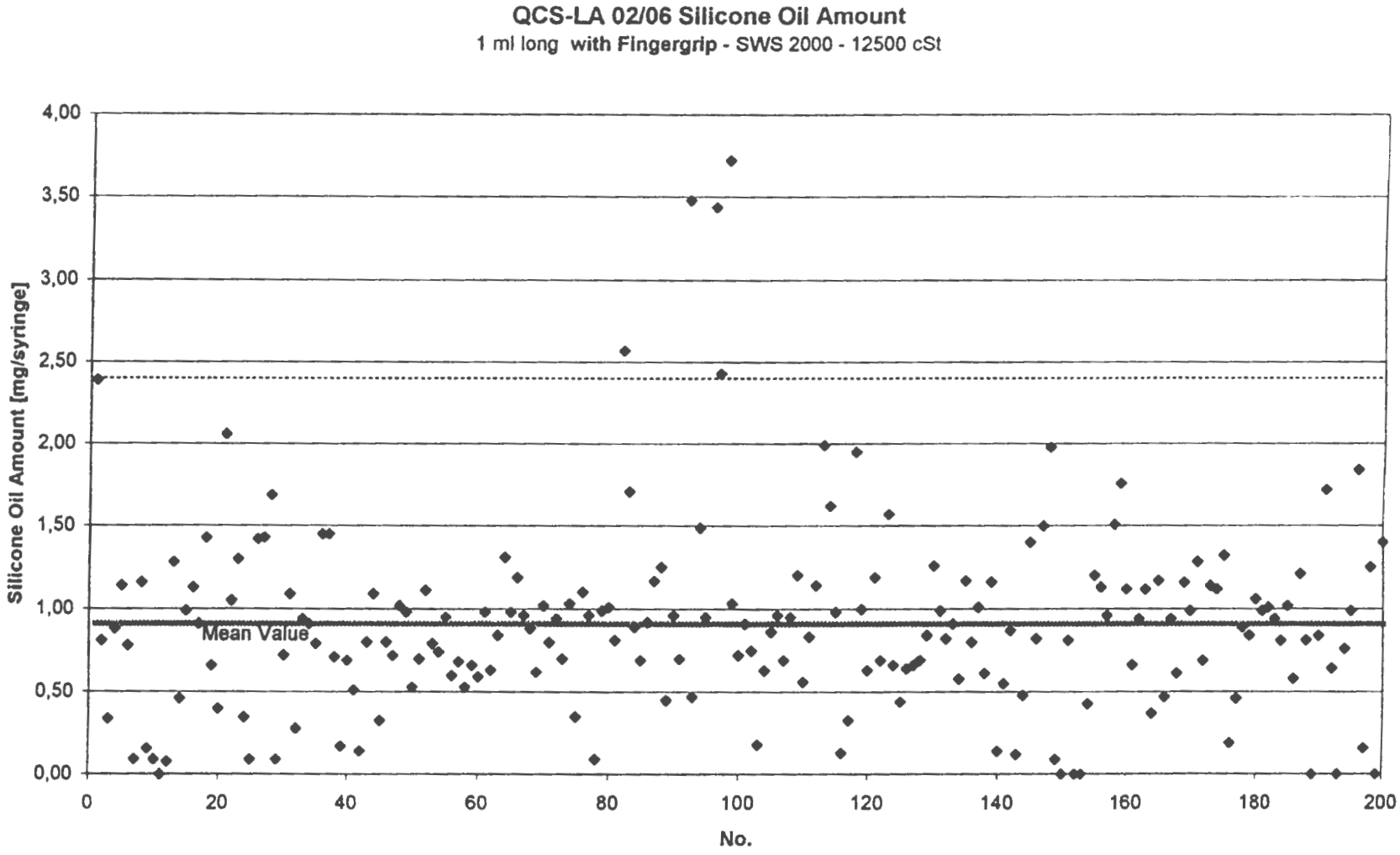
At each batch there were done 200 force measurements (measurements of 200 different syringes per batch)

