Karlo Tomasic BSc

Formulation factor analysis of dosing accuracy from vials

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Supervisor:

Univ.-Prof. Dr.phil.-nat. **Sven Stegemann**
Institute of Process and Particle Engineering, Graz University of Technology

Graz, January 2019

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KURZFASSUNG

Titel: Formelfaktor-Analyse der Dosiergenauigkeit aus Phiolen

Autor: Karlo Tomasic

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Arzneimittel zur Injektion die über Phiole-Spritzen-Systeme appliziert werden bieten einen schnellen Eingriff in gesundheitliche Notfälle. Aufgrund der manuellen Dosierung besteht aber das Risiko einer ungenauen Dosierung. Solche Systeme werden zur parenteralen Medikamentenverabreichung oder insbesondere zur Medikamentenverabreichung in das Gewebe oder das Gefäßsystem verwendet. In dieser Arbeit wurde die Dosiergenauigkeit in Abhängigkeit von der Viskosität der Flüssigkeit und der Nadelgröße bewertet. Daher wurden zwei Datengruppen erhoben und analysiert. Zunächst wurden manuelle Messungen als Ausgangspunkt für diese Analyse durchgeführt. Um den Einfluss menschlicher Faktoren zu minimieren, wurden diese Messungen von einem einzigen Bediener durchgeführt. Die gemessene Dosierungsgenauigkeit war die Volumendifferenz (ΔV) zwischen dem erwarteten und gemessenen Volumen der aus der Phiole in die Spritze entnommene Flüssigkeit. Zu den beobachteten Variablen gehörten der Flüssigkeitstyp (mit drei verschiedenen Formulierungen und Viskositäten) und die Nadelgröße (G19 und G21). Die verwendeten Flüssigkeiten waren wie folgt: Wasser, Wasser mit Glycerin und Wasser, Glycerol und Polysorbat 80 (Tween) -Lösung. Ihre Viskositäten betragen - 1,025 cP, 10,548 cP bzw. 10,474 cP. Hier wurden 60 Messungen durchgeführt, um Daten zu sammeln (10 Messungen pro Kombination von Flüssigkeit und Nadelgröße). Feste Variablen umfassten Phiolengröße (2 ml), Füllvolumen (1,2 ml), Entnahmevolumen (0,9 ml), Spritzengröße (1 ml), Temperatur und Druck (NTP). Eine statistische Analyse, die mit diesem Datensatz durchgeführt wurde, zeigte, dass es nur in der Tensid-haltigen Formulierung (Wasser, Glycerin, Polysorbat) einen statistisch signifikanten Unterschied gibt, der von der Nadelgröße abhängt ($p = 0,0009$). Darüber hinaus wurde der zweite Teil dieser Forschung mit dem Instron ™ 5942 Series®-Prüfsystem durchgeführt, in dem die Entnahme-Kraftprofile untersucht wurden. Die gemessene Variable war die Volumendifferenz zwischen dem erwarteten und dem tatsächlichen Wert (ΔV) mit einer zusätzlichen variablen Geschwindigkeit, mit der die Maschine die Flüssigkeit aus der Phiole entnimmt (Bewegung des Spritzenkolbenkopfes in cm / sec). Es wurden sechs Geschwindigkeiten gemessen - 0,1, 0,2, 0,3, 0,4, 0,5 und 1 cm / s. Hier wurden 36 Messungen durchgeführt (eine für jede Kombination aus Geschwindigkeit, Nadelgröße und Viskosität). Das Ziel dieses Schrittes war die Erstellung eines Vorhersagemodells, das die Variabilität der Dosiergenauigkeit erklärt, die durch die folgenden Variablen - Geschwindigkeit, Nadelgröße und Viskosität - beeinflusst wird. Das erhaltene Modell legte nahe, dass nur die Geschwindigkeit einen statistisch signifikanten Einfluss auf den Wert von ΔV hat ($p = 0,0145$), während Nadelgröße und Viskosität eine geringe statistische Signifikanz für die abhängige Variable aufwiesen ($p = 0,6540$ bzw. $p = 0,6848$). Dieses Modell legt nahe, dass eine höhere Entnahmegeschwindigkeit zu einem höheren Dosierungsfehler führt.

ABSTRACT

Title: Formulation factor analysis of dosing accuracy from vials

Author: Karlo Tomasic

1st keyword: vials
2nd keyword: dosing
3rd keyword: accuracy

Injectable drug formulation using vial-syringe systems provide a rapid intervention in health emergencies, but due to the manual dose measuring the risk for imprecise dosing is possible. Such systems are used for parenteral drug administration, or more specifically for drug administration into the tissue or the vascular system. In this thesis, the dosing accuracy was evaluated depending on liquid viscosity and needle size. Therefore, two groups of data were collected and analysed. First, manual dosing measurements were conducted as starting point for this analysis. To minimize the impact of human factors, these measurements were performed by a single operator. Measured variable was volume difference (ΔV) between the expected and measured volume of liquid expelled from the vial into the syringe. Variables investigated included the type of liquid (with three different formulations and viscosities) and needle size (G19 and G21). Liquids used were as follows – water, water with glycerol and water, glycerol and polysorbate 80 (surfactant) solution. Their viscosities are – 1.025 cP, 10.548 cP and 10.474 cP, respectively. Here, 60 measurements were conducted to collect data (10 measurements per liquid and needle size combination). Fixed variables included vial size (2 mL), vial fill volume (1,2 mL), expelling volume (0,9 mL), syringe size (1 mL), temperature and pressure (NTP). A statistical analysis conducted on that data set showed that there is a statistical significant difference only in surfactant-containing mixture (water, glycerol, polaysorbate) depending on a needle size ($p=0.0009$). Furthermore, second part of this research was conducted on Instron™ 5942 Series® Single Column Table-top Testing System, investigating the expelling force profiles. Measured variable was volume difference between the expected and actual value (ΔV) with an additional variable – speed, with which the machine extracts the liquid from the vial (movement of the syringe plunger head in cm/sec). Six speeds were observed – 0.1, 0.2, 0.3, 0.4, 0.5 and 1 cm/sec. Here, 36 measurements were conducted (one per each combination of speed, needle size and viscosity). The aim of this step was to create a prediction model which explains the variability of dosing accuracy affected by following variables – speed, needle size and viscosity. Obtained model suggested that speed has a statistically significant impact on the value of ΔV ($p=0.0145$), while needle size and viscosity showed low statistical significance on the dependent variable ($p=0.6540$ and $p=0.6848$, respectively). This model also suggests that higher withdrawal speed results in higher dosing error.

PREFACE

This work has been carried out at the Institute of Particle and Process Engineering, Graz University of Technology, under the direction of Univ.-Prof. Dr.phil.-nat. Sven Stegemann.

I would like to thank Prof. Stegemann for his guidance through the process of making this work, and for constructive feedback with suggestions and helpful criticism. Furthermore, I would like to thank all the employees and students from the Institute for Particle and Process Engineering, that had a role in this work and helped me go through this work easier.

Last, but not least, I would like to say a big thank you to my family and my girlfriend, for having understanding and patience through my study path.

Graz, January 2019

Karlo Tomasic

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1 INTRODUCTION

1.1 Motivation and background

Vials are mostly used for parenterally administered drugs or biologics, which means that the active compound is dissolved, emulsified or suspended in an aqueous system which is administered directly into the body. In the majority of cases, such aqueous systems are directly injected into the systemic circulation, thus bypassing the absorption through the skin and mucous membranes. Vials, syringes and needles represent the basic and most commonly used drug delivery devices for such parenteral medicines.

There are different kinds of medical procedures for such systems that include:

- Injection preparations (small volumes of biologics, drug solutions or formulations)
- Infusion preparations (large volumes of drugs in a solution form)

Because of their rapid therapeutic effects, these systems are used for drugs in health emergencies, but as well for large molecules (e.g. biologics) that cannot be administered via the oral route or special applications like ocular administration. Manual dosing, especially in urgency situations or for very low volumes, bear the risk of imprecise or incorrect dosing.

Different kinds of needles and syringes exist to cover the wide range of injection and infusion systems including a collection of tubes used for blood collection. For example, multi-sample, hypodermic and winged infusion (butterfly) needles are used depending on the purpose of the venipuncture blood collection. Winged infusion needles are used on children and poorly accessible veins. For the injection of small volumes to e.g. into the eye, very thin needles in conjunction with low volume syringes (e.g. 1.0 mL) are being used to assure painless injection and precise dosing.

In order to understand the actual knowledge on dosing accuracy of low volume injections from vial-syringe system a literature review was performed.

1.2 Literature research

Literature research (Pubmed) on dosing accuracy from syringe-needle systems show that there are very few publications that focus on the accuracy of dosing in such systems. The available studies suggest that dosing accuracy is highly variable and depends on four major topics:

1. Dosing issues related to human factors (Parshuram et al., 2008.)
2. Dosing issues related to specific device systems (Jarrahian et at., 2017.)
3. Dosing accuracy in infusion preparation (Aguado-Lorenzo et al., 2013.)
4. Comparison of dosing accuracy to pen and pump systems (Gnanalingham et at., 1998.)

Studies found tend to be mainly descriptive and observational, focusing on human factors and specific injection systems, like injection pens. Interestingly, the literature search did not reveal any study on the dependence of formulation factors (e.g. visco-elastic properties and rheology) and needle-syringe factors (e.g. needle size, expelling time/speed).

The objective of this thesis was to close the knowledge gap by performing an investigation into formulation and needle-syringe factors on the dosing accuracy for low volume injections used in general pharmaceutical and medical practice.

1.3 Scientific background

1.3.1 Routes of administration

According to Mosby's Medical Dictionary (2009), route of administration, in pharmacology and toxicology, represents a path in which a substance is taken into the body. Substances can include drugs, poisons, fluids and other.

This chapter will give a short overview of the most commonly used routes of administration, with the purpose to give an introduction into the importance of accurate drug administration. The route of administration for drugs describe how the drug is administered to reach the site of action in a patient convenient way. There are three major underlaying concepts:

1. Parenteral
   The administration of the drug into the systemic circulation not through the mouth (e.g. injection/infusion, intra-ocular, transdermal, etc.)
2. Enteral/gastrointestinal
   Administration of the drug into the systemic circulation or the gastrointestinal tract through the mouth
3. Topical
   Administration of the drug through the body external surfaces or membranes, excluding the mouth (e.g. dermal, intra-nasal, vaginal, pulmonary, etc.)

Different dosage forms are being used of which the major ones are listed below:

- Vials & syringes (e.g. Injection/infusion)
- Tablets & capsules (e.g. oral delivery)
- Dry Powder Inhaler & Metered Dose Inhaler (e.g. pulmonary delivery, inhalation)
- Suppositories (e.g. rectal, vaginal)
- Drops & Solution (e.g. oral delivery, ocular, nasal)
- Patches (e.g. transdermal delivery)

Routes of administration and selection of the dosage forms are chosen according to different factors:

- Properties of the drug
  - Chemical
  - Solubility
  - Stability
- Desired site of action
  - Tissue or organ specific (localized)
  - Blood circulation (generalized)
- Biopharmaceutical properties of drug:
  - Absorption
  - Distribution
  - Metabolism
  - Excretion (ADME)
  - Adverse drug reaction
  - Therapeutic window
- Patient population
  - Paediatric
  - Weight/body surface dosing

According to Nursing Times (NursingTimes.net, 2007.), in emergency situations, drugs are usually given parenterally (IV). In those acute situations, this represents the most reliable route, as patients can be unconscious, cannot swallow or have unpredictable absorption of substances from the tissue and the digestive tract, due to possible alternation in gastric motility and blood flow.

The point of interest of this thesis is the increasing number of biologics and drugs using vials with needle-syringe delivery systems and the impact of formulation and device factors on the accuracy of dosing.

1.3.2 Parenteral drug administration

Parenteral drug administration is every drug administration that bypasses digestive tract by injecting sterile medication directly into the tissue with special hollow needles and other devices. It has a big advantage – medication is entering the bloodstream or a certain place unchanged, thus resulting in complete and rapid effect. On the other hand, because of that directness, there is a danger of infection, vein and arterial damage, embolism, etc. (Terapijske Doze, 1995)

There are 4 basic types of injections used for parenteral drug administration:

1. Intradermal (ID) – into the skin (dermis, under epidermis)
2. Subcutaneous (SC) – into the skin (under dermis)
3. Intramuscular (IM) – into the body of the muscle
4. Intravenous (IV) – into the vein

And others:

5. Intraarterial (IA) – into the artery
6. Intrathecal (IT) – into the spinal canal
7. Intrapleural (IP) – into the lungs
8. Intracardial (IC) – into the heart
9. Intraarticular (IA) – into the joint
10. Intraocular (IO) – into the eye

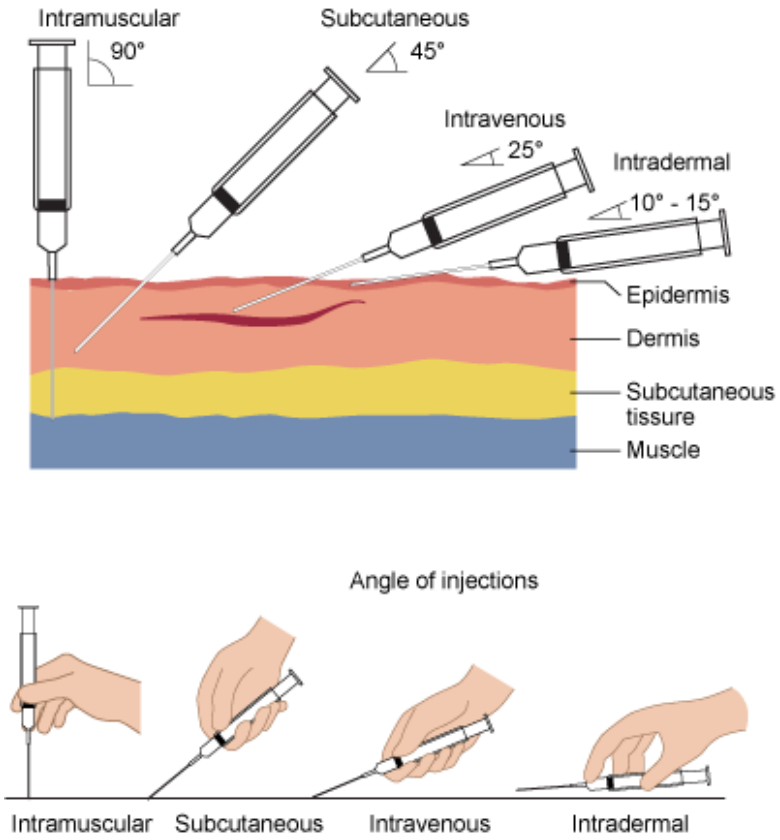


Figure 1-1: Injection angles for different kinds of parenteral injections (Clinical Procedures for Safer Patient Care, 2015)

Only in the case of intravenous and intraarterial injections medication has not to be absorbed, because it is given directly into the bloodstream. But there is a major difference – intravenous drug administration provides faster effects, while slower intraarterial drug administration provides high drug concentration in the tissue supplied by specific artery, and lower drug concentration in the general circulation.

1.3.3 Oral drug administration

Oral delivery is the most used, non-invasive and economical path of drug administration. In oral delivery, the drug is mainly absorbed into the intestines and to some extend in the stomach by diffusion through the epithelial cells into capillaries, portal vein, liver and general circulation. In comparison to parenteral drug administration, effects are delayed, because of the time needed for the absorption process from the digestive tract. (Gauwitz, 2004.)

Oral drug delivery also includes sublingual and buccal delivery, which takes advantage of the relatively fast absorption through the membranes in the oral cavity as well as the bypassing of the first-pass effect.

Medications used for enteral drug administration are either in solid form (powders, tablets, capsules, pills) or liquid form (solutions, mixtures, drops).

1.3.4 Topical drug administration

These drug delivery forms are considered for local effects, tissue targeting or sustained release forms.

Topical drug administration:

1. Pulmonary – inhalation
2. Urogenital – bladder, urinary tube, uterus
3. Ocular
4. Ear
5. Transdermal – percutaneous, intracutaneous, iontophoresis
6. Nasal delivery

The large surface area of the human lungs, together with its rich blood supply, makes pulmonary drug administration a potential choice for treating respiratory diseases (e.g. COPD, asthma). In pulmonary drug delivery the active substance is inhaled in a form of an aerosol which can consist of fine liquid droplets of micronized solids. (Sokota et al., 2008)

2 BASICS

2.1 Drug delivery using vial-syringe device systems

A majority of drug delivery systems for injectable formulations are composed of a vial containing the liquid, a stopper as a vial closure system which can be pierced, a needle and a syringe. The vial is filled with the liquid and closed with the stopper, whereby the needle is connected to the syringe to penetrate the stopper and dip into the liquid. By pulling the piston of the syringe, liquid is expelled from the vial. Since each of these components might have an impact on the accuracy of dosing, their function and role will be described in more details as well as other components and analytical equipment that is used for their performance evaluation.

2.1.1 Vial

Vial (phial, flacon) is a small plastic or glass container, vessel or bottle, most commonly used to store medications in a form of liquid, powder or capsules. Can also be used as scientific sample vessels, for example, autosampler devices for analytical chromatography (Merriam-Webster, 2018).

They can be tubular or have a bottle-like shape with a neck. Neck volume is known as headspace of the vial. The bottom of the vial is usually flat, but for example, test tube vials have rounded bottoms to securely fit into the vial rack with which they are used. Those small test-tube vials, typically used in laboratories, are also known as McCartney's bottles or bijous.

