Low Volume Aseptic Filling of Monoclonal Antibodies

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ZUSAMMENFASSUNG

Die grundlegende Idee von zielgerichteten Medikamenten wurde bereits im späten 19. Jahrhundert von Paul Ehrlich als „magische Kugel“ beschrieben. Diese Idee ist in der heutigen Zeit bereits Wirklichkeit geworden und es existieren viele Therapien welche zielgerichtet gegen eine bestimmte Erkrankung oder Erreger wirken. Eine besondere Therapieform ist die Therapie mit monoklonalen Antikörpern. Monoklonale Antikörper sind Proteinarzneiformen welche gezielt gegen einen Marker (Antigen) gerichtet sind. Aufgrund dieser Selektivität lassen sich oftmals Nebenwirkungen, welche durch eine Wirkung fernab des gewünschten Zielortes hervorgerufen werden, auf ein Minimum reduzieren. Das Anwendungsgebiet der Antikörpertherapie ist nahezu endlos und umfasst neben dem grössten Feld der Krebstherapie auch Autoimmun-, Infektionserkrankungen, Asthma etc. Ein neues, schnell wachsendes Anwendungsgebiet ist die Therapie von Augenerkrankungen mittels monoklonalen Antikörpern welche direkt in das Auge injiziert werden müssen. Das menschliche Auge ist sehr sensitiv was die aufzunehmende Menge an Flüssigkeit betrifft und aus diesem Grund ist das zu injizierende Volumen auf 50 - 100 µL begrenzt. Dieses Volumen muss vor der Applikation unter Einhaltung strenger Sicherheitsvorschiften (bzgl. Sterilität, Partikelanzahl etc.) abgefüllt werden bevor es dem Patienten zur Verfügung gestellt werden kann. Das Ziel dieser Doktorarbeit war es, den gesamten Prozess von aseptischen Abfüllungen im Bereich 50 – 100 µL zu untersuchen und die Lücke zu genauen und präzisen Standardabfüllmenge im höheren Volumenbereich zu schliessen.

Während des Abfüllprozesses kommt es zu vier kritischen Prozessschritten, welche in dieser Arbeit detailliert untersucht wurden. Der untersuchte Prozess beginnt mit der Genauigkeit der aseptischen Abfüllung von Füllvolumina < 200 µL, sowie dem Einfluss physikalischer Kräfte auf den empfindlichen Antikörper. Im Anschluss wird ein System benötigt, welches die kleinen abgefüllten Volumina zuverlässig detektieren und als Kontrollsystem fungieren kann. Der letzte Prozessschritt ist die Partikelanalyse in den geringen Volumina und die damit verbundenen Methoden welche für Füllvolumen im Mililiterbereich entwickelt sind.

In einer ersten Studie wurde eine neuartige und zum Patent angemeldete Abfülltechnologie verwendet, welche im direkten Vergleich zu traditionellen Abfüllsystemen genauer und präziser im