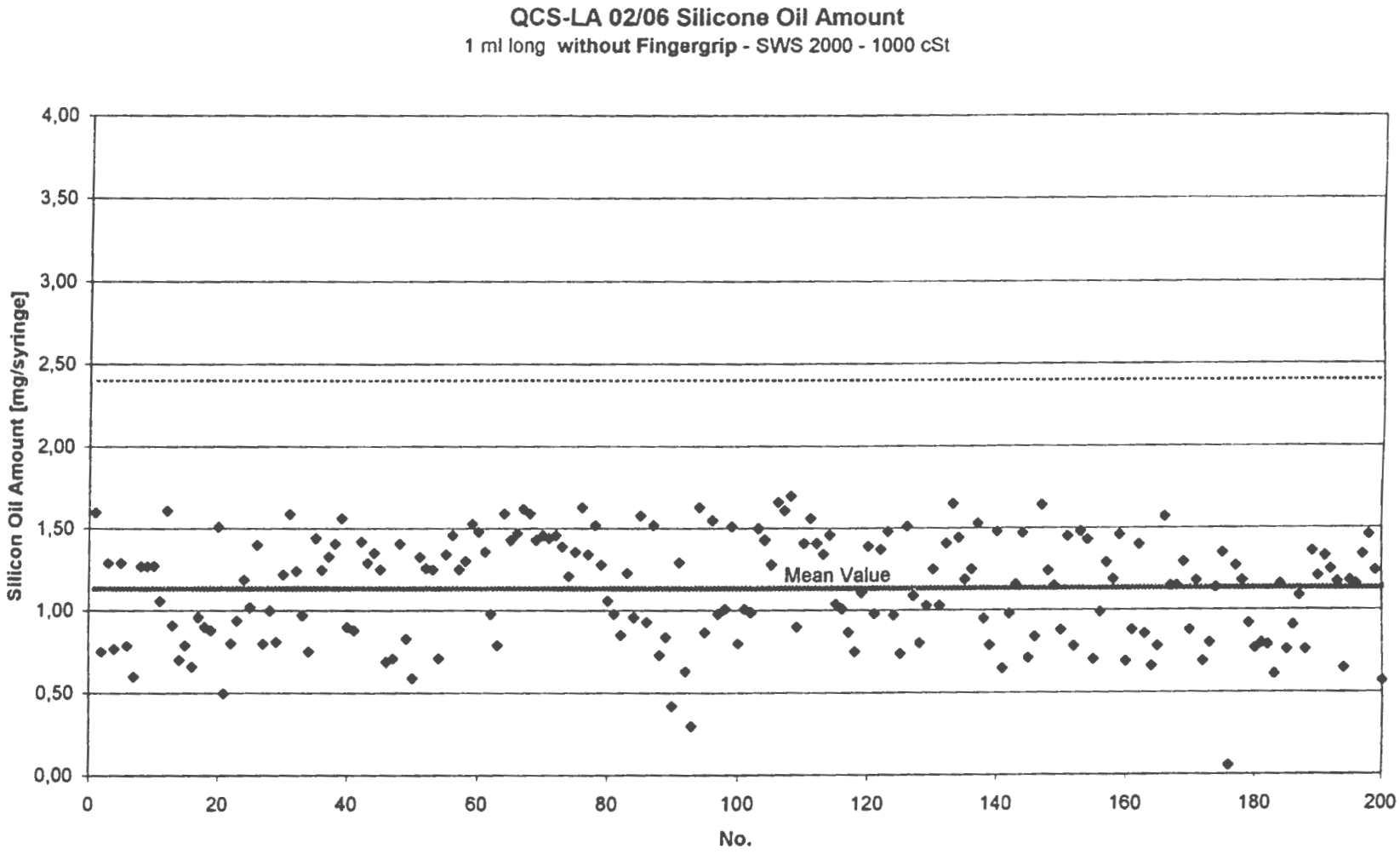


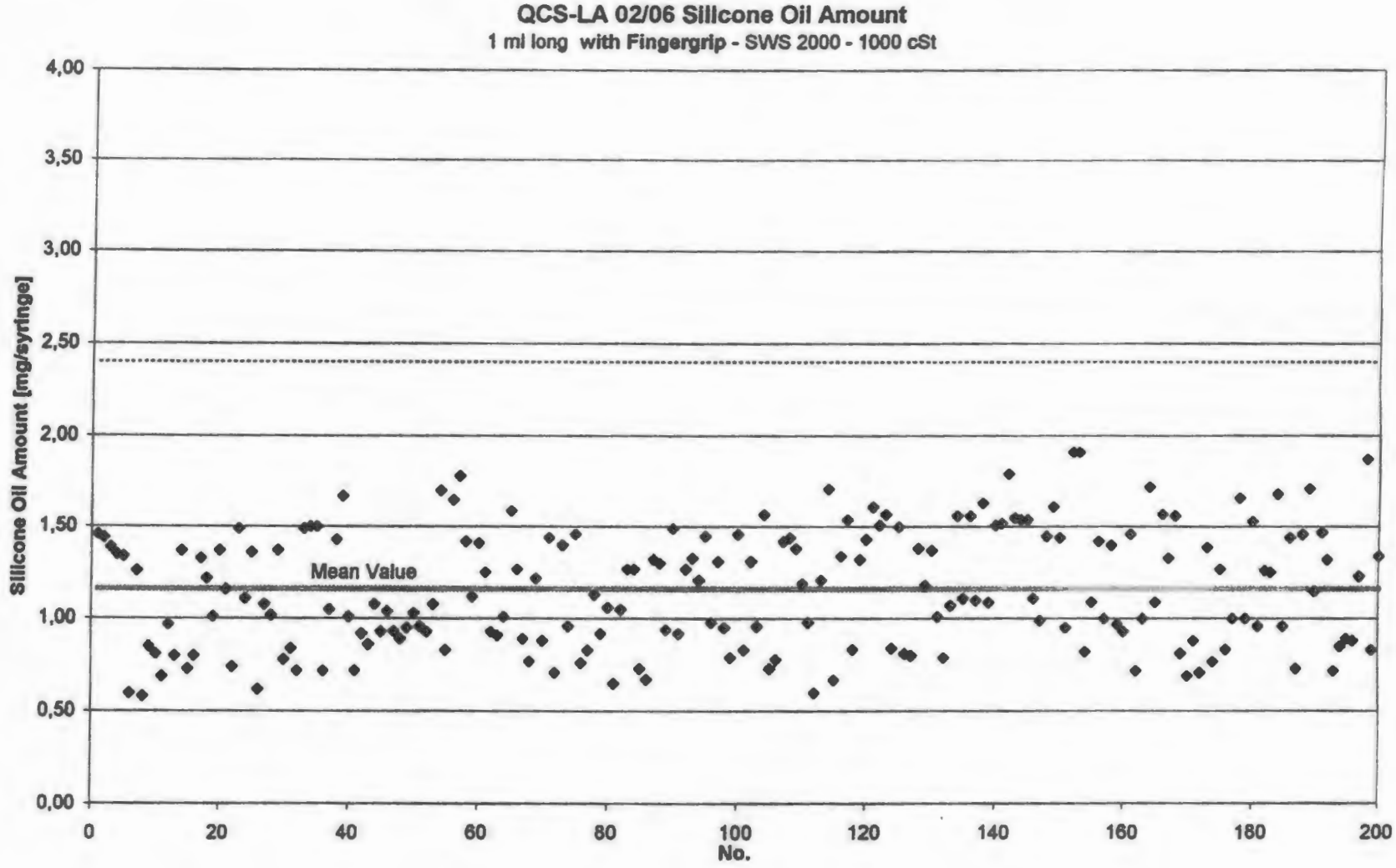
Mean Value: 11,48 N
Standard Deviation: 2,17 N



Mean Value: 7,66N
Standard Deviation: 0,90N


QCS-LA 02/06 Silicone Oil Amount
1 ml long with Fingergrip - SWS 2000 - 12500 cSt
Silicone Oil Amount [mg/syringe]
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Mean Value
0
20
40
60
80
100
120
140
160
180
200
No.