Modern vials are usually made from plastic and glass. The materials used for vials are borosilicate glass, HDPE (high density polyethylene) and PET (polyethylene terephthalate).

Vials can be in different colours, depending on the content inside the vial – e.g. for light-sensitive content amber colour vials are used. It is critical that the material used is chemically resistant, neutral, strong and impermeable. (Schoot, 2017)

There are different types of closing mechanisms:
- screw cap or dropper/pipette – screw vials
- cork or plastic stopper – lip vials
- rubber stopper and a metal cap – crimp vials
- hinge caps (snap-caps, flip-tops) – hinge vials

2.1.2 Vial closing and stopper systems

There are different types of closing mechanisms:
- screw cap or dropper/pipette – screw vials
- cork or plastic stopper – lip vials
- rubber stopper and a metal cap – crimp vials
- hinge caps (snap-caps, flip-tops) – hinge vials

Vial stoppers are small, ready-to-sterilize (RS) pharmaceutical components made from elastomer used to safely-seal the vial and protect its content from bacteria and unwanted particles. It is also meant to be self-sealing, which means that it should withstand multiple needle punctures and keep its protective purpose.

2.1.3 Syringe

Syringe is a simple medical device or instrument consisting of a hollow barrel fitted with a plunger, used to inject fluids and gasses, or withdraw them from the body or cavities. It is usually fitted with a hollow needle onto its hollow barrel.

The first syringes were made from metal, but with the improvement of glass making capabilities, later were made from glass. Today they are usually made from plastic with a tuber piston - plunger, which eases volume control of the content inside and disposability.

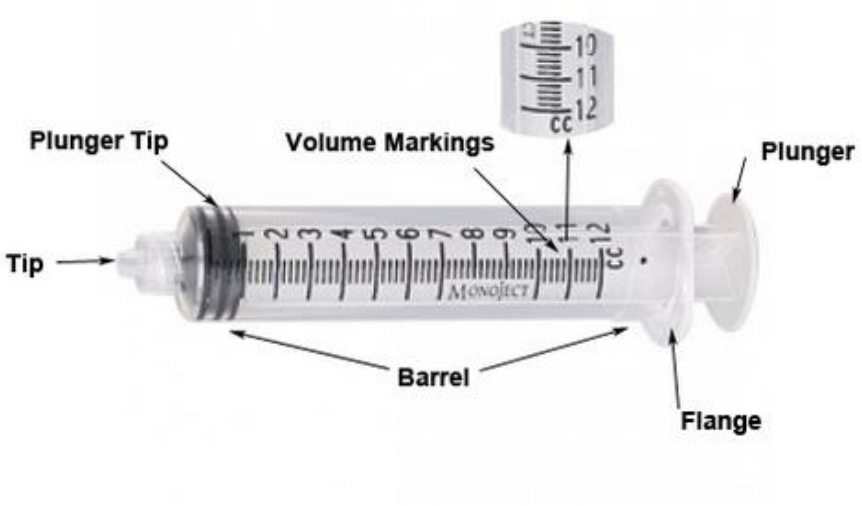


Figure 2-1: Standard syringe parts (Cancaster, 2015)

Syringe sizes can vary from 1 to 70 ml. They are selected based on the intended volumes to be administered whereby, a first larger syringe capacity is selected. For example, a 1 mL syringe should be selected to measure e.g.0.8 ml. This secures that volume markings on the syringe have the smallest possible increments for that measurement. On the other hand, a 'safety' space should be left between the volume measured and syringe capacity to make sure that the plunger does not get dislodged during extraction. The larger syringe capacity, the larger is the interval between volume markings (The Pharmaceutics and Compounding Laboratory, 2018).

Syringe tip designs can vary according to their application (BP, 2017):

- Luer Lock Tip – the tip is threaded to enable the needle to be locked, used when secure connection in needed
- Luer Slip-Tip – needle hub is simply pushed-and-twisted to insert and accomplish a friction-fit
- Eccentric Luer Slip Tip – off center tip used for surface veins or artery injections, allows closer proximity to the skin
- Catheter Tip – longer and tapered slip tip design used with tubing or for irrigation
- Permanently attached needle

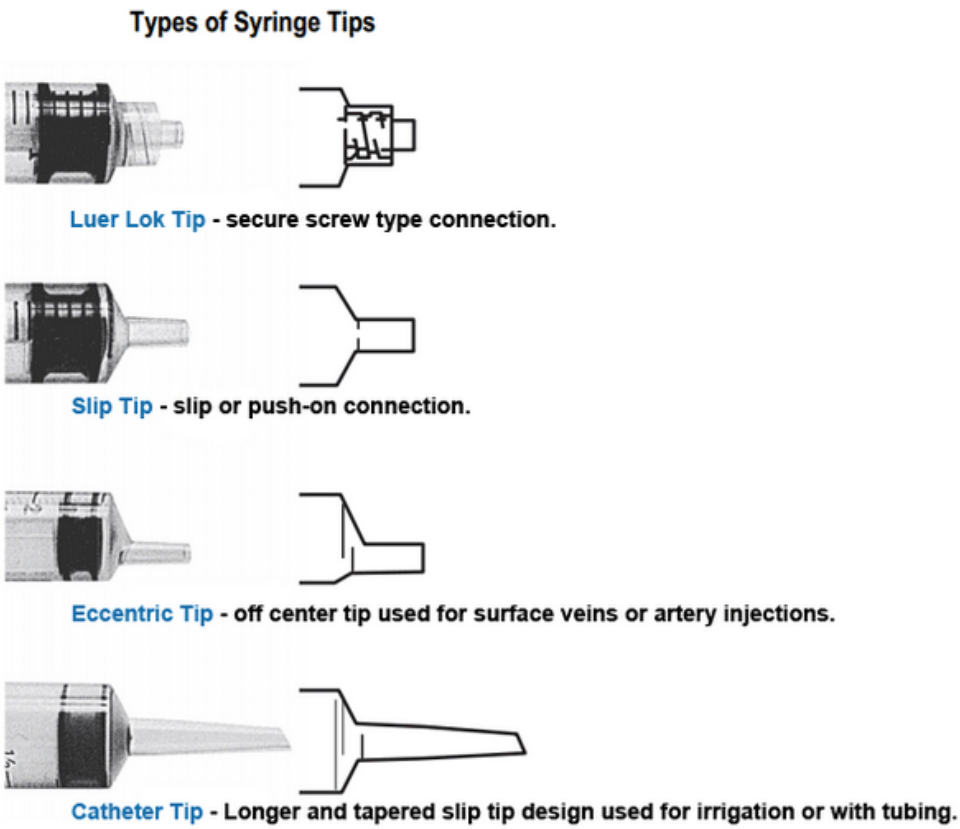


Figure 2-2: Different types of syringe tips and belonging fitting mechanisms (Cancaster, 2015)

Permanently attached needle syringes, most commonly used in insulin and tuberculin syringes, have the lowest tip dead volume, which reduces the amount of medication waste and allows accurate mixing of different medications into one syringe. (BP, 2017)

Dead volume represents the amount of liquid that cannot be expelled from the syringe. That liquid remains within the needle and between the plunger and the syringe hub. It is one of the main causes for inaccurate dosing, disease transmission and medication waste.

It can be minimized 2 ways, using a:

- modified syringe – plunger is extended, entering the syringe neck and thus expelling more fluid from the syringe
- modified needle – has a plastic neck that fits inside the neck of a standard syringe hub

According to Oramasionwu (2016.), high dead volume syringe (HDSS) is accountable for large amount of medical waste and related excess cost of injectable medication waste. Some biologics (e.g. Mabs) are so expensive that cost is a main driver for production and drug selection. First, patients pay that high price through their insurances. Moreover, the waste might limit the number of patients being able to receive the drug (e.g. vaccine), due to a limited production.

According to Strauss et al. (2006.), switching to a low dead space syringe-needle system provides between 2 and 19% more vaccine doses per vial.

Impact of syringe dead volume will be further discussed later in this thesis.

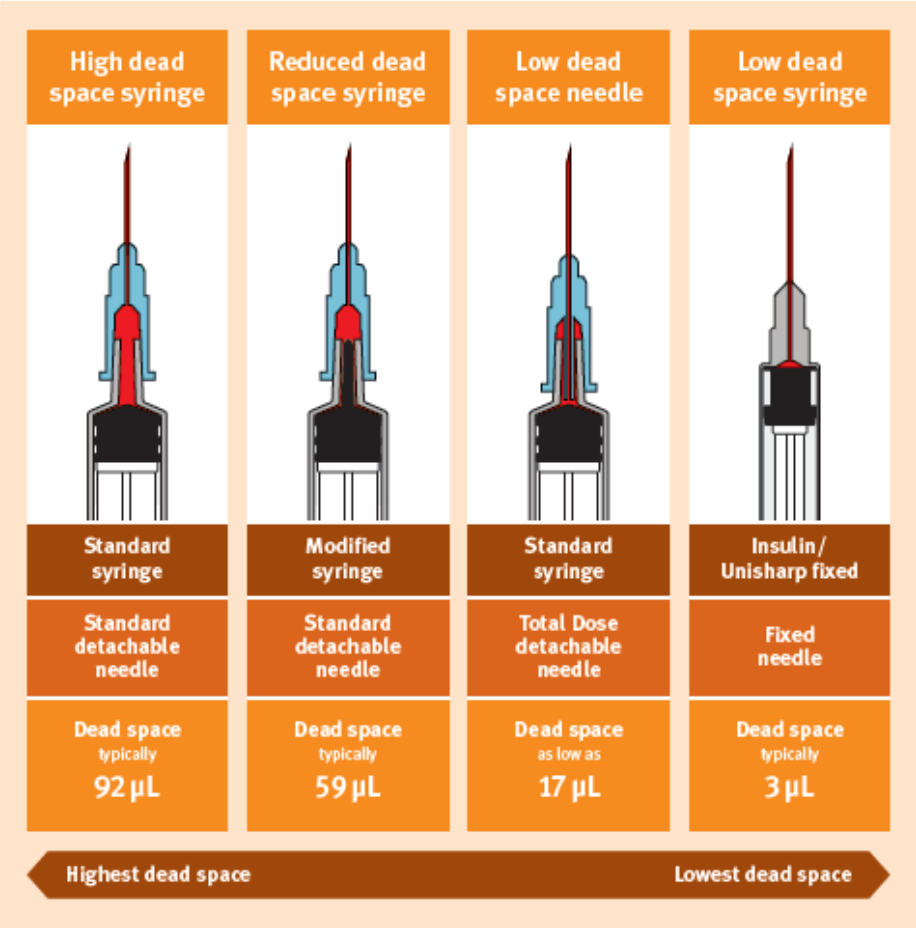


Figure 2-3: Different combinations of syringe-needle designs and their average measured dead space (Southampton University, Department of Engineering and Microfluidics, 2018)

2.1.4 Hypodermic Needles

Hypodermic needle is medical device or equipment used to enter the skin. In pair with a syringe, it I used to inject fluids and gasses, or withdraw them from the body or cavities. It is used when the substance cannot be ingested, or the substance administration should be done so it enters directly into the bloodstream. Usually they are made from stainless-steel.
It serves also an important role in laboratory and clinical environment, where sterile conditions are required. There are two main reasons hypodermic needle is suitable for that conditions. First, because of its extremely smooth surface, which prevents the retention of airborne pathogens on the surface. Second, needle tip is extremely sharp, which reduces the puncture diameter on the skin, preventing microbes in contaminating the substrate.

Standard hypodermic needle parts, as shown in Figure 2-5 on the next page:

- Shaft
- Hub
- Bevel
- Lumen

A needle cover (cap, sheath) is necessary to keep it sterile.

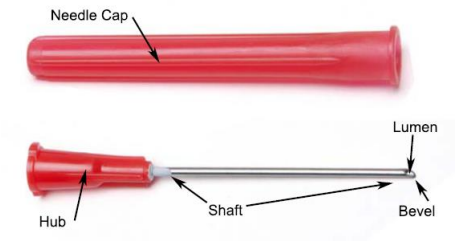


Figure 2-4: Hypodermic needle anatomy (Vitality Medical, 2013.)

Standard used for needle denotation is Birmingham gauge. French gauge is mainly used for catheters, so it will not be explained here. Birmingham gauge is also referred to as Stubs Iron Wire Gauge or Birmingham Wire gauge, because it is generally used as a wire gauge system, to specify the outside diameter of a wire or fine tubing product. Withal, for larger mechanical tubing it specifies the wall thickness (independent of the overall tube size).

Regarding hypodermic needles, gauge number describes outer diameter of the needle. Gauge number and outer diameter are inversely proportional, meaning that the smallest gauge number represents the largest outer diameter of a hypodermic needle.

Standardly used hypodermic needles range from G34 to G18 (BD, 2017).
Outer diameter ranges from 4,572 mm (G7) to 0,00725 mm (G34).
Needle dimensions, along with gauge number, are described with wall thickness, which can range from 0,381 mm (G7) to 0,0508 mm (G36).

Hypodermic needles with same gauge number can have different needle shaft lengths, e.g. G21 needle length can vary from 16 to 50 mm (16, 25, 32, 38 and 50 mm).

2.1.5 Forces – plunger pulling

To give a short introduction to forces that are included into medicine withdrawal, or to be more precise, pulling of the plunger inside a syringe, an explanation of the event is necessary.

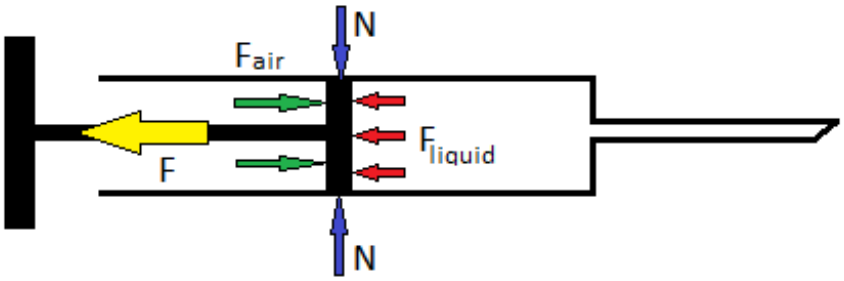


Figure 2-5: Forces exerted on a plunger

From Figure 2-5., it can be assumed that the force (F), needed to be exerted on a plunger, is equal:

$$F = F_{air} + F_{friction} - F_{liquid}$$

F_{air} - created by the air inside the syringe affecting a plunger stopper
$F_{friction}$ - frictional force of a plunger to a syringe barrel wall
F_{liquid} – created by substance inside the syringe barrel affecting a plunger stopper

From these formulas it can be concluded that to move the plunger, force exerted on the plunger needs to overcome force from air in the syringe barrel and frictional force between plunger stopper and syringe barrel.

To help reduce friction appearing between plunger stopper and syringe barrel wall, inside walls and plunger stoppers are usually coated with medical grade silicone one (silicone free syringes in development). (BD, 2017.)

To graphically present forces mentioned, force-extension diagrams were gathered from the machine during experiment. In Figure 2-6, an example of expected force distribution is shown.

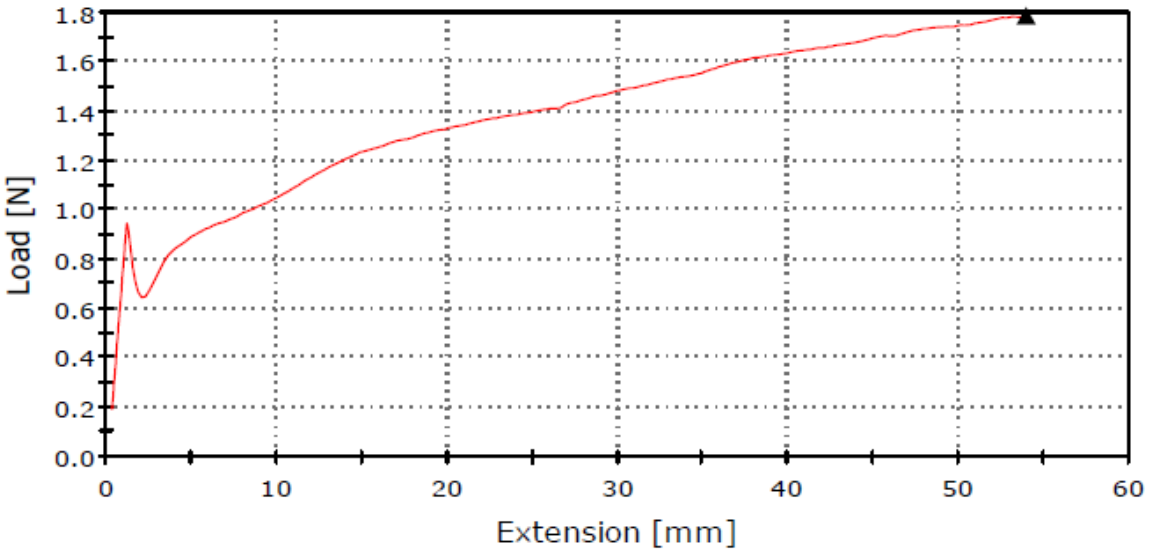


Figure 2-6: A force-extension diagram gathered from Instron during plunger-pulling experiment (water/glycerol/polysorbate 80; 21G; 2mm/s)

From diagram above, a peak in force value at the beginning is noticeable. Force responsible for it is called **break-loose** or activation force. This is the force that needs to be exceeded to start the plunger from moving, therefore overcoming frictional force between the plunger stopper and syringe barrel wall and air force on plunger stopper. After that, force decreases shortly, preceding a continuous increase until the maximum force. This force is called **gliding force**, extrusion force or propagation force. Gliding force is usually lower than break-loose force and enables continuous movement of the plunger.