Abfüllen kleiner Volumina ist. Diese Technologie basiert auf dem Prinzip einer linearen Peristaltik, welche für den gewünschten Volumenbereich charakterisiert und weiterentwickelt wurde. Es konnte gezeigt werden, dass die lineare Peristaltik einen Volumenbereich von 12 – 450 µL problemlos bei gleichbleibender Genauigkeit und Präzision abdeckt. Des Weiteren ist ein kontinuierlicher Betrieb über acht Stunden möglich ohne Einbußen bezüglich Materialermüdung machen zu müssen. Im zweiten Teil der Arbeit, wurde der Einfluss der linearen Peristaltik auf monoklonale Antikörperlösungen untersucht und mit traditionellen Abfüllsystemen verglichen. Hintergrund der Studie ist, dass nicht nur die Genauigkeit eines Abfüllsystems entscheidend für dessen Anwendbarkeit ist, sondern auch die Eigenschaft die Qualität des Arzneimittels nicht negativ zu beeinflussen. Monoklonale Antikörper gehören zu den Proteinarzneiformen und sind daher sehr empfindlich in ihrer Handhabung. Man benötigt besondere Produkt- und Prozesskenntnis um diese Arzneiformen für den Patienten sicher abzufüllen. Innerhalb der Studie wurden zwei verschiedene Modelle entwickelt um den Einfluss auf das Protein quantifizieren und zukünftig abschätzen zu können. Diese Modelle lassen sich nach einer Weiterentwicklung in der Zukunft auch auf anderen Bereiche (zB. Mischen) anwenden. Nach erfolgter, oder simultan während der Abfüllung kommt es zu einer Kontrolle des Füllvolumens mittels Gravimetrie. Diese Technologie ist für Füllvolumina < 200 µL ungeeignet, da die Störanfälligkeit (zB. Durch Vibrationen oder unidirektionalen Luftstrom auf die Wägezelle) zu hoch ist. In einer weiteren Studie wurden neue Sensoren untersucht, welche im Bereich 10 – 150 µL zur Füllvolumenkontrolle eingesetzt werden können. Im letzten Kapitel der Arbeit wurde analog dem letzten Schritt während der aseptischen Herstellung die Partikelanalyse von nicht-sichtbaren Partikeln untersucht. Das Arzneibuch beschreibt zwei Methoden, wovon die Methode der Lichtverdunklung als Standard eingesetzt wird. Diese Methode benötigt Testmengen im Mililiterbereich und stellt somit für die Analyse von Volumina von 50 – 100 µL eine grosse Herausforderung dar. Um die Anwendbarkeit dieser Methode besser für den Kleinstvolumenbereich zu verstehen wurde die Methode in ihren einzelnen Bestandteilen analysiert und ein Ausblick auf neuartige Technologien ermöglicht.

Die hier realisierte Arbeit stellt eine komplette Betrachtung des aseptischen Abfüllprozesses dar. Aufgrund der gezeigten Erfolge ist es möglich einige der Projekte in Zukunft weiterzuführen und auf neue Anwendungsgebiete auszuweiten.

SUMMARY

The basic idea of targeted drug therapy was already described in the late 19th century by Paul Ehrlich referred as "magic bullet". This idea is already reality today and there are many therapies targeted against a specific disease or pathogen. A special form of this is the therapy of monoclonal antibodies. Monoclonal antibodies are protein drugs which are specifically directed against a distinct marker (antigen). Due to this selectivity, side effects, which are often caused by an effect far away from the desired target, can be reduced to a minimum. The field of application of antibody therapy is endless and includes cancer therapy as well as autoimmune diseases, infectious diseases, asthma, etc. A new, rapidly growing field of application is the therapy of eye diseases using monoclonal antibodies which have to be injected directly into the eye. The human eye is very sensitive to the amount of fluid to be administered and for this reason the volume to be injected is limited to 50 - 100 µL. This volume must be filled before application under strict safety regulations (sterility, particle count etc.) before it can be made available to the patient. The aim of this PhD thesis was to investigate the entire process of aseptic filling < 200 µL and to close the gap to accurate and precise standard filling volumes in the higher volume range.

During the filling process there are four critical process steps, which were investigated in detail in this thesis. The process under investigation starts with the accuracy of aseptic filling of filling volumes < 200 µL, and the influence of physical forces on the sensitive antibody. Subsequently, a system is required that can reliably detect the small filled volumes and act as a control system. The last process step is the particle analysis in the small volumes and the associated methods which are developed for filling volumes in the milliliter range.

In a first study a new and patent pending filling technology was used, which is more accurate and precise in filling small volumes in direct comparison to traditional filling systems. This technology is based on the principle of linear peristalsis, which was characterized and further developed for the desired volume range. It was shown that linear peristaltic covers a volume range of 12 - 450 µL while maintaining the same accuracy and precision. Furthermore, continuous operation for eight hours is possible without any occurence of material fatigue. In the second part of the work, the influence of linear peristaltic on monoclonal antibody solutions was investigated and compared with traditional filling