QCS-LA 02/06 Silicone Oil Amount
1 ml long without Fingergrip - SWS 2000 - 1000 cSt
Silicon Oil Amount [mg/syringe]
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Mean Value
0
20
40
60
80
100
120
140
160
180
200
No.


QCS-LA 02/06 Silicone Oil Amount
1 ml long with Fingergrip - SWS 2000 - 1000 cSt
Silicone Oil Amount [mg/syringe]
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
Mean Value
0
20
40
60
80
100
120
140
160
180
200
No.

APPENDIX D

Appendix D contains

1. Comparison of needle penetration profile graphs of Liquid Avonex® luer-slip and staked needles (figure 1-3)
2. The Fmax at 32 mm extension for the luer-slip and staked needles (Table 1).

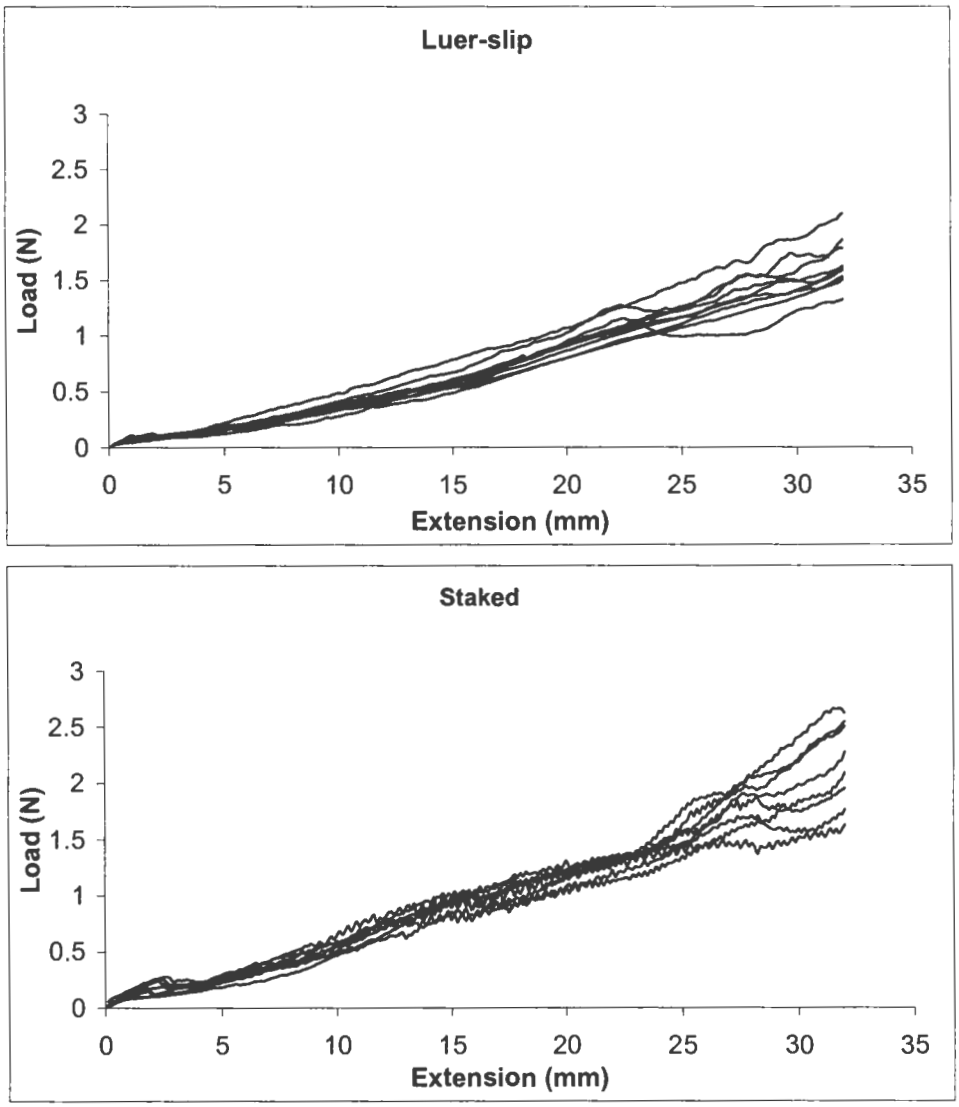


Figure 1. Comparison of needle penetration profiles of Liquid Avonex® luer-slip and staked needles, n=10, Set # 1.