Note that, to fully understand the impact of these values, forces should be interpreted keeping in mind suggested values for safe and accurate using. These are adduced as pressures (force as a function of area). According to Cilurzo et al., suggested maximum pressure (from maximum force) values for smooth gliding of the plunger are following:

- Fmax over 250 mPa – practically impossible
- Fmax between 160 and 250 mPa – very difficult
- Fmax between 125 and 160 mPa – feasible, but with some difficulty
- Fmax lower than 125 mPa – smooth

2.1.6 Viscosity

Viscosity is fluid's resistance to flow. It represents the friction created by fluid's motion under a shear force. That friction is created between two surfaces of the fluid moving at different velocities. For example, when a fluid is forced through a tube, fluid's molecules touching the tube's wall surface travel slower that the ones closer to the middle of the tube's axis. Therefore, some stress is needed to overcome that difference in velocities between different layers of the fluid.

It is important to mention that dynamic viscosity is completely unrelated to density! Viscosity appears because of the intermolecular forces inside the fluid, while density tracks its origins to the molecular weight of the fluid. On the other hand, kinematic viscosity is described as dynamic viscosity divided by density. It is so because the kinematic viscosity describes how fast does the momentum diffuse in the fluid. (Elert)

Standard measuring unit for dynamic viscosity is pascal-second [Pa s] or poise [P]. Pascal-second is often used with metric prefix mili-, while poise with centi-. One centipoise is equal to one millipascal-second.

1 mPa•s = 1 cP

- In some literature, variations of notations cps or cPs are also used and are considered corresponding to cP

In further work, dynamic viscosity will be referred to as just viscosity, and as the unit of measurement centipoise will be used.

Standard SI unit of kinematic viscosity is square-meter-per-second [m^2/s]. This unit tends to be so large that it is rarely used. More commonly used is square-centimeter-per-second [cm^2/s], which is also referred to as stokes [St]. Most literatures consider dynamic viscosity (also referred to as absolute viscosity or simple viscosity) as just viscosity. (Elert)

Newtonian fluids are the ones that have a linear relationship between shear stress exerted on the fluid and its viscosity. In other words, viscosity remains constant independent on the shear stress applied on the fluid. On the other hand, fluids that don't follow this law are considered as non-Newtonian fluids. Their viscosity can change under different shear rates and forces exerted on the fluid.

Liquids used in this experiment are considered to be Newtonian fluids, which is verified by rheometer experiment and its graphical output, which confirms that these liquids have a linear relationship between viscosity and shear stress.

2.1.7 Laboratory glass bottles

Containers with narrow openings used to store samples. Can be made from different types of glass, according to their purpose (e.g. borosilicate, quartz, etc.).

2.1.8 Pipette

Laboratory tool used in medicine, pharmacy, etc. to transport exactly defined volume of liquid. They come in different designs according to their purpose. They work by creating a vacuum over the liquid chamber to absorb liquid and releasing it to eject liquid.

They are used with disposable tips to ensure sterility of every sample.

2.1.9 Analytical balance

Laboratory class of balance used to measure small mass in laboratory conditions, with a readability in sub-milligram range. The balance consists of a measuring pan inside a draft shield. Shield is used to eliminate the impact of possible air current in the room and to prevent dust collecting on the measuring pan, thus affecting balance's operation.

2.1.10 Single Column Tabletop multiple force Testing System

This machine is used as a universal static testing system which can perform wide range of tests: tensile, compression, shear, flexure, peel, tear, cyclic and bend. Column stiffness ensures precise aligning, testing efficiency and repeatability. (INSTRON, 2018)

2.1.11 Rheometer

Rheometer is an instrument used for measuring rheological properties of a certain substance. Rheological properties show how a liquid (suspension, slurry) reacts to applied forces.

There are 2 basic types of rheometers (Malkin, 2012):

- Rotational/shear rheometers – control applied shear stress/strain
    - Linear shear rheometer
    - Pipe/capillary rheometer
    - Dynamic shear rheometer
        - Spindle type
        - Concentric cylinder
        - Double cone and plate
        - Cone and plate
        - Plate and plate
        - Cone and cone
- Extensional rheometer – control extensional stress/strain
    - Capillary breakup rheometer
    - Opposed jet devices
    - Contraction flow systems
    - Filament stretching rheometers
    - Constant-length devices
    - Acoustic rheometer
    - Falling plate
    - Capillary/contraction flow

3 MATERIALS AND METHODS

For the experimental part of this study, different apparatus was used to prepare and execute tests needed to the gather data which will be processed later in this thesis.

Materials used:

- Vials – Schoot™ 2.00 mL Fiolax® clear StandardLine
    - Item No. VCDIN2R
    - Loot no. 6104394393

- Stoppers- West™ Envision ® 13 mm
    - Item No 7001-5250
    - Batch 5162018601

- Syringes – BD™ Plastipak® 1.00 mL Luer Slip-Tip
    - REF 303172
    - LOT 1804001

- Hypodermic Needles –
    - BD™ Microlance® 19G x 50 mm Luer
        - REF 301750
        - LOT 140519
    - BD™ Microlance® 21G x 50 mm Luer
        - REF 301155
        - LOT 131018

- Laboratory glass bottles – Schoot™ Duran® 250 mL
    - Retrace Code 10020990

- Pipette - Eppendorf™ Research® plus Single-Channel; Blue; 100-1000 µL
    - R39404F
    - Last verification 08/2018.
    - Eppendorf™ epT.I.P.S.® 1 000 µL

- 1 L Laboratory wash bottle with distilled water

- 50 mL polystyrene universals

- Different fixation devices

Formulations used:

- Glycerol 85% PHE ACM (CAS 56-82-5, HBK1016; 10-692-0009-7)
- Water
- Polysorbate (Tween) 80 (CAS 9005-65-6; P4780-100ML)

Formulations (for future reference):

1. W - water
2. WG – water + glycerol
3. WGP -water + glycerol + polysorbate 80

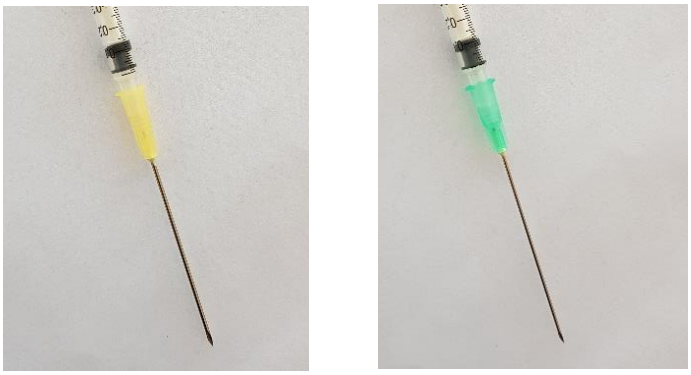
Equipment used used:

- Analytical balance - KERN™ ABS 120-4N®
- Bench scale – KERN™ GAB-12K0.1N®
- Instron™ 5942 Series® Single Column Table-top Testing System
- Anton Paar™ Physica® MCR 300 Rheometer
    - Anton Paar™ CP50-2® Measuring Cone

Figure 3-1: Schoot™ 2.00 mL Fiolax® clear, used in this experiment

Figure 3-2: A vial with West™ Envision ® 13 mm stopper, used in this experiment

Figure 3-3: BD™ Plastipak® 1.00 ml Luer Slip-Tip, used in this experiment



a. b.

Figure 3-4: a. BD™ Microlance® 19G x 50 mm Luer ; b. BD™ Microlance® 21G x 50 mm Luer, used in this experiment

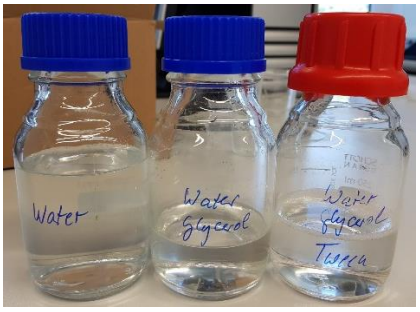


Figure 3-5: Schoot™ Duran® 250 mL, used in this experiment

Figure 3-6: Eppendorf™ Research® plus Single-Channel; Blue; 100-1000 µL with Eppendorf™ epT.I.P.S.® 1 000 µL, used in this experiment

Pipette, which was used in this experiment, is calibrated according to ISO/UEC 17025:2005 Accredited Pipette Calibration Program standard and was last proofed in August 2018.

According to Eppendorf (2018), mentioned pipette used with original tips has a systematic error of ±0.92% (±5.2 µL) and a random error of ±0.2% (±1.2µL) when used for 0.6 ml transfer (total of 1.2 ml was transferred by two 0.6 ml pipette transfers).

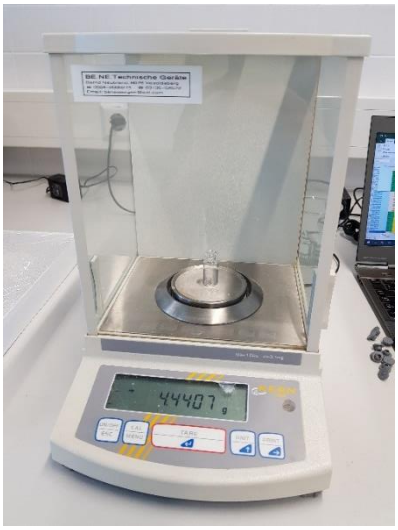


Figure 3-7: Analytical balance - KERN™ ABS 120-4N®, used in this experiment

Analytical balance used in this experiment is declared to be conformed with the standards included in EU Directives: 2004/108/EC and 2006/95/EC.

Technical specifications (KERN, 2018):

- weighing capacity (max) - 120 g
- readability - 0,1 mg
- repeatability – 0,2 mg
- linearity 0,3 mg

Figure 3-8: Bench scale - KERN™ GAB-12K0.1N®, used in this experiment

Technical specifications (KERN, 2018.):

- weighing capacity (max) - 12 kg
- readability - 0,1 g

Figure 3-9: Instron™ 5942 Series® Single Column Table-top Testing System, used in this experiment

In this experiment this machine was used to measure mechanical force needed to fill a syringe, as well as some cover data like time, etc. for which data will be shown in further chapters.

Figure 3-10: Anton Paar™ Physica® MCR 300 Rheometer with Anton Paar™ CP50-2® Measuring Cone, used in this experiment

For this experiment, cone and plate rheometer is used to determine dynamic viscosity of 3 liquids used in the experiment. Data related to these tests can be found in further chapters.

3.1 Preparation of mixtures

The first step of this experiment was to prepare 3 mixtures (distilled water and 2 mixtures) that were used for it. They were chosen and prepared to show (different) behaviour of liquids with different viscosities.

1. Distilled water was chosen as a liquid with the lowest viscosity
2. Glycerol was added to the second mixture to increase mixture's viscosity
3. Polysorbate was added to water and glycerol mixture to see possible behavioural differences with this kind of surfactant

Mixtures were prepared for roughly 100 measurements per liquid in total and additional safety volume in case additional measurements are needed.

For further simplicity, liquids will be referred to as the initials of their names:

- Distilled water → W
- Distilled water + glycerol → WG
- Distilled water + glycerol + polysorbate 80 → WGP

Preparation was done using following equipment:

- bench scale weight balance
- 3 250 mL laboratory glass bottles
- laboratory wash bottle with distilled water
- 2 50 mL polystyrene universals with:
    - Glycerol 85%
    - Polysorbate 80

PROCEDURE – 1st liquid – W (distilled water):

1. Take an empty and clean laboratory glass bottle
2. Put it on the bench scale
3. Tare the scale
4. With a laboratory wash bottle add 200 grams of distilled water
5. Cover the bottle with a screw

PROCEDURE – 2nd liquid – WG (distilled water + glycerol 85%):

1. Take an empty and clean laboratory glass bottle
2. Put it on the bench scale
3. Tare the scale
4. From a polystyrene universal add 137 grams of glycerol 85%
5. Tare the scale
6. With a laboratory wash bottle add 63 grams of distilled water
7. Cover the bottle with a screw

PROCEDURE – 3rd liquid – WGP (distilled water + glycerol 85% + polysorbate 80):

1. Take an empty and clean laboratory glass bottle
2. Put it on the bench scale
3. Tare the scale
4. From a polystyrene universal add 137 grams of glycerol 85%
5. Tare the scale
6. With a laboratory wash bottle add 63 grams of distilled water
7. Tare the scale

8. From a polystyrene universal add 0,1 grams of polysorbate 80
9. Cover the bottle with a screw

3.2 Rheometer

To characterize the obtained liquids formulations for the study the dynamic viscosity was measured after the liquids were prepared, which was done using a rheometer.

Experiment was done using following equipment:

- Rheometer
- PC
- Pipette
- Cleaning wipes

PROCEDURE (the same for all three samples):

1. Turn on rheometer's power unit
2. Adjust the settings on the PC (appropriate software needed)
3. Insert measuring cone into the head of rheometer
4. With a pipette, place 1mL of liquid on the plate
5. Start the measurement on the PC
6. Export results
7. Clean rheometer surface with a cleaning wipe

The liquid placed on the plate is pressed by a shallow cylindrical cone (the one used has an angle of 1.994º and a diameter of 49.972 mm). The cylindrical cone is rotated at a set speed.

Following data can be collected:

- Shear rate
- Shear stress
- Viscosity
- Speed
- Torque

Detailed output data and findings can be found it the following pages.

NOTE

- Machine is set to produce shear rate up to 100 1/s, which is consistent with 31 measurement points which can be seen in the output (every point – roughly 3,33 more with every measurement point)
- First four viscosity values for each liquid were excluded from the graphical representation because it is not a real value, but a step needed for the machine to start the measurement, so average value was calculated based on 27 measurement points

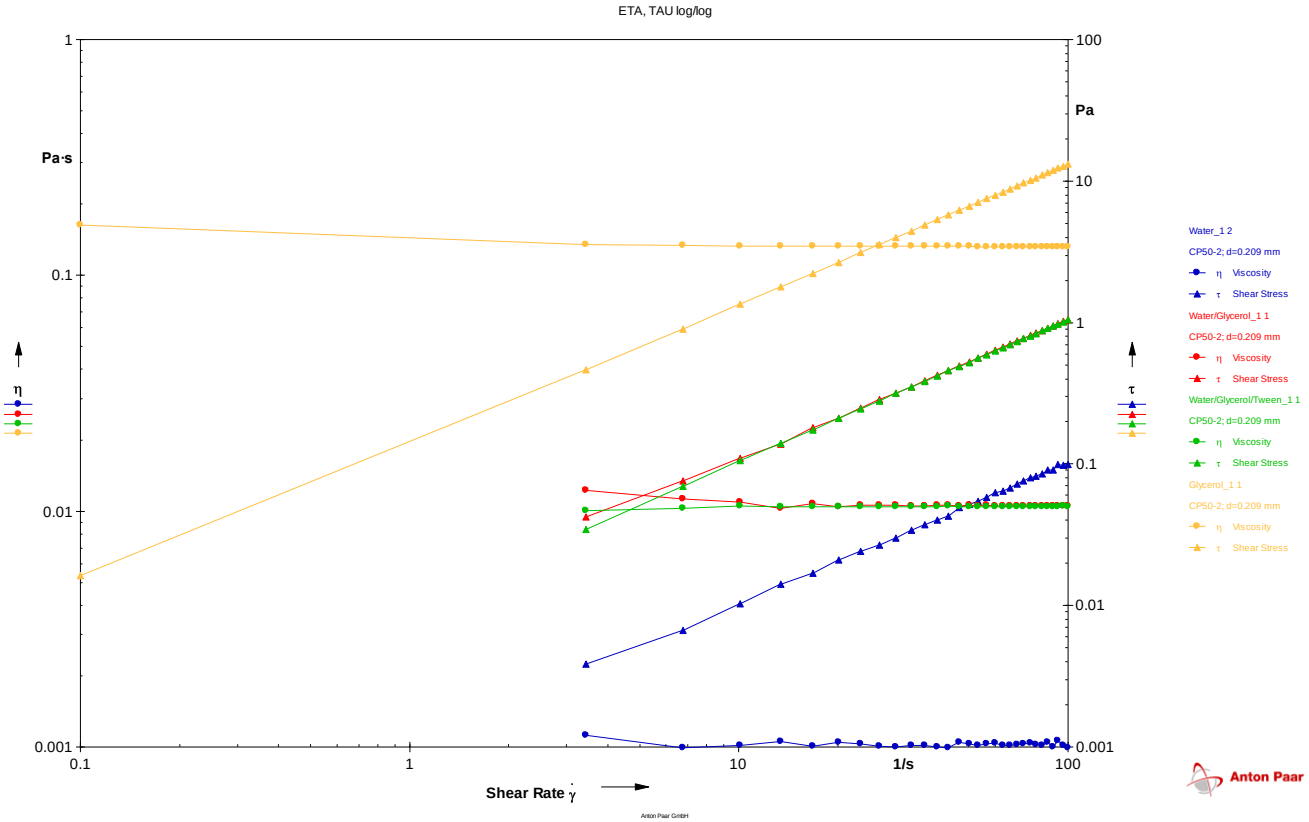


Figure 3-11: Graphical output obtained from the rheometer

This graphical output shows next:

1. YELLOW – glycerol
2. RED – water and glycerol mixture
3. GREEN – water, glycerol and polysorbate 80 mixture
4. BLUE - water

Steep lines represent viscosities, while flat lines represent the shear stress exerted on the liquid during the experiment.