systems. The background of the study is that not only the accuracy of a filling system is crucial for its applicability, but also the ability not to negatively influence the quality of the drug. Monoclonal antibodies belong to the group of protein drugs and are therefore very sensitive in their handling. Special product and process knowledge is required to fill these dosage forms safely for the patient. Within the study two different models were developed to quantify the influence on the protein and to estimate the future impact. These models can be applied to other areas (e.g. mixing) in the future after further development. After filling or simultaneously during filling, the filling volume is gravimetrically controlled. This technology is unsuitable for filling volumes because its susceptibility to interferences (e.g. vibrations or unidirectional air flow onto the load cell) is too high. In a further study, new sensors were investigated which can be used in the range 10 - 150 µL for fill volume control. In the last chapter of the work, the particle analysis of subvisible particles was investigated, analogy to the last step during aseptic production. The pharmacopoeia describes two methods, of which the light obscuration method is used as standard. This method requires test quantities in the milliliter range and thus poses a great challenge for the analysis of volumes of < 200 µL. In order to better understand the applicability of this method for the smallest volume range and to reduce the sample volume as much as possible, the method was analyzed in its individual sequences and a preview of novel technologies was given.

The work carried out here represents a holistic view of the aseptic filling process. Due to the shown successes it is possible to continue some of the projects in the future and to extend them to new fields of application.

INTRODUCTION

In the late 1800s the German scientist and physician Paul Ehrlich describes the development of a therapy called “magic bullet”, which selectively targets a disease causing organism [1]. In the late 1970s, the discovery of the structure of antibodies paved the way for Paul Ehrlichs vision to become reality and the era of biopharmaceuticals started in pharmaceutical industry. Antibodies are affinity proteins, which play a central role in humoral immunity. A central ability of their function is their binding to xenobiotic substances as they are part of the immune system of higher vertebrates. This ability brought antibodies in the spotlight for their medicinal use. The development of the hybridoma technology – a discovery awarded by the Nobel Prize in 1984 to César Milstein and Georges Köhler, paved the way for the production of monoclonal antibodies (mAb) [2]. Their high specificity with simultaneous reduction of toxicity was one of the advantages over the state-of-the-art therapy options. The first commercially mAb antibody therapy, muromonab-CD3, was released in 1986 for the treatment of organ transplant rejection. Up to now there are 92 mAb drug products approved with another 13 in review in the European Union or in the United States [3]. Most of the currently approved therapeutic antibodys belong to the IgG class (Fig. 1), but there are novel antibody formats (e.g. bispecific antibodies or antibody fragments) entering the market.

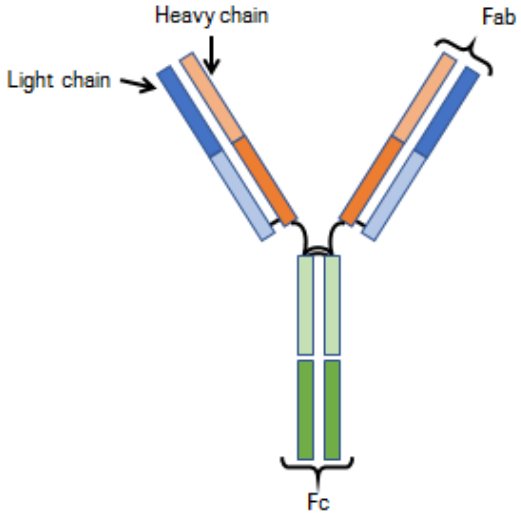

Figure 1. Structure of an IgG antibody
The IgG antibody is a large globular protein with a molecular weight of ~150 kDa. The antibody structure is composed of four peptide chains. It contains two identical heavy chains (~50 kDa) and two identical light chains (~25 kDa). The heavy chains are bound to each other and to one light chain by disulfide bonds.