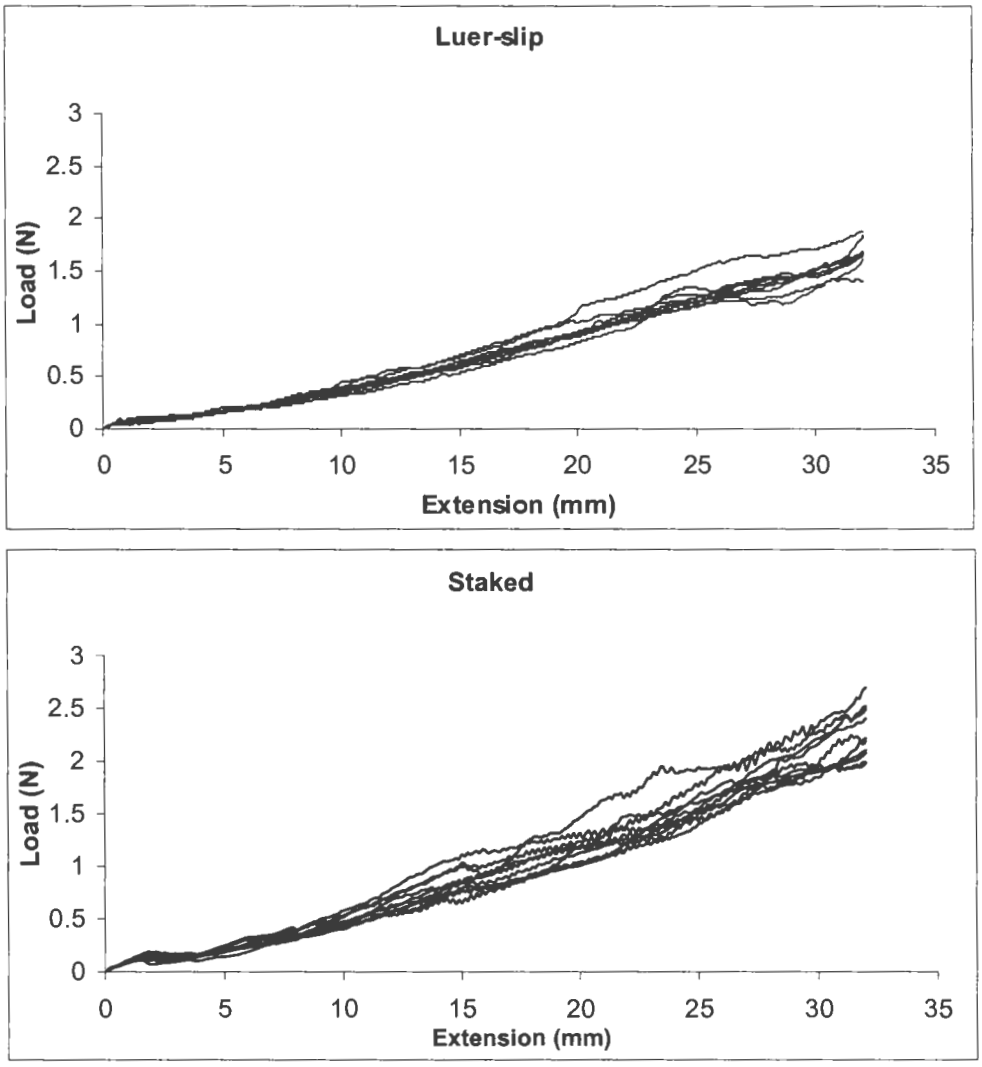


Figure 2. Comparison of needle penetration profiles of Liquid Avonex® luer-slip and staked needles, n=10, Set # 2.