In the graphical representation obtained from the rheometer – dynamic viscosity, shear rate and shear stress can be followed. For this thesis, dynamic viscosity is the only part taken into consideration. Therefore, in the next chart, only viscosity trends for our samples will be shown.

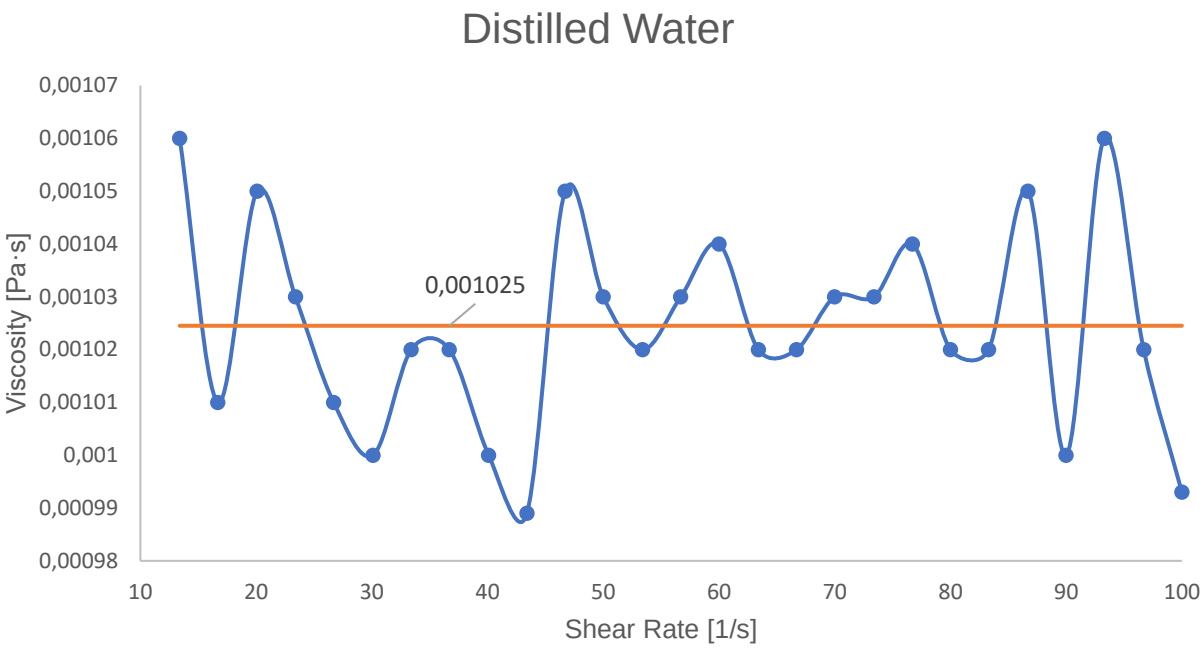


Figure 3-12: Viscosity data for W gathered from the rheometer with its average value (η_{avg}=1,025 cP)

As already explained, standard unit was used – centipoise (equal to mili pascal-second).

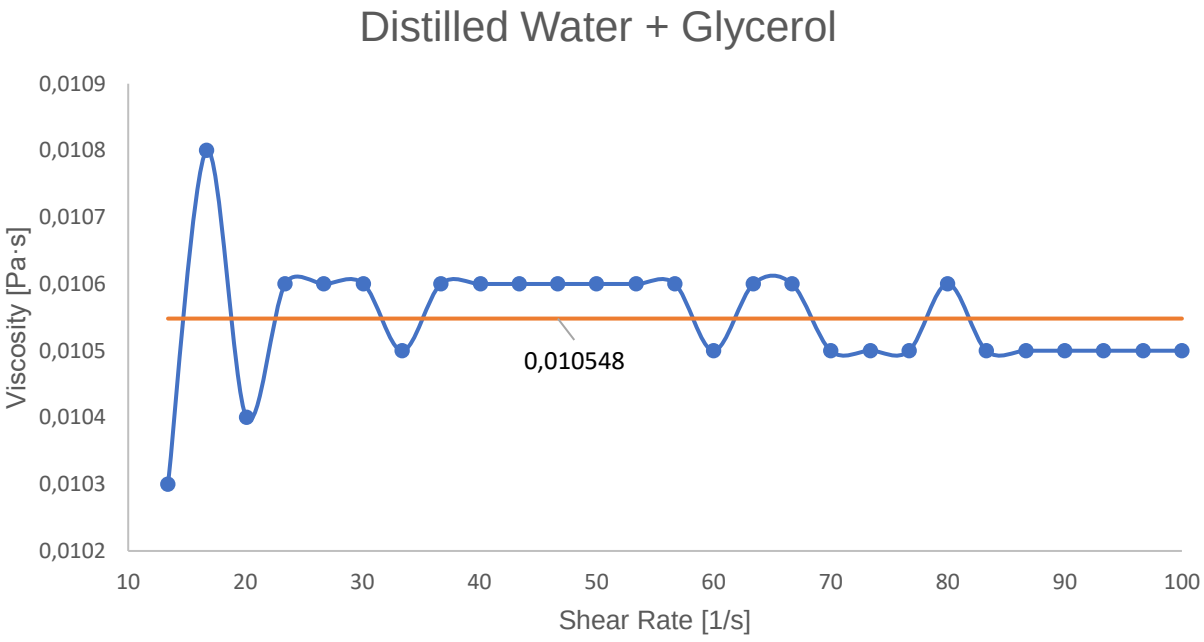


Figure 3-13: Viscosity data for WG gathered from the rheometer with its average value (η_{avg}=10,548 cP)

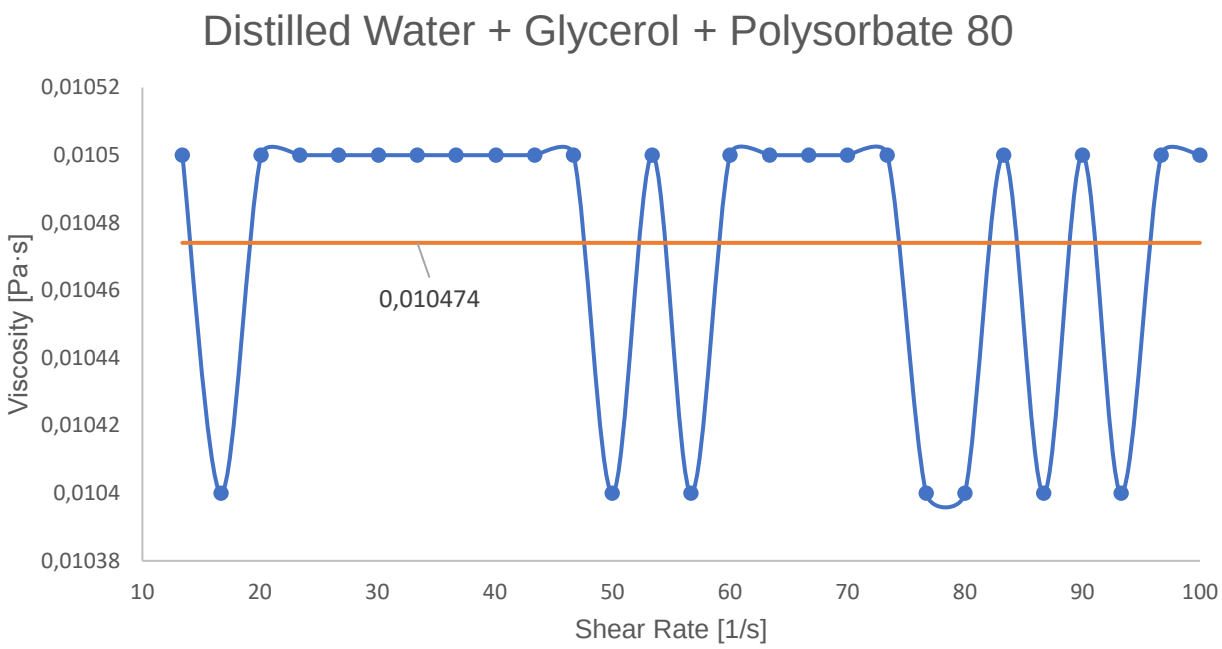


Figure 3-14: Viscosity data for WGP gathered from the rheometer with its average value (η_{avg}=10,474 cP)

From these results, it can be concluded that the preparation of mixtures was successful – it was intended to have 'different' liquids, and average values show that the difference in viscosity between W and WG is 10 times, while WGP has an unnoticeable lower viscosity than WG (0,7% difference in average values).

Also, it is important to mention again that first 4 measurement points were excluded from calculation of average value of viscosity because they were not considered as actual values, but auxiliary values needed for the machine to have a steady output. Only after those values, viscosity shows a trend which can be used for further measurement.

3.3 Manual preparation and procedures

Manual preparation procedures were the base. On standard procedure known from general practice of physicians and nurses. In addition, some alternations will be evaluated, together with data gathered from laboratory measurements to show their performance.

3.3.1 Generally accepted procedure

In most of the literature that has been researched, similar versions of syringe filling procedures are mentioned.

According to Gauwitz (2004):

1. Cleanse the seal (vial stopper) with an alcohol wipe, firmly in a circular motion
2. Because pressure in the vial is lower than the one outside, air needs to be injected into the vial to make it possible to withdraw medication from the vial
    a. Place the needle (with a cover on) on the syringe – for Luer Slip-tip firmly insert syringe tip into needle hub

    b. With a needle cover still on, inject the volume of air inside the needle which is corresponding to the volume of medication needed
3. Remove the needle cover and take the vial in your hand, positioning it between thumb and index finger
4. With a vial upside down, and needle bevel pointing up – insert needle tip through the middle of the vial stopper (this part can also be done with the vial sitting on a table)
5. Inject air from the needle into the vial
6. If the vial is still not inverted, turn it upside down, with a needle tip inside the medication in the vial (below the liquid's surface) to avoid taking in air
7. Grasp syringe barrel with the middle finger and base of your thumb
8. Slowly but steadily pull on the syringe plunger until the marking on the syringe corresponding to the medication volume wanted
9. With a needle bevel still in the vial, check for air bubbles inside the syringe
    a. If found, tap the syringe sharply with your finger (this causes the bubbles to collect at the tip of the syringe
    b. Inject part of the volume back into the vial to get the bubbles out and repeat from step 8.
10. If you are not administering that medication immediately, place needle cover loosely back onto the needle

NOTE: for measurements in this thesis, steps 1-5 were omitted because they don't represent parts of the procedure considered to be prone to errors. Enough air was enabled inside the syringe, to keep a focus on filling the needle with medications.

3.3.2 Procedure

Experiment was done using following equipment:

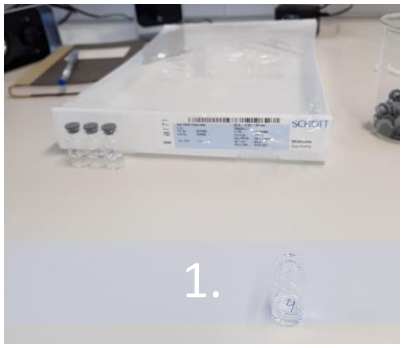
- 2 mL vials
- 1 mL syringes
- Hypodermic needles
    - 19G
    - 21G
- Vial stoppers
- Analytical balance
- Pippete with appropriate tips
- Laboratory glass bottles with 3 previously prepared liquids (W, WG and WGP)

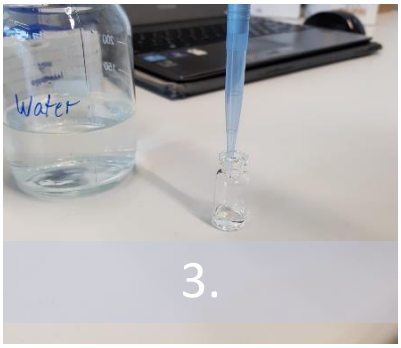
<u>PROCEDURE</u>

1. Take a new vial
2. Weigh it on an analytical scale
3. With a pipette, fill 1.2 mL of liquid into the vial
4. Weigh it again
5. Weigh vial stopper (vial cap)
6. Close the vial
7. Mark it
8. Take a new syringe and put a needle on it,
    a. Remove needle cover
9. With a vial upside down, and needle bevel pointing up – insert needle tip through the middle of the vial stopper (this part can also be done with the vial sitting on a table)

10. If the vial is still not inverted, turn it upside down, with a needle tip inside the medication in the vial (below the liquid's surface) to avoid taking in air
11. Grasp syringe barrel with the middle finger and base of your thumb
12. Slowly but steadily pull on the syringe plunger until the marking on the syringe corresponding to the medication volume wanted
13. With a needle bevel still in the vial, check for air bubbles inside the syringe
    a. If found, tap the syringe sharply with your finger (this causes the bubbles to collect at the tip of the syringe
    b. Inject part of the volume back into the vial to get the bubbles out and repeat from step 13.
14. Weigh the vial
15. Take a new vial
16. Weigh it
17. Empty the content of the syringe into it
18. Weigh the vial
19. Dispose used vials, syringe and needle properly

All these steps are shown in the graphics below:





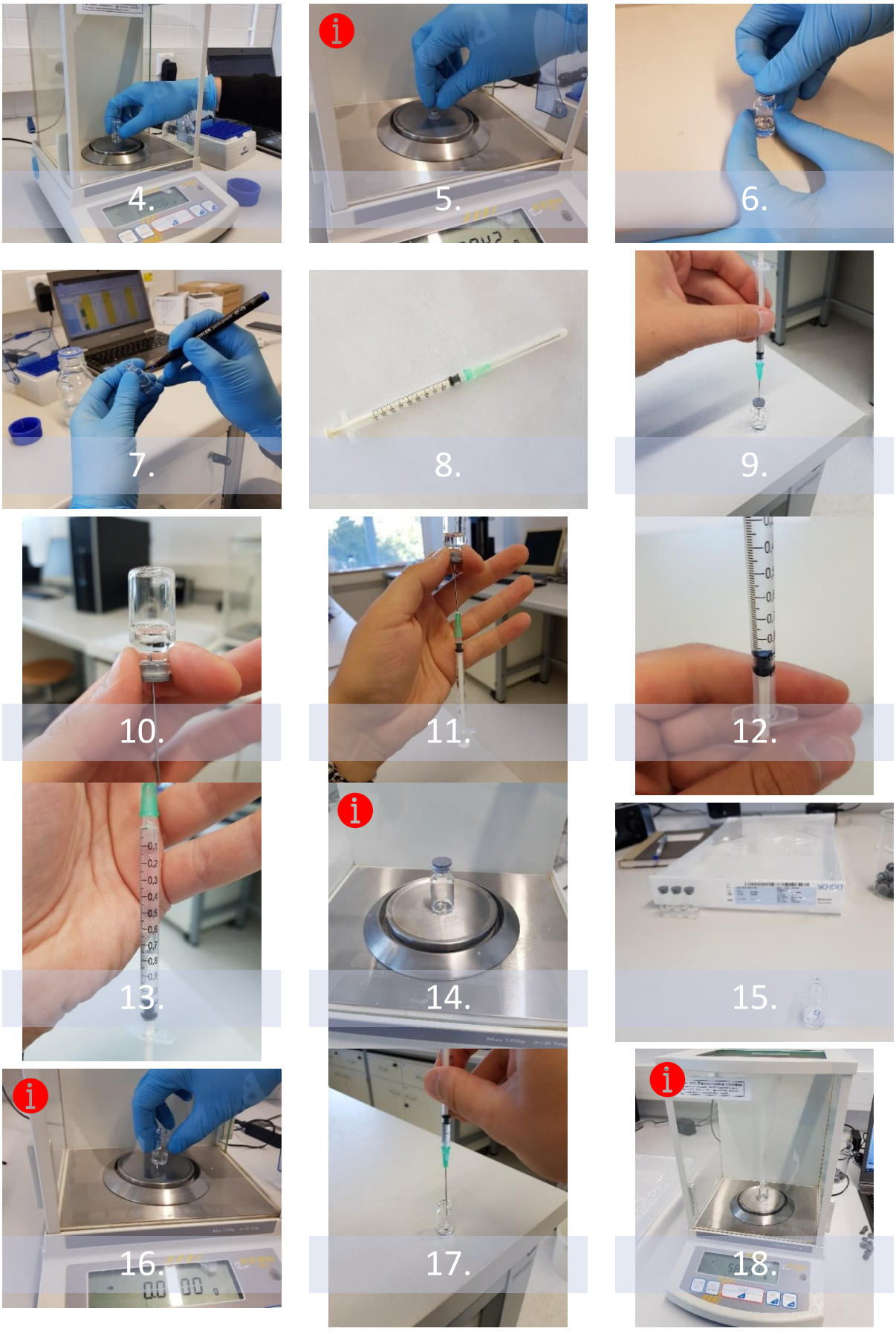


Figure 3-15: Procedure steps (steps with a red information sign are the ones in which data is collected)

The data gathered from these measurment should give us an answer to a few questions:

1. How much liquid is actually left in the vial?
2. How much liquid can be extracted from a syringe?
3. How much liquid is left in a syringe (waste)?

volume_remaining → volume that remained in the vial which was filled by 1.2 mL of liquid after 0.9 mL of liquid was taken out with a syringe

volume_syringe →volume that was extracted from a syringe that was filled to 0.9 mL mark

Density is included in this table because all the volumes were calculated from mass that was measured through a density function:

$$\rho = \frac{m}{V}$$

This was done because, at the beginning, exact volume of liquid put into a vial is known (this is done with a pipette which has an error of 1,92%, which can be neglected).