The medical treatment with mAbs ranges from diseases like cancer, autoimmune diseases to rare inherited diseases. Regarding delivery, mAbs only have a limited oral bioavailability around < 1-2 % and are therefore administered parenterally [4]. The administration routes of choice are intravenous (i.v.), subcutaneous (s.c.), intramuscular (i.m.) or intravitreal (ivt.) [5]. Most mAb drug products, mainly oncology products, are administered i.v. The i.v. administration always needs the presence of a health care professional and is therefore time consuming and complex. The second major route of administration is the s.c. delivery. The s.c. administration has the advantage that it may be performed at the patients home by the patient itself, family member or a health care professional reducing the time needed for travelling and spending time at a healthcare facility. Unless the benefit for the patient is very high, new challenges for the pharmaceutical industry arise with the increasing development of mAb formulation for s.c. use. The typical maximum volume for s.c. administration is limited to < 1.5 mL at concentrations between 50 to 200 mg/mL) to prevent injection pain or adverse effects at the injection site [6, 7]. Intravitreal administration is done by injecting a drug product formulation into the vitreous body. The main field of therapy is the treatment for chronicle eye diseases. Chronicle eye diseases like cataract, glaucoma and the age-dependent macula-degeneration are responsible for vision loss in 80 %. Statistics provided by Bourne et. al state that approximately 400 million people worldwide are suffering with some form of blindness caused by ocular diseases [8]. The intravitreal injectables market shows a stable growth of 4.8 % during the forecast to 2026, highlighting the urgent need of more innovative medicines to treat patients worldwide [9]. For the treatment of these diseases, there must be a defined therapeutic concentration in the retina, the fovea or the choroidea [10, 11]. In the United States there are an estimated 5.9 million intravitreal injections based on an annual growth rate of 10 % [12]. The therapy of choice is the antineovascular therapy with the most common drugs Ranibizumab (Lucentis®), Aflibercept (Eylea®) and Bevacizumab (Avastin® as off-lable use) [13, 14]. These three drugs inhibit the vascular endothelial growth factor VEGF and therefore prevent or slow down the growth of choroidal vessels in the retina. Besides these three drugs, there are other active pharmaceutical ingredients (API) for intravitreal injection for other diseases (Tab. 1).

As highlighted in Tab. 1 the application volume of the intravitreal drug products ranges between 50 – 100 µL and is therefore far below any standard application volume known for intravenous administration and even lower than for subcutaneous administration (<1.5 mL) [7].

Table 1. Overview of approved intravitreal medications and the corresponding application volume per dose

Active Pharmaceutical Ingredient	Application Volume / Dose
Bevacizumab	1.25 mg/0.05 mL
Ranibizumab	0.5 mg/0.05 mL
Aflibercept	2.0 mg/0.05 mL
Triamcinolon acetonide	0.4 mg/0.1 mL
Foscarnet Intravitreal	1.2 mg/0.05 mL
Vancomycin	1 mg/0.1 mL
Ceftazidime	2.25 mg/0.1 mL
Amikacin	0.4 mg/0.1 mL
Amphotericin B	5 µg/0.1 mL
Voriconazole	50-100 µg/0.1 mL

With the development of more and more high-concentrated s.c. and new ivt. formulations, pharmaceutical industry is facing multiple new challenges in the field of aseptic manufacturing. During aseptic manufacturing, the filling system plays an essential role in the whole aseptic process. The filling system is responsible to deliver the desired and right amount of liquid drug product into the primary packaging container (i.e vial or syringe). With the reduction in the administration volume, there is a need for new filling technologies which are capable of reliably filling volumes < 200 µL as the state-of-the art filling systems like radial peristaltic pumps or time-pressure fillers lack dose accuracy and consistency in the lower microliter range [15]. The piston pump is a valid alternative in terms of accuracy and precision but shows an elevated risk of protein damage. Using piston pumps, it is standard to allocate dedicated piston and cylinders to each product to prevent any risk of

cross-contamination. This process results in high material and maintenance costs and effort and requires long change-over times. In terms of increasing patient individual therapies, the batch sizes will be decreasing because the therapy will be more specialized towards a smaller population of patients. This requires fast format changes during manufacturing to supply patients globally. Therefore, peristaltic pumps offer an advantage by using the single use tubing during aseptic processing. When treated as disposable, peristaltic pumps do not require any cleaning and each batch can be produced with a new fluid path with rapid change-over times at the filling lines with almost no risk of cross-contamination. The current method of choice for the administration of a 50-µL dose to a patient is the use of pre-filled syringes (PFSs), which facilitates the practice of down-dosing from a larger fill volume (e.g., 0.5 mL to 50 µL), based on dose marks on the PFS. Unfortunately this practice results in a large volume fraction of waste for the precious drug product and bears the risk of misuse like the reuse for multiple treatments. Therefore a reduction in the fill volume closer to the administration volume is inevitable. Inappropriate excess volume and labeled vial fill sizes are factors that may contribute to unsafe handling. Therefore, each product is filled with a volume that slightly exceeds the content indicated on the labeling to allow enough withdrawal and administration of the labeled volume [16]. Recommendations on limits regarding excess volume are published in USP General Chapter <1151> [17]. The best case scenario will be to fill only the required administration volume and the volume lost in the dead space of the syringe which is about 87 µL for one ivt. injection of Lucentis® (currently filled with 230 µL for an application volume of 50 µL) [18].