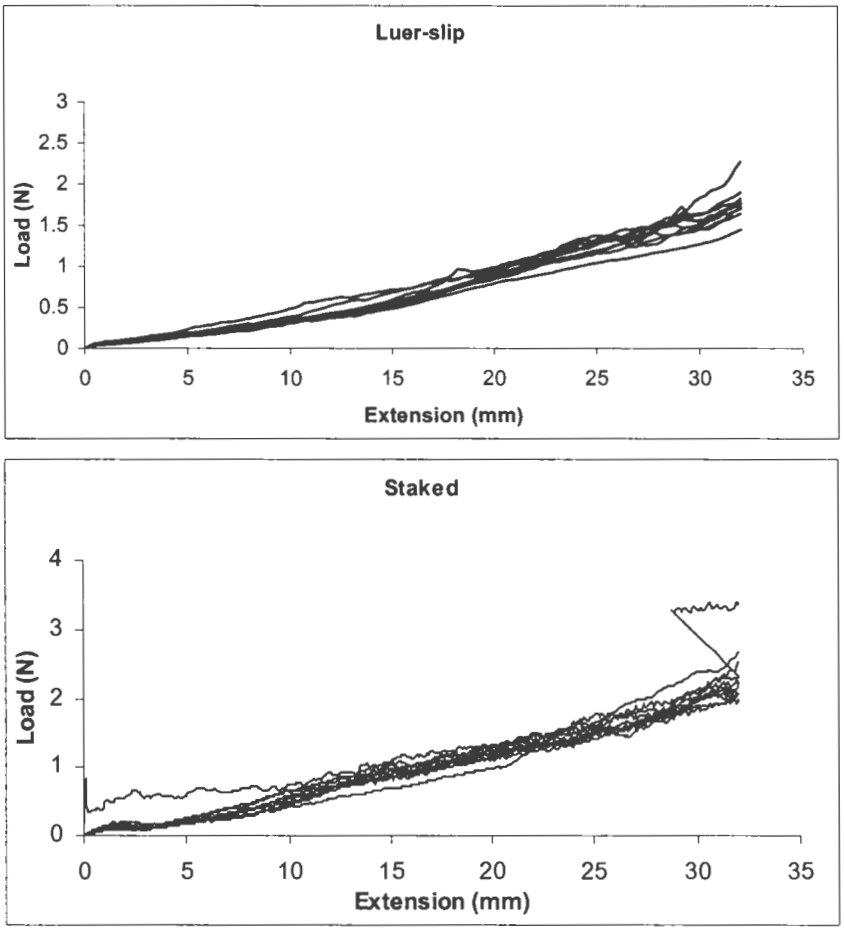


Figure 3. Comparison of needle penetration profiles of Liquid Avonex® luer-slip and staked needles, n=10, Set # 3

Syringe number	Fmax (32 mm extension)	
	Luer-slip needles	Staked needles
1	2.2736	2.0998
2	1.8971	2.4011
3	1.7860	2.4904
4	1.8209	2.2158
5	1.7233	1.9771
6	1.6399	2.6931
7	1.4493	2.1881
8	1.7001	1.9877
9	1.7704	2.5161
10	1.7545	2.0784
11	1.7594	1.9334
12	1.3248	1.9979
13	1.6225	1.6256
14	1.5301	1.7651
15	1.6090	1.9517
16	1.8621	2.6236
17	1.5861	2.0907
18	1.7867	2.5457
19	2.1026	2.2740
20	1.5005	2.5050
21	1.6884	2.6622
22	1.6641	2.0735
23	1.6651	2.1540
24	1.8341	2.1723
25	1.6848	2.5185
26	1.6045	2.2073
27	1.6520	2.1900
28	1.4055	1.9967
29	1.8741	2.0617
30	1.6657	3.3899
AVG	1.7079	2.2462
SD	0.1902	0.3442

Table 1. Fmax for luer-slip and staked needles at 32 mm extension

APPENDIX E

Appendix E contains

1. Details of the size exclusion chromatography method used to quantify the percentage aggregates of Inteferon beta-1a.
2. Percent peak area of all the samples subjected to SEC (Table 1).
3. Mean values of the percent peak area of the samples subjected to SEC (Table 2).