3.4 Expelling force measurements

The procedure itself was similar to manual measurements until step 8. shown in Figure 3-15. Procedure after the named step is explained and shown in Figure 3-16.

The equipment set-up was modified to enable vial positioning at the base, under the crosshead. Crosshead was fitted with a string, which is intended to be bound to plunger head and therefore enable pulling of the plunger. Computer that was used was equipped with certified Instron BlueHill® software.

3.4.1 Procedure

Experiment was done using following equipment:

- 2 mL vials
- 1 mL syringes
- Hypodermic needles
    - 19G
    - 21G
- Vial stoppers
- Analytical balance
- Pippete with appropriate tips
- Laboratory glass bottles with 3 previously prepared liquids (W, WG and WGP)
- Different auxiliary fixation equipment

Before the experiment, computer's software was adjusted for this purpose:

- Test method: tension
- Sample: samples were named 'waterG19', 'waterG21', etc.
- Pre-tension: 0.2 N – this was done to make sure that the string is tensed enough before the machine starts collecting data
- Test stop: 5.4 cm (syringe length from 0 – 0.9 mL)

- Test speed: 0.1, 0.2, 0.3, 0,4, 0.5 and 1 cm/s
- Desired output: Force
- Live display:
    - Force
    - Time
    - Length

After the syringe was prepared and vial filled up and weighted (step 1-8, page 26-27):

9. Place a filled vial on the base plate of the machine
10. Take a syringe and pierce vial stopper with the needle
11. Fix the syringe to the machine – a improvised fixation device was made to fixate the syringe
12. Attach the string (from machine's crosshead) to the head of the syringe plunger
13. Balance the machine (automatic procedure to reset force meter to 0N)
14. Start the procedure on the computer
15. Bring back the machine to zero-value position
16. Detach the vial from the needle
17. Weigh the vial
18. Take a new vial
19. Weigh it
20. Detach the syringe from the string and fixation device
21. Empty the content of the syringe into the vial weighted in step 19.
22. Weigh the vial
23. Dispose used vials, syringe and needle properly

Figure 3-16: Procedure steps - Instron (steps with a red information sign are the ones in which data is collected); steps 1-8 in Figure 3-5

The data gathered from these measurment should give us an answer to a few questions:

1. The relationship between needle size and expelling force
2. The relationship between expelling force and viscosity
3. The relationship between expelling force and expelling speed
4. The correlation between needle size, expelling force and expelling time on dosing accuracy

Speed → for each liquid, different plunger speeds were used to investigate a possible impact of that factor to the dosing accuraccy

- Note that because of already mentioned air, visible error was noticable – syringe was usually filled between 0.8 and 0.9 mL, which was unavoidable because of machine's constant pulling speed and its inability to 'tap' the syringe or go the opposite way and push the plunger (at least during the duration of the test with pulling) to eliminate air entrapped in the syringe

volume_remaining → volume that remained in the vial which was filled by 1.2 mL of liquid after 0.9 mL of liquid was withdrawn

volume_syringe → volume extracted from a syringe that was filled to 0.9 mL mark

Note that because of already mentioned air, visible error was noticable – syringe was usually filled between 0.8 and 0.9 mL, which was unavoidable because of machine's constant pullung speed and its inability to 'tap' the syringe or go the opposite way and push the plunger (at least during the duration of the test with pulling) to eliminate air entrapped in the syringe.

Density is included in this table because all volumes were calculated from mass that was measured through a density function:

$$\rho = \frac{m}{V}$$

This was done because, at the beginning, exact volume of liquid injected into a vial is known (this is done with a pipette which has an error of 1,92%, which is negligible).

Following tables (separate for W, WG and WGP) show values for:

- Volume – exact amount of liquid in the vial
- Speed – machine's pulling speed
- Density – for accuracy, it was calculated for each sample separately
- Volume remaining in the vial after filling the syringe
- Volume extracted from the syringe

From machine output, maximum force needed for pulling the plunger and belonging charts were gathered.

4 RESULTS

4.1 Manual preparation measurements

The following table shows average values of:

- Density – for accuracy, it was calculated for each sample separately
- Average volume remaining in the vial after filling the syringe
- Average volume extracted from the syringe

Detailed table with separate values can be found in Appendix A.

mixture	needle size	volume [ml]	average density [kg/m^3]	average volume_remaining [ml]	average error [%]	average volume_syringe [mL]	average error [%]
water	19G	1,2	989,8333	0,179066	-40,3112	0,9121	1,3498
	21G	1,2	988,4167	0,181475	-38,6251	0,9144	1,8714
water+glycerol	19G	1,2	1135,4750	0,185925	-38,0250	0,9144	1,5949
	21G	1,2	1135,3480	0,186145	-37,8714	0,9143	1,5734
water+glycerol+polysorbate 80	19G	1,2	1117,8917	0,166858	-44,3805	0,9258	2,8623
	21G	1,2	1126,8440	0,180968	-34,5041	0,9176	0,9657

Table 4-1: Table with average values used for further analysis

Errors were calculated based on 10 measurements each (60 in total). Even though not a focus of this thesis, volume left in the vial was measured and its error calculated, because it shows less liquid present in the vial, which can affect filling of the syringe (because of the needle tip - bevel length).

Results show that:

1. In the vial an average of 34,50-44,38% less liquid was found than expected (expected value was 0,3 mL)
2. From a syringe, 0,96-2,86% more liquid in average was extracted

Only from these values no connection to liquid type (formulation) or needle size can be found. More detailed statistical analysis found in the following subchapter shows more interesting results.

4.1.1 Statistical analysis

In order to show more detailed results, statistical analysis from values found in Appendix A has been conducted. For this purpose, special statistical software was used - SAS®OnDemand (SAS Institute Inc., Cary, NC, USA).

A database was created (shown in Table 3-2). Variables in the database are as following:

- Obs – observation – unique data location in the database
- Mix – type of liquid:
    - 1 – water
    - 2 – water + glycerol
    - 3 – water + glycerol + polysorbate 80
- Needle – needle size:
    - 1 – G19
    - 2 – G21

- ΔV – difference between the volume expelled from a syringe and volume needed (expected) – 0.9 mL

Obs	mix	needle	ΔV	Obs	mix	needle	ΔV	Obs	mix	needle	ΔV
1	1	1	0,00931113	21	2	1	0,00888563	41	3	1	0,02765060
2	1	1	0,01403568	22	2	1	0,02228117	42	3	1	0,01451684
3	1	1	0,01353402	23	2	1	0,02317969	43	3	1	0,05381785
4	1	1	0,01450227	24	2	1	0,04176883	44	3	1	0,02213808
5	1	1	0,00827169	25	2	1	0,00212704	45	3	1	0,03688822
6	1	1	0,01028211	26	2	1	0,01980964	46	3	1	0,03379424
7	1	1	0,00717944	27	2	1	0,01471038	47	3	1	0,02522282
8	1	1	0,01533474	28	2	1	-0,00915254	48	3	1	0,02040982
9	1	1	0,00717655	29	2	1	0,00509909	49	3	1	0,01341301
10	1	1	0,02185363	30	2	1	0,01482890	50	3	1	0,00975556
11	1	2	0,02012844	31	2	2	0,01163061	51	3	2	0,01096433
12	1	2	0,00733675	32	2	2	0,02444314	52	3	2	0,00742236
13	1	2	0,01239251	33	2	2	0,00532596	53	3	2	0,01171840
14	1	2	0,01135408	34	2	2	-0,02100633	54	3	2	0,00922466
15	1	2	0,01768886	35	2	2	0,05989011	55	3	2	0,00557783
16	1	2	0,00979664	36	2	2	0,01081996	56	3	2	0,01142020
17	1	2	0,01698591	37	2	2	0,02107201	57	3	2	0,01398918
18	1	2	0,02219595	38	2	2	0,01770427	58	3	2	0,00660039
19	1	2	0,03237337	39	2	2	0,00649804	59	3	2	0,00141787
20	1	2	0,01817487	40	2	2	0,00523243	60	3	2	0,00857773

Table 4-2: Database used for statistical analysis from the 6 variations (10 measurements each)

After running these values through the named statistical analysis software, 30-pages long output was obtained. Following are most important data collected from this procedure.

First step was to do a univariate analysis of data – just one factor (liquid type - mix, needle size and both simultaneously-still a unambiguous factor) at the time.

Measurements were first classified according to the type of liquid (factor = mix). A total of 20 measurements per liquid were made and included in the statistical analysis (3 liquids, 2 variations per liquid, a total of 60 measurements).

Level of mix	N	delta_v	
		Mean	Std Dev
W	20	0.01449543	0.00638518
WG	20	0.01425740	0.01696469
WGP	20	0.01722600	0.01283863

Table 4-3: Mean and standard deviation values of ΔV for different types of liquids used in this experiment (W, WG, and WGP)

From values in Table 4-3, a volume surplus can be noticed in all liquids. Standard deviation, as a measure of central tendency (spread of the data), shows that very little standard data deviation. On the other hand, W shows lowest standard deviation, while WG shows the largest one. Mean values are roughly the same. All of this can be seen graphically in Figure 4-1.

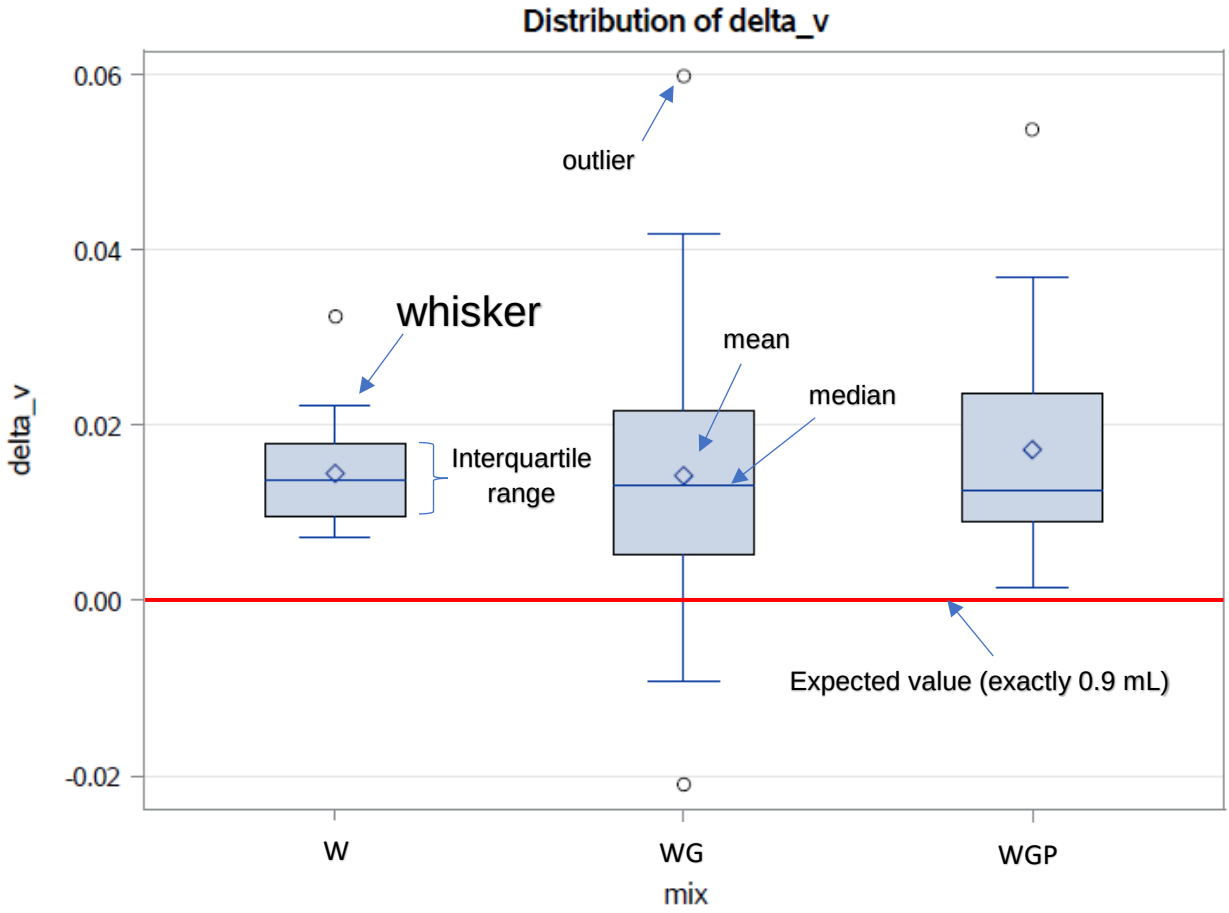


Figure 4-1: Boxplot for ΔV according to the type of liquid (pooled data from W, WG, and WGP)

Boxplot is used in descriptive statistics for graphically depicting groups of numerical data through their quartiles. Data between whiskers represent all data except outliers (extremes) which are represented as circles outside whiskers. Whisker endings present maximum (upper) and minimum (lower) value. Interquartile range box represents medium 50% of the data.

Furthermore, the same group of data was statistically analyzed according to 2 different needle sizes, to see if there is a significant difference in mean values.

Level of needle	N	delta_v	
		Mean	Std Dev
G19	30	0.01742087	0.01253302
G21	30	0.01323168	0.01268315

Table 4-4: Mean and standard deviation values of ΔV for different needle sizes used in this experiment

A small, but noticeable difference can be seen in mean values of those needles. In Figure 4-2, data is presented by a boxplot to see those values graphically.

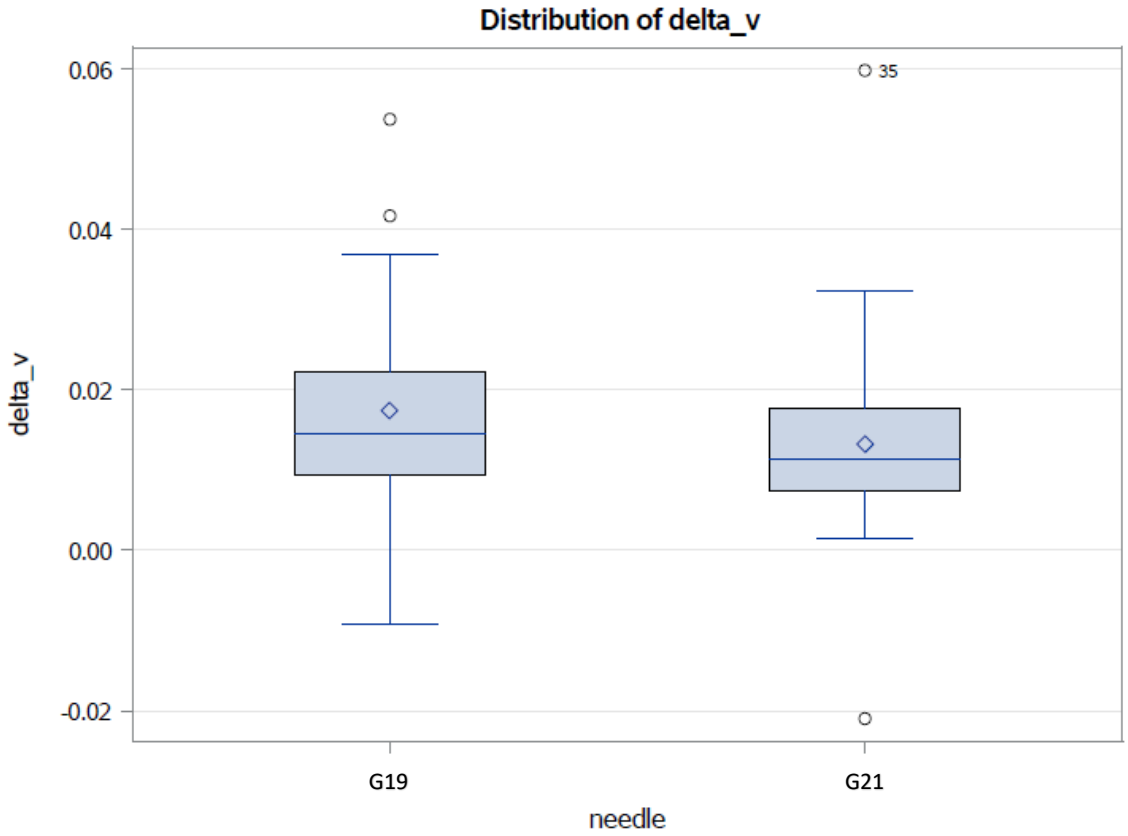


Figure 4-2: Boxplot for ΔV according to needle size – pooled date from all 3 liquids depending on the needle (1-G19; 2-G21)

Interquartile range boxes are quite small which shows a small variability of data. If put differently, 50% of data (presented by a box) do not vary much. Despite this, both needles had some measurements which are considered as outliers (small circles outside the boxes), which tend to have a strong impact on data.

Next, all factors mentioned (type of liquid, needle size) were considered in the same analysis. For that purpose, 3-factor ANOVA analysis was used.

Level of mix	Level of needle	N	delta_v	
			Mean	Std Dev
W	G19	10	0.01214813	0.00461084
W	G21	10	0.01684274	0.00725033
WG	G19	10	0.01435378	0.01390457
WG	G21	10	0.01416102	0.02035244
WGP	G19	10	0.02576070	0.01314971
WGP	G21	10	0.00869130	0.00363196

Table 4-5: Mean and standard deviation values of ΔV for different needle sizes and different liquids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	0.00167604	0.00033521	2.32	0.0558
Error	54	0.00780743	0.00014458		
Corrected Total	59	0.00948347			

Table 4-6. General model – discrimination power of the model

Source	DF	Anova SS	Mean Square	F Value	Pr > F
mix	2	0.00010884	0.00005442	0.38	0.6881
needle	1	0.00026324	0.00026324	1.82	0.1829
mix*needle	2	0.00130397	0.00065198	4.51	0.0154

Table 4-7: Separate models – discrimination power of the model (mix – type of liquid; needle – needle size)

In ANOVA analysis, 3 factors were considered – mix, needle, and interaction between mix and needle. That model was statistically significant which can be seen from p value (p=0,0558). This value shows is there a statistical significance of the model. Only factor concluded as statistically significant was interaction between needle size and type of liquid because of its p-value (p=0,0154).