Obviously, there is a gap between existing filling systems and the requirements on dosing accuracy and precision for fill volumes < 200 µL for e.g intravitreal application. For a more detailed overview of filling systems please refer to the introduction section of **Chapter-I**. During manufacturing, mAbs are exposed to various stresses (e.g shear stress, interfacial stress) which can cause denaturation and inactivation of the protein [19-25]. The filling system is beyond any point of control for particulate matter in the manufacturing process (e.g. filters), therefore it must be verified that the filling parameters do not have a negative impact on the drug product quality.

To ensure that the right fill volume is filled in the primary packaging container, there is an active in-process control (IPC) in each filling line. This should prevent from falsely filled vials (either under- or overfilled) which may end up in a wrong dose administration to the patient. Aseptic filling of mAbs is performed mostly on a gravimetric basis, which is easy

to measure with good accuracy and quick measurement times. The gravimetric inprocess-control is a key part during manufacturing to assure process robustness and stability, since it allows direct feedback for filling system adjustment. Fill weights can be monitored in two different ways: In-line and off-line weight checks. For in-line weight check, the fill weight is evaluated by the equipment software and checked against the configured acceptance criteria. The filling system adjustment is done automatically. Off-line fill weight checks require additional operator handling. The fill weight is checked manually at a specified frequency and the filling systems is adjusted manually [26]. Whatever fill weight check method is used, each filling operation has a target fill weight with upper and lower acceptance criteria. The choice of acceptance criteria should account for machine capabilities, as well as the required amount of volume to meet the lable claim [17, 27]. Typical fill requirements are ± 0.5 % of the target fill for each and every filling needle but may vary with the fill volume [28].

After filling and capping the finished drug product has to be visually inspected. Visual inspection is the final unit step during drug product manufacturing. It is mandatory to conduct a 100 % inspection of every unit of filled containers and to inspect for critical, major or minor defects including the presence of visible particles. Visual inspection is described in the USP chapter <1> and chapter <790> [27, 29]. The requirement for parenteral products is to be essentially free from visible particles (≥ 50 µm). Therefore, any unit with visible particles in it will be removed from the rest of the batch. There is the possibility to use manual, semi-automated or fully automated inspection equipment. The conditions during visual inspection are defined with 2000-3750 lux, viewing at least 5 s against black and white backgrounds. Potential defects besides particle contamination are described as cracks, scratches, under/overfills, fogging and bad crimping. Sometimes air-bubbles can falsely be detected as particles which leads to false positive rejects. Besides air-bubbles, the viscosity of the solution as well as the refractive index may be a more decisive factor on the analytical results [30]. In addition to visible particles, subvisible particles (≥ 25 µm, ≥ 10 µm) are also required to be measured and specified. Light obscuration is the method of choice for subvisible particle analysis, nevertheless there are cases where microscopy may serve as alternative method. Products for intravitreal injection present a special case for subvisible particle analysis. USP <789> describes limits for particulate matter in ophthalmic solutions which are 120x less compared to injections < 100 mL [31]. These low limits are challenging regarding statistically relevant results for

the low fill volumes of intravitreal products and high sample volumes are needed to distinguish similar particle concentrations [32, 33].

Looking at all the different unit operations under the umbrella of aseptic manufacturing the production of biopharmaceutical is rather complex. With the newly introduced challenges for intravitreal application or high concentrated formulations, a new filling system with suitable IPC and analytics is heavily required.