Quantification of aggregate percentage of Interferon beta-1a was done using Size Exclusion Chromatography (SEC). The analysis was carried out according to the "*Standard operating procedure for performing size exclusion chromatography on Interferon beta-1a liquid formulation process intermediate and finished product*" (SOP # 22d.320).

The HPLC details are as follows:

HPLC system	Waters Alliance 2690
Column	Superdex® G-75
Column Temperature	Ambient
Detection	Wavelength: 215 nm
Bandwidth	4 nm
Injection volume	200 µL (syringe samples and Blank) 50 µL (Process intermediate samples and control) 40 µL (resolution std)
Flow rate	0.8mL/min
Initial conditions	100% mobile phase
Elution mode	Isocratic
Maximum system pressure	270 psi plus the pressure from pump to autosampler outlet
Run stop time	30 min
Post time	4 min (Waters Alliance HPLC)

Injection queue:

1. Blank-column wash
2. Blank
3. First Resolution Standard of Duplicate
4. First Control of Triplicate- column conditioning
5. Second control of triplicate
6. First sample of Triplicate
7. Second sample of Triplicate
8. Blank
9. Third Control of Triplicate
10. Second Resolution Standard of Duplicate

ID No.	Percent Peak Area				
	Sample Condition	Monomer (%)	Dimer (%)	Aggregate (%)	% Total Aggregate
7576-66-03	Control-A	99.9	0.1	-	-
7576-66-03	Control-B	99.7	0.1	-	-
7576-66-03	Control-C	99.9	0.1	-	-
7576-66-04	Control-A	99.5	0.1	0.2	0.3
7576-66-04	Control-B	99.7	0.1	0.2	0.3
7576-66-04	Control-C	99.7	0.1	0.2	0.3
7576-66-07	Manual-A	99.9	0.1	-	-
7576-66-07	Manual-B	100	-	-	-
7576-66-07	Manual-C	99.3	0.1	-	-
7576-66-07	Manual-D	99.5	-	-	-
7576-66-07	Manual-E	99.6	-	-	-
7576-66-07	Manual-F	99.5	-	-	-
7576-66-08	Manual-A	99.3	-	0.2	-
7576-66-08	Manual-B	99.5	0.1	0.2	0.3
7576-66-08	Manual-C	99.8	0.1	0.2	0.3
7576-66-08	Manual-D	99.5	0.1	0.2	0.3
7576-66-08	Manual-E	99.8	-	0.2	0.2
7576-66-08	Manual-F	99.7	0.1	0.2	0.3
7576-66-07	Instron-A	100	-	-	-
7576-66-07	Instron-B	99.7	-	-	-
7576-66-07	Instron-C	99.6	-	-	-
7576-66-07	Instron-D	99.7	-	-	-
7576-66-07	Instron-E	98.6	-	-	-
7576-66-07	Instron-F	99.5	-	-	-
7576-66-08	Instron-A	99.7	-	0.3	0.3
7576-66-08	Instron-B	99.7	-	0.3	0.3
7576-66-08	Instron-C	99.9	-	0.1	0.1
7576-66-08	Instron-D	99.7	-	0.3	0.3
7576-66-08	Instron-E	99.5	-	0.2	0.2
7576-66-08	Instron-F	99.7	-	0.3	0.3

Table 1. Percent peak area of all the samples subjected to SEC

		Percent Peak Area				
Tween 20	ID No.	Sample Condition	Monomer (%)	Dimer (%)	Aggregate (%)	% Total Aggregate
Present	7576-66-03	Control (n=3)	99.8	0.1	-	-
	7576-66-07	Instron (n=6)	99.5	-	-	-
	7576-66-07	Manual (n=6)	99.6	-	-	-
Absent	7576-66-04	Control (n=3)	99.6	0.1	0.2	0.3
	7576-66-08	Instron (n=6)	99.7	-	0.3	0.3
	7576-66-08	Manual (n=6)	99.6	0.1	0.2	0.2

Table 2. Mean values of the percent peak areas of the samples subjected to SEC

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