Noticeable differentiation in mean value can be seen for WGP. This difference can be seen even better in Figure 4-4.

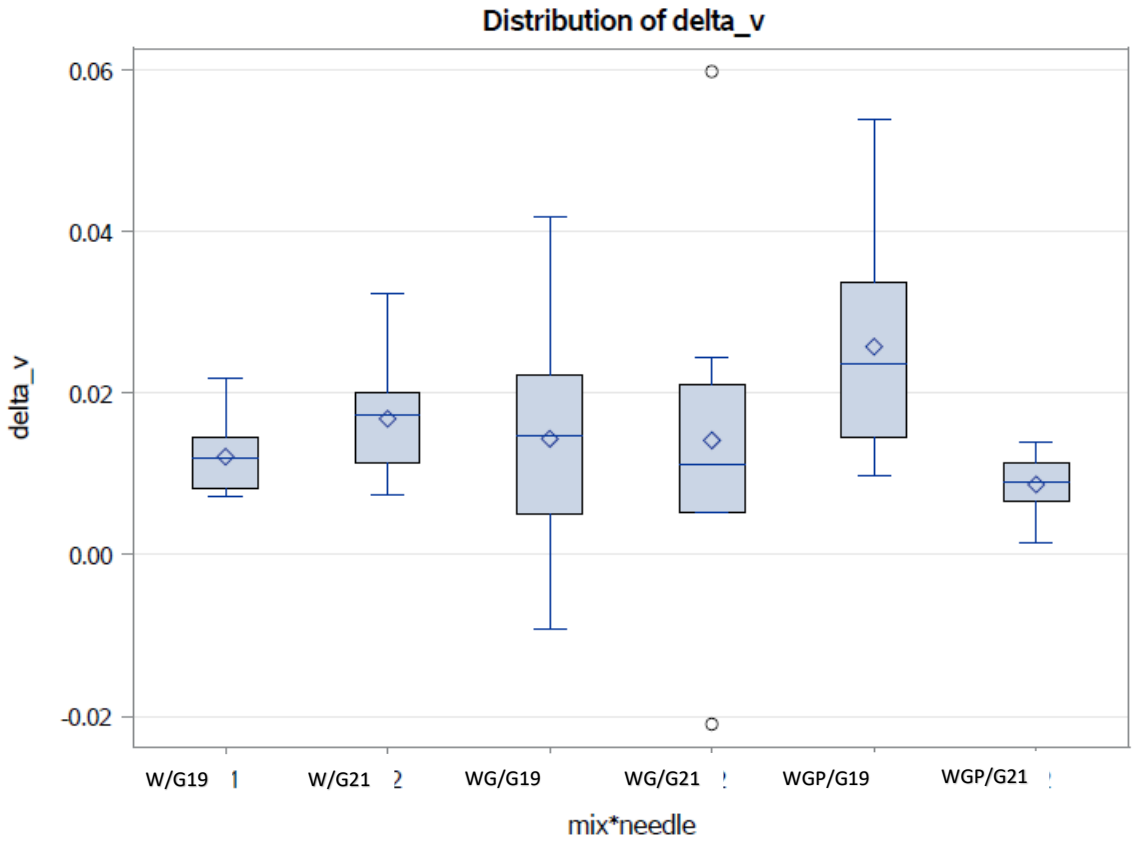


Figure 4-4: Boxplot for ΔV according to liquid type and needle size simultaneously

Here, a noticeable differentiation in mean value can be seen for WGP. As seen in the boxplot, interquartile range boxes for liquid 3 have a distinguished differentiation in size and position of the values in the range.

To further investigate that difference for WGP, and to see if that difference is statistically significant, a t-test was conducted. Named test can show if there is a statistical significance between the mean values.

The result of t-test was $p=0.0009$, which is considered as extremely significant difference in mean values ($p<0.001$), shows that different needles in that type of liquid influence the general accuracy of the system.

4.2 Expelling force measurements

4.2.1 Expelling force graphical output (force diagram)

4.2.1.1 Water

mixture	needle size	volume [ml]	speed [cm/s]	density [kg/m^3]	volume_remaining [ml]	error [%]	volume_syringe [mL]	error [%]
water	19G	1,2	0,1	0,9820	0,364053	21,3510	0,825356	-9,0438
	19G	1,2	0,2	0,9838	0,327014	9,0047	0,854485	-5,3266
	19G	1,2	0,3	0,9833	0,323316	7,7718	0,844750	-6,5405
	19G	1,2	0,4	0,9859	0,320311	6,7703	0,862953	-4,2930
	19G	1,2	0,5	0,9888	0,325259	8,4197	0,852592	-5,5605
	19G	1,2	1	0,9946	0,522028	74,0092	0,654244	-37,5634
	21G	1,2	0,1	0,9905	0,345381	15,1270	0,827562	-8,7532
	21G	1,2	0,2	0,9831	0,309536	3,1788	0,867373	-3,7616
	21G	1,2	0,3	0,9857	0,382178	27,3926	0,799662	-12,5476
	21G	1,2	0,4	0,9812	0,316868	5,6226	0,876304	-2,7041
	21G	1,2	0,5	0,9805	0,302397	0,7989	0,873942	-2,9817
	21G	1,2	1	0,9768	0,328641	9,5470	0,855490	-5,2028

Table 4-8: Values gathered from measurements with W (constant vial volume, 6 different speeds of measurement, 2 needle sizes)

From these measurements it can be noticed that the error, compared to manual measurements, is significantly higher. That can be attributed to high amount of air that was pulled into the syringe. As already mentioned, machine does not have the ability to mitigate the effects of air entrapment, but with human factor removed from the procedure, effects of speed can be investigated.

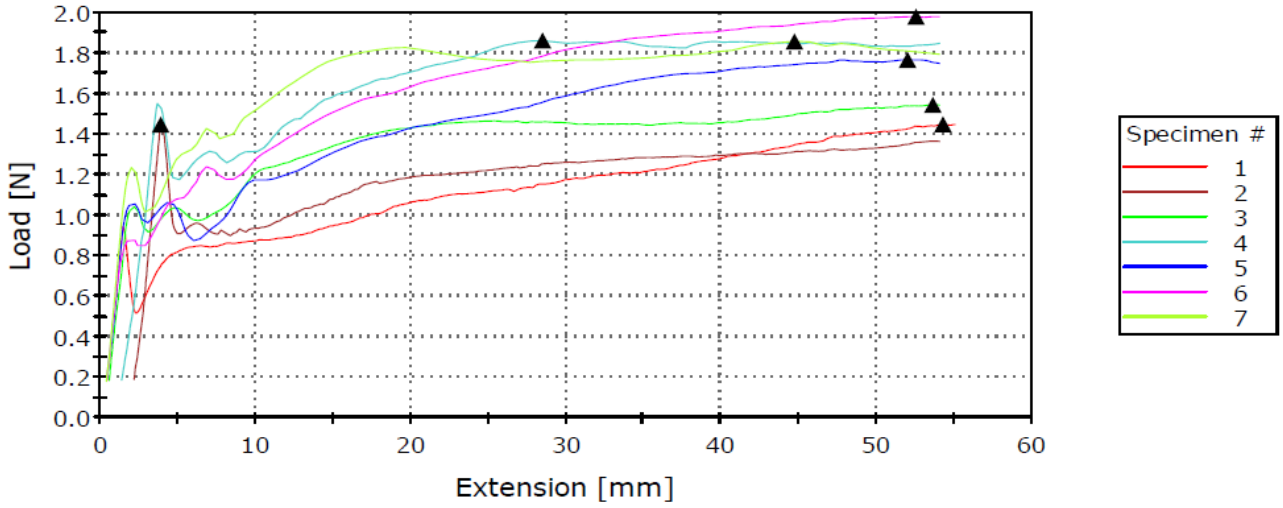


Figure 4-5: Distribution of force needed to pull the plunger and withdraw liquid (W, G19, η_{avg}=1,025 cP)

Speed [cm/s]		Maximum Load [N]
0,1	1	1.44909
0,2	2	1.44845
0,3	3	1.54532
0,4	4	1.86226
	X 5	1.76707
0,5	6	1.97908
1,0	7	1.85758

Table 4-9: Maximum load needed to withdraw liquid with a syringe (W, G19)

In Figure 4-5, it is noticed that during withdrawal of liquid in a syringe, force increases rapidly until one point, after which it decreases and continues to gradually rise again. This is due to the force that needs to be exerted to pull the plunger called break-loose force, after which comes a force called gliding force. Break-loose force and all other forces will be explained in detail in the next chapter.

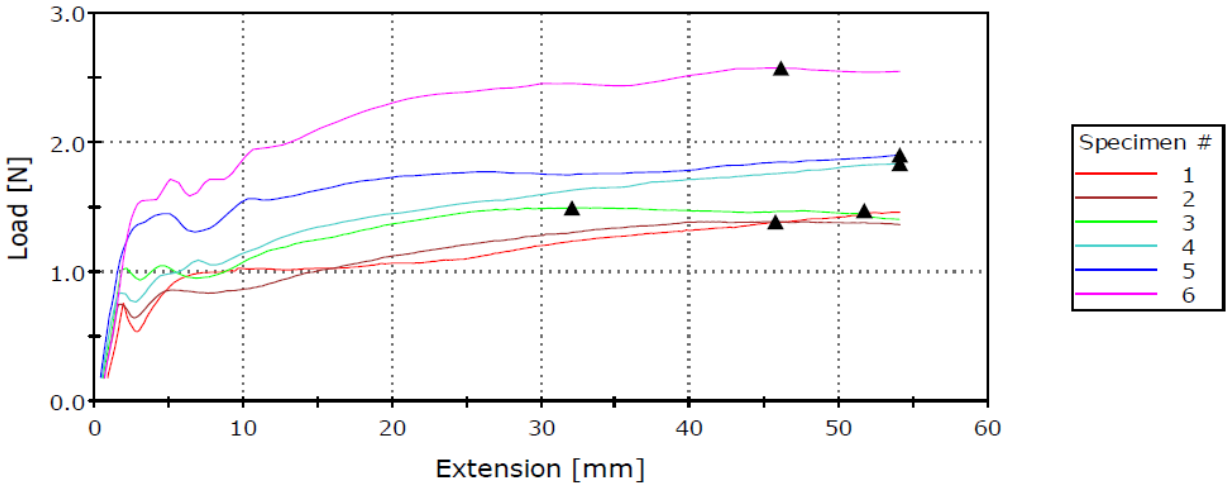


Figure 4-6: Distribution of force needed to pull the plunger and withdraw liquid (W, G21, η_{avg}=1,025 cP)

Speed [cm/s]		Maximum Load [N]
0,1	1	1.47828
0,2	2	1.39307
0,3	3	1.49930
0,4	4	1.83933
0,5	5	1.90658
1,0	6	2.57562

Table 4-10: Maximum load needed to withdraw liquid with a syringe (W, G21)

It is noticeable that the load (force) increases gradually with the increase of speed. Also, range of forces differentiates from the one with a bigger needle (19G). That difference becomes more obvious with the increase of speed, as well as in the maximum value (1,98N compared to 2,58N).

Next, liquid was changed. Same procedure, just with distilled water and glycerol mixture, which has a viscosity roughly 10 times higher than distilled water. Again, 12 measurements, 6 with each needle.

4.2.1.2 Water/glycerol

mixture	needle size	volume [ml]	speed [cm/s]	density [kg/m^3]	volume_remaining [ml]	error [%]	volume_syringe [mL]	error [%]
water+glycerol	19G	1,2	0,1	1,1180	0,302236	0,7454	0,860733	-4,5620
	19G	1,2	0,2	1,1053	0,298575	-0,4750	0,860620	-4,5758
	19G	1,2	0,3	1,1403	0,315259	5,0862	0,848699	-6,0446
	19G	1,2	0,4	1,1514	0,342535	14,1782	0,821510	-9,5544
	19G	1,2	0,5	1,1456	0,327344	9,1147	0,820193	-9,7302
	19G	1,2	1	1,1638	0,326164	8,7212	0,839210	-7,2438
	21G	1,2	0,1	1,1262	0,323132	7,7105	0,832648	-8,0889
	21G	1,2	0,2	1,2319	0,381438	27,1460	0,777731	-15,7212
	21G	1,2	0,3	1,1269	0,301265	0,4215	0,850640	-5,8027
	21G	1,2	0,4	1,1427	0,319341	6,4469	0,837427	-7,4720
	21G	1,2	0,5	1,1594	0,320247	6,7491	0,836110	-7,6413
	21G	1,2	1	1,1528	0,395460	31,8202	0,760040	-18,4147

Table 4-11: Values gathered from measurements with WG (constant vial volume, 6 different speeds of measurement, 2 needle sizes)

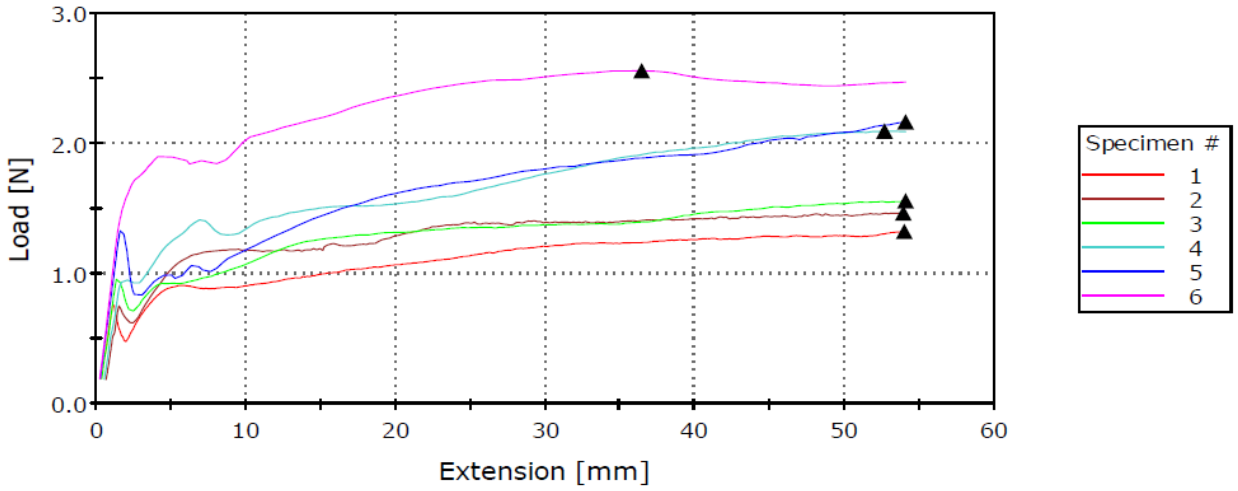


Figure 4-7: Distribution of force needed to pull the plunger and withdraw liquid (WG, G19, η_{avg}=10,064667 cP)

The forces observed for the WG had similar trajectories, as when speed is increased, forces are increased as well. But in Table 4-12, additional increase in maximum load can be noticed. This can be attributed to an increase in liquid viscosity, which directly impacts (and increases) force needed to pull the plunger. When compared to the values gathered for water with the same needle, an increase of 0,57907 N is visible.

Speed [cm/s]		Maximum Load [N]
0,1	1	1.32864
0,2	2	1.46838
0,3	3	1.56160
0,4	4	2.09747
0,5	5	2.16737
1,0	6	2.55815

Table 4-12: Maximum load needed to withdraw liquid with a syringe (WG, G19)

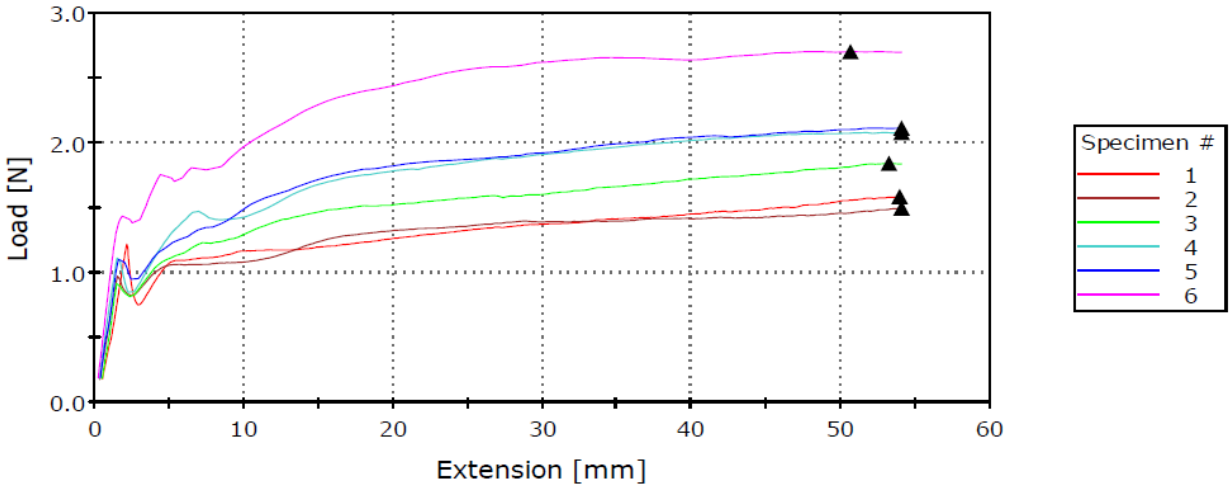


Figure 4-8: Distribution of force needed to pull the plunger and withdraw liquid (WG, G21, η_{avg}=10,064667 cP)

Speed [cm/s]		Maximum Load [N]
0,1	1	1.58891
0,2	2	1.50222
0,3	3	1.84390
0,4	4	2.08200
0,5	5	2.11625
1,0	6	2.70311

Table 4-13: Maximum load needed to withdraw liquid with a syringe (WG, G21)

The data confirms that with the growth of the speed force needed for liquid withdrawal is increased.