AIM OF THE THESIS

As outlined above, low volume filling of mAbs describes a complete new chapter within aseptic manufacturing. Not only the filling systems, but also IPC and analytics are only capable to work reliably for larger fill volumes ≥ 200 µL. The aim of this thesis was to provide an end-to-end investigation in the field of low volume filling of monoclonal antibodies. This includes an overview of the current state-of-the art filling systems, and the development and characterization of a novel low volume (≤ 200 µL) filling system. To provide a holistic view over the whole aseptic manufacturing process the focus of the thesis was set on four work packages:

- **Low Volume Filling Using a linear Peristaltic Pump (Chapter I)**
  - How does the linear peristaltic pump performe in the desired volume range of 10 – 100 µL in terms of accuracy and precision? What are the boundaries regarding fill volumes?
  - Is there a possibility in prototype optimization towards a future application in routine production?
- **Low Volume Aseptic Filling: Impact of Pump Systems on Shear Stress (Chapter II)**
  - Is the linear peristaltic pump a more gentle filling system compared the state of the art filling systems?
  - What mechanism is causing product stress during aseptic manufacturing of low fill volumes?
- **Assessment of sensor concepts for a 100 % in-process control of low volume aseptic fill finish processes (Chapter III)**
  - What in-process control technologies could serve as an alternative for the state of the art gravimetric in-process control for fill volumes ≤ 200 µL?
- **Subvisible particle analysis by light obscuration for low fill volumes and high concentrated protein formulations**
  - How are different measurement parameters influencing the accuracy and precision of the light obscuration analysis?
  - Which measurement parameters can be adapted to reduce the sample volume as much as possible without compromising analytical quality?

RESULTS

The presented PhD project consists of four major work packages and has led to three peer reviewed publications and one patent filing. Each of these publications is assigned to Chapter I-III. Each chapter is presented separately.

Chapter I:

Low Volume Filling of mAbs Using a Linear Peristaltic Pump

Chapter II:

Low Volume Aseptic Filling: Impact of Pump Systems on Shear Stress

Chapter III:

Assessment of sensor concepts for a 100 % in-process control of low volume aseptic fill finish processes

Chapter IV: Unpublished results

Subvisible particle analysis by light obscuration for low fill volumes and high concentrated protein formulations

Patent:

Microdosing – WO2020/079236A1 - A dosing system for transferring an aseptic fluid in dosages into a container, comprising a peristaltic pump configured such that the filling accuracy for fill volumes < 100 µL is ± 3 µL

Chapter I

Low Volume Aseptic Filling Using a Linear Peristaltic Pump

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Highlights: The linear peristaltic pump is a promising novel and innovative technology for the aseptic filling of fill volumes < 200 µL. This study evaluated the fill volume range of the linear peristaltic pump as well as the filling accuracy for up to 8 hours, which is an important aspect in pharmaceutical manufacturing. In a final step the possibility of size reduction without compromising the filling accuracy of the filling system was investigated.

RESEARCH

Low-Volume Aseptic Filling Using a Linear Peristaltic Pump

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ABSTRACT: The pharmaceutical industry has been confronted with new and complex challenges, particularly with regard to the aseptic filling of parenterals, including monoclonal antibodies and ophthalmologic drugs designed for intravitreal injections, which often require fill volumes <200 µL. In addition to intravitreal administration, microliter doses may be required for applications using highly concentrated formulations and cell and gene therapies. Many of these therapies have either a narrow or unknown therapeutic window, requiring a high degree of accuracy and precision for the filling system. This study aimed to investigate the applicability of a linear peristaltic pump as a novel and innovative filling system for the low-volume filling of parenterals, compared with the state-of-the-art filling systems that are currently used during pharmaceutical production. We characterized the working principle of the pump and evaluated its accuracy for a target fill volume of 50 µL. Our results demonstrated that the linear peristaltic pump can be used for fill volumes ranging from 12 to 420 µL. A deeper investigation was performed with the fill volume of 50 µL, because it represents a typical clinical dose of an intravitreal application. The filling accuracy was stable over an 8 h operation time, with a standard deviation of +/−4.4%. We conclude that this technology may allow the pharmaceutical industry to overcome challenges associated with the reliable filling of volumes <1 mL during aseptic filling. This technology has the potential to change aseptic filling methods by broadening the range of potential fill volumes while maintaining accuracy and precision, even when performing microliter fills.