4.2.1.3 Water/glycerol/polysorbate 80

mixture	needle size	volume [ml]	speed [cm/s]	density [kg/m^3]	volume_remaining [ml]	error [%]	volume_syringe [mL]	error [%]
water+glycerol+polysorbate 80	19G	1,2	0,1	1,1292	0,301107	0,3690	0,827513	-8,7596
	19G	1,2	0,2	1,1340	0,316049	5,3498	0,839153	-7,2509
	19G	1,2	0,3	1,1379	0,337986	12,6620	0,803574	-11,9997
	19G	1,2	0,4	1,1383	0,309927	3,3089	0,837628	-7,4463
	19G	1,2	0,5	1,1167	0,305642	1,8806	0,826119	-8,9431
	19G	1,2	1	1,1250	0,337511	12,5037	0,797600	-12,8385
	21G	1,2	0,1	1,1394	0,330169	10,0563	0,825773	-8,9887
	21G	1,2	0,2	1,1489	0,335011	11,6704	0,827127	-8,8104
	21G	1,2	0,3	1,1426	0,319714	6,5714	0,838451	-7,3408
	21G	1,2	0,4	1,1498	0,324395	8,1316	0,834556	-7,8418
	21G	1,2	0,5	1,1433	0,318391	6,1302	0,841373	-6,9680
	21G	1,2	1	1,1283	0,339882	13,2939	0,815539	-10,3564

Table 4-14: Values gathered from measurements with WGP (constant vial volume, 6 different speeds of measurement, 2 needle sizes)

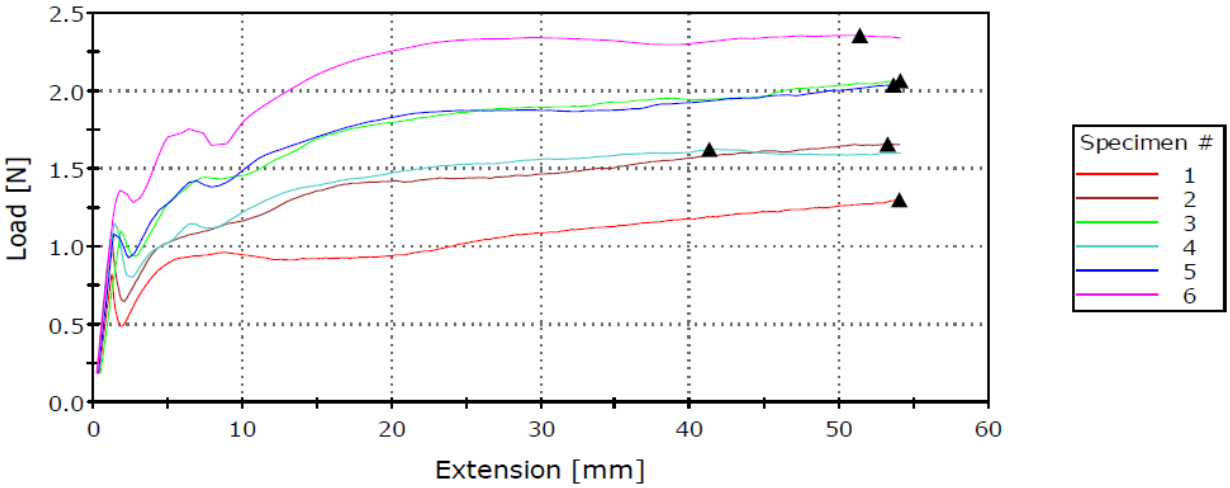


Figure 4-9: Distribution of force needed to pull the plunger and withdraw liquid (WGP, G19, η_{avg}=10,453333 cP)

Speed [cm/s]		Maximum Load [N]
0,1	1	1.30354
0,2	2	1.66015
0,3	3	2.06689
0,4	4	1.62576
0,5	5	2.03885
1,0	6	2.35542

Table 4-15: Maximum load needed to withdraw liquid with a syringe (WGP, G19)

With the growth of density and withdrawal speed, fine distinction between different forces exerted on a syringe is more and more lost, which can be seen in Figure 4-10.

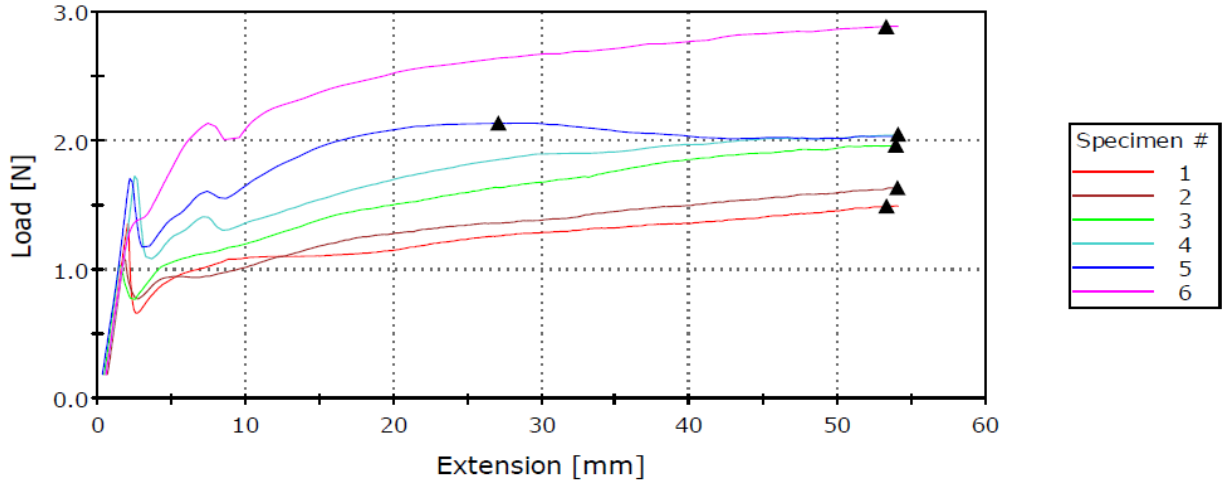


Figure 4-10: Distribution of force needed to pull the plunger and withdraw liquid (WGP, G21, η_{avg}=10,453333 cP)

Speed [cm/s]		Maximum Load [N]
0,1	1	1.49968
0,2	2	1.63888
0,3	3	1.96870
0,4	4	2.05352
0,5	5	2.13968
1,0	6	2.88643

Table 4-16: Maximum load needed to withdraw liquid with a syringe (WGP, G21)

4.2.2 Results – statistical analysis

A more detailed statistical analysis was conducted. First step was to check if any of the variables mentioned and measured influences the outcome (difference in volume or error), and is that connection statistically significant.

A database with values for ΔV was created as an input for the analysis (Table 4-17). Data that is included in this database was collected from initial measurements (36 observations).

In this case, instead of categorizing liquids as 1 (W), 2 (WG) and 3 (WGP), measured value of their viscosities was used as an input for the analysis. This was done to facilitate further model discussion with numerical values, not categories.

Also, an exact needle diameter was used in the database to gain a more precise model.

A database was created (shown in Table 4-17). Variables in the database are as following:

- Obs – observation – unique data location in the database
- Mix – type of liquid:
    - 1 – water
    - 2 – water + glycerol
    - 3 – water + glycerol + polysorbate 80
- Speed – ranging from 0,1 cm/sec to 1 cm/sec
- Needle – needle size:
    - 0,686 mm – G19
    - 0,514 mm – G21
- Viscosity – exact value the viscosity if each liquid
- ΔV – difference between the volume expelled from a syringe and volume needed (expected) – 0.9 mL

Obs	mix	speed	needle	viscosity	ΔV	Obs	mix	speed	needle	viscosity	ΔV
1	1	0,1	0,686	1,025	-0,07464358	19	2	0,1	0,514	10,548	-0,06735238
2	1	0,2	0,686	1,025	-0,04551461	20	2	0,2	0,514	10,548	-0,12226882
3	1	0,3	0,686	1,025	-0,05525044	21	2	0,3	0,514	10,548	-0,04936035
4	1	0,4	0,686	1,025	-0,03704674	22	2	0,4	0,514	10,548	-0,06257293
5	1	0,5	0,686	1,025	-0,04740834	23	2	0,5	0,514	10,548	-0,06388989
6	1	1	0,686	1,025	-0,24575618	24	2	1	0,514	10,548	-0,13995952
7	1	0,1	0,514	1,025	-0,07243816	25	3	0,1	0,686	10,474	-0,07248708
8	1	0,2	0,514	1,025	-0,03262694	26	3	0,2	0,686	10,474	-0,06084656
9	1	0,3	0,514	1,025	-0,10033818	27	3	0,3	0,686	10,474	-0,09642622
10	1	0,4	0,514	1,025	-0,02369628	28	3	0,4	0,686	10,474	-0,06237189
11	1	0,5	0,514	1,025	-0,02605813	29	3	0,5	0,686	10,474	-0,07388060
12	1	1	0,514	1,025	-0,04450985	30	3	1	0,686	10,474	-0,10240000
13	2	0,1	0,686	10,548	-0,03926655	31	3	0,1	0,514	10,474	-0,07422658
14	2	0,2	0,686	10,548	-0,03938023	32	3	0,2	0,514	10,474	-0,07287300
15	2	0,3	0,686	10,548	-0,05130079	33	3	0,3	0,514	10,474	-0,06154912
16	2	0,4	0,686	10,548	-0,07849027	34	3	0,4	0,514	10,474	-0,06544427
17	2	0,5	0,686	10,548	-0,07980650	35	3	0,5	0,514	10,474	-0,05862672
18	2	1	0,686	10,548	-0,06079049	36	3	1	0,514	10,474	-0,08446086

Table 4-17: Database used for this statistical analysis (mix: 1-W; 2-WG; 3-WGP)

After running these values through the named statistical analysis software output was obtained. Following are most important data collected from this procedure.

In this part of the statistical analysis linear regression method was used to get the most appropriate model describing our dependent variable (ΔV). Independent variables used are speed (cm/sec), needle diameter (mm) and viscosity (cP). Furthermore, the procedure that was used is STEPWISE procedure. This procedure selects the variables according to their impact to the significance of the model. This is done by adding and removing variables step-by-step and monitoring their impact on the model in each step. The results are shown in the following table (Table 4-18).

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	1	0.00871	0.00871	6.64	0.0145
Error	34	0.04465	0.00131		
Corrected Total	35	0.05336			

Table 4-18: Linear regression results for a general model containing p-values

Variable	Parameter Estimate	Standard Error	Type II SS	F Value	Pr > F
Intercept	-0.04843	0.01055	0.02769	21.09	<.0001
speed	-0.05345	0.02075	0.00871	6.64	0.0145

Table 4-19: Linear regression results containing p-values

The intercept is an expected value of the dependent variable when all independent variables are equal to 0. Model created by stepwise procedure, according to its p-value (0,0145), is statistically significant. It contains variables intercept and speed as variables that have the most impact on the model itself, which can be seen from the further output.

Additionally, a linear regression with all independent variables was conducted to show each of variable significances for the model (p-values).

Parameter Estimates					
Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-0.02473	0.04566	-0.54	0.5919
needle	1	-0.03256	0.07197	-0.45	0.6540
visc	1	-0.00056701	0.00138	-0.41	0.6848
speed	1	-0.05345	0.02126	-2.51	0.0172

Table 4-20: p-values of all independent variables

From this table, high p-values of needle and viscosity can be seen (0,6540 and 0,6848), which shows that these independent variables have very low impact on clarification of the variability of the dependent variable (ΔV).

By analysing the influence of independent variables on the dependent variable, based on this database, a model which is chosen as the best one is presented by this formulation.

$$\Delta V = intercept + parameter\ estimate(speed) \cdot speed$$

$$\Delta V = -0{,}02473 - 0{,}05345 \cdot speed\ (\frac{cm}{\sec})$$

This formula is graphically shown in the following diagram (FIT PLOT), on the next page.

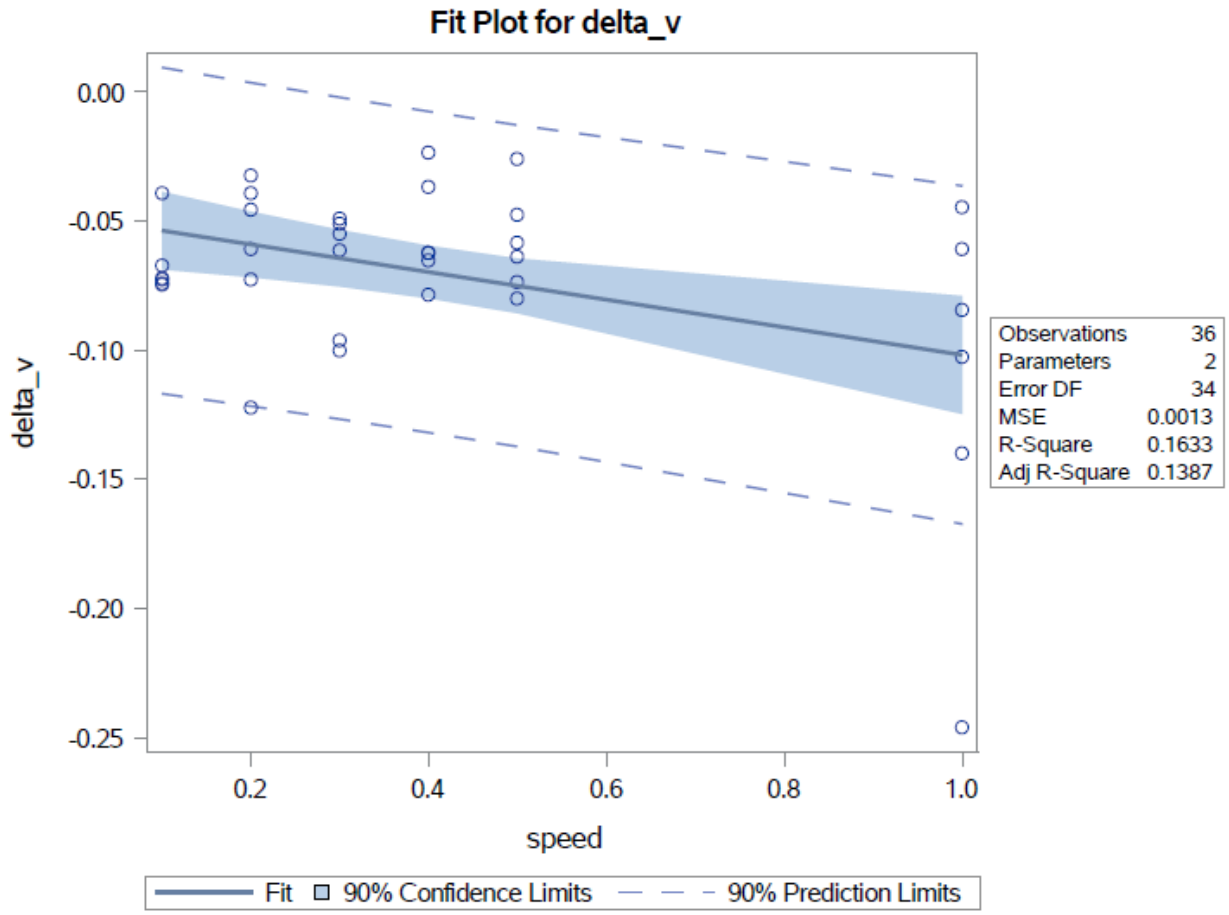


Figure 4-11. Fit plot diagram

This diagram shows that a rise in speed causes rise in dosing accuracy error (based on the available measurements). The result of the regression analysis is a line chosen by the software using LMS (Least Mean Squares) method. In addition, it can be seen that all measurements fulfil the 90% confidence limit.

5 DISCUSSION

The delivery of an accurate dose of an active pharmaceutical ingredient is of major importance for the efficacy and safety of drug therapy. Biologic drugs like monoclonal antibodies and large proteins have to be delivered parenterally due to their molecular size and nature, which is not suitable for oral administration. As biologic compounds are increasingly developed, more emphasize is given to their delivery form and device component to ensure usability as intended. Vials are often used as a primary packaging for lyophilized or dissolved biologics. For administration, the liquid solution is expelled from the vial using a needle-syringe device system, whereby the exact dose is visually determined by the grading of the syringe. The literature research revealed the impact of human factors and the device components as a major source for inaccurate dosing. As we hypothesize that formulation factors like viscoelastic properties and the mode of expelling like needle size, force and time might also contribute to the achievable dose accuracy, we performed this study. This laboratory study was based on one typical vial-syringe device system and preparation procedure investigation three formulations with different physical properties and two different needle sizes as variables.

The viscoelastic properties of the formulation affected the extent of variability of the expelling volume. Water (W) with the lowest viscosity had the lowest, while WG had the highest variability. Since the viscosity of WG and WGP are similar, the addition of the surfactant (polysorbate 80) suggest the increase in the dosing accuracy (Figure 4-1). The effect might be explained by a lower surface tension of the WGP formulation compared to WG and a less steep concave meniscus in the neck of the vial on the liquid surface assuring that the needle tip remains in the liquid phase until the end of the expelling.

The needle size show to have an impact on the variability, whereby the average statistical variability was highest for the larger needle size, but the minimum and maximum outlier range was higher for the smaller needle size based on pooled data of all three formulations (Figure 4-2). When the variability of the needle size was analyzed for each formulation (W, WG and WGP), the variability of the larger needle size (G19) seem to correlate with the higher viscose formulation WG and WGP (Figure 4-4).

As expected, the expelling forces depend on the viscosity, needle size and expelling speed. No difference was observed between WG and WGP suggesting that the addition of the surfactant does not influence the expelling forces.

Statistical analysis using a linear regression model provided evidence that expelling speed had a major impact on the dosing accuracy (Figure 4-11). The faster the liquid is expelled from the vial, the higher the variability. This observation might be explained by different theories.

1. The faster the formulation is expelled and depending on the viscosity, air bubbles might be formed and remain inside the syringe.
2. The increasing expelling force with speed suggests a limit or maximum flow of liquid transfer through the needle requiring a longer holding time at the end of the expelling to complete the dosing.
3. The faster expelling speed might also form a steeper concave meniscus on the liquid surface with the potential risk that the bevel of the needle tip expels air towards the end of the dosing (which remains undetected insight the needle
4. The lower expelling force for the larger needle size might lead to faster expelling reducing the expelling time and/or expelling air from the surface, which would explain the higher variability observed with larger needle for WG and WGP.

5. A lower expelling speed might be more forgiving, with regard to the needle tip position after piercing the stopper in the vial upside-down
6. The expelling time might be faster than the flow rate of the formulation from the vial surfaces into the bottom of the vial might increase the expelling of air towards the end of dosing

The results of this laboratory study confirm the hypothesis that formulation and device system factors influence dosing accuracy from vials. By using a typical vial and needle-syringe device system as well as formulation properties as the only variables under constant and optimal conditions, the study provides relevant information on additional factors contributing to dosing accuracy and risk mitigation in drug product development.

The results of the study are based on a small, yet statistically significant set of experimental data, a single operator and the limited number of formulation and device system variables. Moreover, the study was performed under ideal laboratory conditions to exclude human factors and real-world conditions. Therefore, additional studies will be required to better understand the impact of formulation and device system factors on dosing accuracy from vials as well as how these affect the dosing accuracy in real world clinical practice.

6 CONCLUSION

The accuracy of dosing of pharmaceutical formulation from vials is an emerging area of interest to assure the best benefit to risk profile of a drug product. The measurement and delivery of a parenterally administered from vials is a manual process, involving human factors as well as product factors. Since previous research has focused on the importance of human factors in dosing accuracy, a study was performed to investigate the product related factors. Using three different formulations and two device systems as product variable, the study provided evidence that these factors influence the achievable dose accuracy. Even though the study was limited in terms of potential formulation and device system variables and require additional studies, the results contribute to the considerations on risk mitigation of parenteral product requiring a high dose precision.

Future studies may include additional variables:

- Different vial sizes
- Different fill volume
- Different formulations
- Additional formulation viscosities
- Different expelling volume
- Additional needle sizes
- Different syringe sizes
- Different formulation temperatures

Even though the study was limited in terms of potential formulation and device system variables and requires additional studies, the results contribute to the considerations on risk mitigation of parenteral product requiring a high dose precision.

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APPENDIX A

mixture	needle size	volume [ml]	density [kg/m^3]	volume_remaining [ml]	error [%]	volume_syringe [mL]	error [%]
water	19G	1,2	990,7500	0,181681	-39,4398	0,909311	1,0346
	19G	1,2	990,3333	0,177011	-40,9963	0,914036	1,5595
	19G	1,2	988,2500	0,177283	-40,9056	0,913534	1,5038
	19G	1,2	989,5000	0,177868	-40,7108	0,914502	1,6114
	19G	1,2	991,3333	0,182381	-39,2065	0,908272	0,9191
	19G	1,2	989,5833	0,178257	-40,5811	0,910282	1,1425
	19G	1,2	992,4167	0,187119	-37,6270	0,907179	0,7977
	19G	1,2	989,5833	0,173406	-42,1979	0,915335	1,7039
	19G	1,2	989,3333	0,180425	-39,8585	0,907177	0,7974
	19G	1,2	987,2500	0,175234	-41,5886	0,921854	2,4282
average			989,8333	0,179066	-40,3112	0,9121	1,3498
water	21G	1,2	986,1667	0,174818	-41,7272	0,920128	2,2365
	21G	1,2	991,5833	0,188688	-37,1040	0,907337	0,8152
	21G	1,2	988,5000	0,187051	-37,6496	0,912393	1,3769
	21G	1,2	990,8333	0,192061	-35,9798	0,911354	1,2616
	21G	1,2	985,0833	0,185264	-38,2455	0,917689	1,9654
	21G	1,2	987,5833	0,191275	-36,2417	0,909797	1,0885
	21G	1,2	987,5833	0,194718	-35,0941	0,916986	1,8873
	21G	1,2	986,6667	0,179899	-40,0338	0,922196	2,4662
	21G	1,2	983,8333	0,167305	-44,2317	0,932373	3,5970
	21G	1,2	980,7500	0,180168	-39,9439	0,918175	2,0194
average			988,4167	0,181475	-38,6251	0,9144	1,8714
water+glycerol	19G	1,2	1136,6667	0,184311	-38,5630	0,908886	0,9873
	19G	1,2	1131,0000	0,167374	-44,2087	0,922281	2,4757
	19G	1,2	1122,7500	0,162280	-45,9066	0,923180	2,5755
	19G	1,2	1142,0000	0,179247	-40,2510	0,941769	4,6410
	19G	1,2	1140,0833	0,214283	-28,5725	0,902127	0,2363
	19G	1,2	1120,6667	0,175699	-41,4337	0,919810	2,2011
	19G	1,2	1143,7500	0,186754	-37,7486	0,914710	1,6345
	19G	1,2	1155,4167	0,224940	-25,0198	0,890847	-1,0169
	19G	1,2	1122,7500	0,192207	-35,9311	0,905099	0,5666
	19G	1,2	1139,6667	0,172156	-42,6148	0,914829	1,6477
average			1135,4750	0,185925	-38,0250	0,9144	1,5949
water+glycerol	21G	1,2	1147,8333	0,193582	-35,4726	0,911631	1,2923
	21G	1,2	1137,3333	0,176729	-41,0903	0,924443	2,7159
	21G	1,2	1145,3333	0,196100	-34,6333	0,905326	0,5918
	21G	1,2	1146,0833	0,190737	-36,4211	0,878994	-2,3340
	21G	1,2	1092,0000	0,140934	-53,0220	0,959890	6,6545
	21G	1,2	1132,1667	0,217989	-27,3370	0,910820	1,2022
	21G	1,2	1131,8333	0,182094	-39,3020	0,921072	2,3413
	21G	1,2	1131,0833	0,179297	-40,2343	0,917704	1,9671
	21G	1,2	1146,5000	0,189097	-36,9676	0,906498	0,7220
	21G	1,2	1141,9167	0,197300	-34,2334	0,905232	0,5814
average			1135,3480	0,186145	-37,8714	0,9143	1,5734
water+glycerol+polysorbate 80	19G	1,2	1106,6667	0,155512	-48,1627	0,927651	3,0723
	19G	1,2	1133,1667	0,194940	-35,0199	0,914517	1,6130
	19G	1,2	1087,0000	0,136155	-54,6151	0,953818	5,9798
	19G	1,2	1122,5000	0,167127	-44,2910	0,922138	2,4598
	19G	1,2	1103,3333	0,165861	-44,7130	0,936888	4,0987
	19G	1,2	1108,9167	0,151950	-49,3500	0,933794	3,7549
	19G	1,2	1122,0000	0,163012	-45,6625	0,925223	2,8025
	19G	1,2	1130,5833	0,169205	-43,5984	0,920410	2,2678
	19G	1,2	1129,5000	0,185215	-38,2618	0,913413	1,4903
	19G	1,2	1135,2500	0,179608	-40,1307	0,909756	1,0840
average			1117,8917	0,166858	-44,3805	0,9258	2,8623
water+glycerol+polysorbate 80	21G	1,2	1135,5000	0,197534	-34,1553	0,910964	1,2183
	21G	1,2	1135,0833	0,191352	-36,2161	0,907422	0,8247
	21G	1,2	1122,1667	0,187227	-37,5910	0,911718	1,3020
	21G	1,2	1132,8333	0,191908	-36,0306	0,909225	1,0250
	21G	1,2	1142,9167	0,211651	-29,4495	0,905578	0,6198
	21G	1,2	1138,3333	0,191245	-36,2518	0,911420	1,2689
	21G	1,2	1140,1667	0,197164	-34,2786	0,913989	1,5544
	21G	1,2	1140,0833	0,206564	-31,1454	0,906600	0,7334
	21G	1,2	1146,0833	0,192394	-35,8685	0,901418	0,1575
	21G	1,2	1133,7500	0,197839	-34,0537	0,908578	0,9531
average			1126,8440	0,180968	-34,5041	0,9176	0,9657

APPENDIX B

mixture	needle size	volume [ml]	speed [cm/s]	density [kg/m^3]	volume_remaining [ml]	error [%]	volume_syringe [mL]	error [%]
water	19G	1,2	0,1	0,9820	0,364053	21,3510	0,825356	-9,0438
	19G	1,2	0,2	0,9838	0,327014	9,0047	0,854485	-5,3266
	19G	1,2	0,3	0,9833	0,323316	7,7718	0,844750	-6,5405
	19G	1,2	0,4	0,9859	0,320311	6,7703	0,862953	-4,2930
	19G	1,2	0,5	0,9888	0,325259	8,4197	0,852592	-5,5605
	19G	1,2	1	0,9946	0,522028	74,0092	0,654244	-37,5634
	21G	1,2	0,1	0,9905	0,345381	15,1270	0,827562	-8,7532
	21G	1,2	0,2	0,9831	0,309536	3,1788	0,867373	-3,7616
	21G	1,2	0,3	0,9857	0,382178	27,3926	0,799662	-12,5476
	21G	1,2	0,4	0,9812	0,316868	5,6226	0,916256	1,7742
	21G	1,2	0,5	0,9805	0,302397	0,7989	0,873942	-2,9817
	21G	1,2	1	0,9768	0,328641	9,5470	0,855490	-5,2028
water+glycerol	19G	1,2	0,1	1,1180	0,302236	0,7454	0,860733	-4,5620
	19G	1,2	0,2	1,1053	0,298575	-0,4750	0,860620	-4,5758
	19G	1,2	0,3	1,1403	0,315259	5,0862	0,848699	-6,0446
	19G	1,2	0,4	1,1514	0,342535	14,1782	0,821510	-9,5544
	19G	1,2	0,5	1,1456	0,327344	9,1147	0,820193	-9,7302
	19G	1,2	1	1,1638	0,326164	8,7212	0,839210	-7,2438
	21G	1,2	0,1	1,1262	0,323132	7,7105	0,832648	-8,0889
	21G	1,2	0,2	1,2319	0,381438	27,1460	0,777731	-15,7212
	21G	1,2	0,3	1,1269	0,301265	0,4215	0,850640	-5,8027
	21G	1,2	0,4	1,1427	0,319341	6,4469	0,837427	-7,4720
	21G	1,2	0,5	1,1594	0,320247	6,7491	0,836110	-7,6413
	21G	1,2	1	1,1528	0,395460	31,8202	0,760040	-18,4147
water+glycerol+polysorbate 80	19G	1,2	0,1	1,1292	0,301107	0,3690	0,827513	-8,7596
	19G	1,2	0,2	1,1340	0,316049	5,3498	0,839153	-7,2509
	19G	1,2	0,3	1,1379	0,337986	12,6620	0,803574	-11,9997
	19G	1,2	0,4	1,1383	0,309927	3,3089	0,837628	-7,4463
	19G	1,2	0,5	1,1167	0,305642	1,8806	0,826119	-8,9431
	19G	1,2	1	1,1250	0,337511	12,5037	0,797600	-12,8385
	21G	1,2	0,1	1,1394	0,330169	10,0563	0,825773	-8,9887
	21G	1,2	0,2	1,1489	0,335011	11,6704	0,827127	-8,8104
	21G	1,2	0,3	1,1426	0,319714	6,5714	0,838451	-7,3408
	21G	1,2	0,4	1,1498	0,324395	8,1316	0,834556	-7,8418
	21G	1,2	0,5	1,1433	0,318391	6,1302	0,841373	-6,9680
	21G	1,2	1	1,1283	0,339882	13,2939	0,815539	-10,3564

APPENDIX C

Code used in computer software (SAS®OnDemand) for statistical analysis including the actual database used for the analysis.

MANUAL MEASURMENTS

```
data manual_measurments;
input mix needle delta_v;
cards;
1 1 0.009311128
1 1 0.014035678
1 1 0.013534025
1 1 0.014502274
1 1 0.008271688
1 1 0.010282105
1 1 0.007179444
1 1 0.015334737
1 1 0.00717655
1 1 0.021853634
1 2 0.020128443
1 2 0.007336751
1 2 0.012392514
1 2 0.011354079
1 2 0.017688859
1 2 0.009796642
1 2 0.016985908
1 2 0.022195946
1 2 0.032373369
1 2 0.018174866
2 1 0.00888563
2 1 0.022281167
2 1 0.023179693
2 1 0.041768827
2 1 0.002127037
2 1 0.019809637
2 1 0.014710383
2 1 -0.009152542
2 1 0.005099087
2 1 0.014828897
2 2 0.011630608
2 2 0.024443142
2 2 0.00532596
2 2 -0.021006326
2 2 0.05989011
2 2 0.010819962
2 2 0.021072007
2 2 0.017704266
2 2 0.006498038
2 2 0.005232431
3 1 0.027650602
```

```
3 1 0.014516841
3 1 0.053817847
3 1 0.022138085
3 1 0.036888218
3 1 0.033794244
3 1 0.025222816
3 1 0.020409818
3 1 0.013413015
3 1 0.00975556
3 2 0.010964333
3 2 0.007422363
3 2 0.011718402
3 2 0.009224658
3 2 0.005577834
3 2 0.011420205
3 2 0.013989183
3 2 0.006600395
3 2 0.001417872
3 2 0.008577729
;
proc print;

proc univariate data= manual_measurments;
var delta_v;
class mix needle;
run;

proc univariate data= manual_measurments;
var delta_v;
class mix;
run;

proc univariate data= manual_measurments;
var delta_v;
class needle;
run;

proc anova data= manual_measurments;
class mix needle;
model delta_v=mix needle mix*needle;
means mix needle mix*needle/alpha=0.1;
means mix needle mix*needle/tukey alpha=0.1;
run;
```

MACHINE MEASURMENTS

```
data machine_measurments;
input mix speed needle delta_v visc;
cards;
1 0.1 1 -0.094643585 1.025
1 0.2 1 -0.045514612 1.025
```

```
1 0.3 1 -0.055250445 1.025
1 0.4 1 -0.047046742 1.025
1 0.5 1 -0.057408344 1.025
1 1 1 -0.265756179 1.025
1 0.1 2 -0.072438163 1.025
1 0.2 2 -0.032626939 1.025
1 0.3 2 -0.110338181 1.025
1 0.4 2 0.016256158 1.025
1 0.5 2 -0.036058134 1.025
1 1 2 -0.044509854 1.025
2 0.1 1 -0.039380231 10.548
2 0.2 1 -0.039380231 10.548
2 0.3 1 -0.061300789 10.548
2 0.4 1 -0.078490266 10.548
2 0.5 1 -0.089806503 10.548
2 1 1 -0.070790491 10.548
2 0.1 2 -0.067352375 10.548
2 0.2 2 -0.132268822 10.548
2 0.3 2 -0.049360349 10.548
2 0.4 2 -0.072572929 10.548
2 0.5 2 -0.073889887 10.548
2 1 2 -0.15995952 10.548
3 0.1 1 -0.082487085 10.474
3 0.2 1 -0.070846561 10.474
3 0.3 1 -0.096426218 10.474
3 0.4 1 -0.082371889 10.474
3 0.5 1 -0.083880597 10.474
3 1 1 -0.1224 10.474
3 0.1 2 -0.074226578 10.474
3 0.2 2 -0.072872996 10.474
3 0.3 2 -0.071549121 10.474
3 0.4 2 -0.065444267 10.474
3 0.5 2 -0.078626722 10.474
3 1 2 -0.084460857 10.474
1 0.2 1 -0.060481988 1.025
1 0.2 1 -0.06135026 1.025
1 0.2 1 -0.043555021 1.025
1 0.2 1 -0.112541851 1.025
1 0.2 2 -0.051745938 1.025
1 0.2 2 -0.039639529 1.025
1 0.2 2 -0.032045598 1.025
1 0.2 2 -0.106750534 1.025
2 0.2 1 -0.074469558 10.548
2 0.2 2 -0.130395557 10.548
3 0.2 1 -0.093525557 10.474
3 0.2 2 -0.080626727 10.474
;
proc print;
proc reg data= machine_measurments;
model delta_v=needle visc speed /selection=STEPWISE;
run;
```

```
proc reg data= machine_measurments;
model delta_v=needle visc speed ;
run;
```