KEYWORDS: Low-volume filling, Microdosing, Proteins, Aseptic filling, Filling systems, Pumps, Single-use technology.

Introduction

The potency of new drugs has resulted in the use of smaller volumes for therapeutic applications (1). Additionally, many pharmaceutical companies focus their portfolio on intraocular injections. Therefore, the drug manufacturers must adapt to changes and challenges encountered with the filling of small volumes during the completely aseptic process. Existing commercial state-of-the-art filling lines were typically designed for the high-throughput production of hundreds of thousands of vials or syringes during every shift. Each aseptic filling suite supports different unit operations with distinct purposes that contribute to the aseptic manufacturing process, such as sterile filtration, filling into the primary container, container closure, and sealing. Figure 1 provides a schematic overview of an aseptic filling process.

The current trend toward increasingly personalized and highly potent therapies has resulted in the need to develop microliter aseptic fill-finish systems, to deliver low fill volumes, such as the 50 µL volumes required for intravitreal injections, with the high level of accuracy and precision that characterize current filling systems. A flow diagram describing a representative aseptic drug product manufacturing process is shown in Figure 2.

The United States Pharmacopeia (USP) General Chapter <1151> describes the acceptance criterion that the average contents of all tested samples must not be <100% of the labeled amount (2). It is therefore common industry practice to have a small overage in vials to allow for the correct withdrawable volume using a syringe for administration. The volume in the vial required to obtain the appropriate withdrawable volume is determined in laboratory studies. The industry

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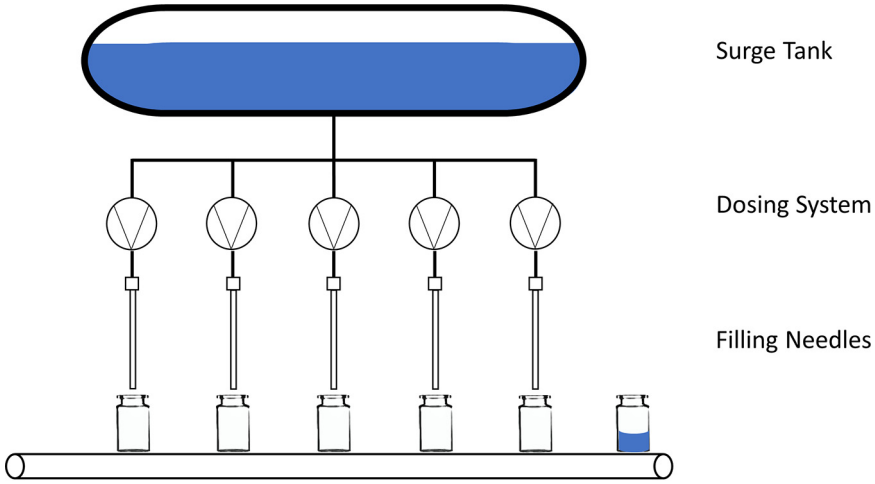

Figure 1

Schematic view of a filling process, with five filling systems and filling needles. Single-use tubing connects the central filling systems to the surge tank, which contains a reservoir of the drug product solution (in blue), and to the filling needles.

as a whole is concerned that the process of overfilling could lead to the repeated use of a single vial or the pooling of leftover drug products from multiple vials to obtain a single dose. Additionally, there is the risk of users misusing the product and injecting the entire contents rather than dosing from a higher volume. These practices would expose patients to adverse events caused by microbial contaminations and overdoses (2–5).

The current method of choice for the administration of a drug representing a 50 µL volume to a patient is the use of prefilled syringes (PFSs), which facilitates the application of the target dose from a larger fill volume (e.g., 50 µL out of 0.5 mL), based on dose marks on the PFS. Intravitreal administrations using PFSs resulted in a 50% reduction in infectious endophthalmitis cases when compared with administrations from a vial (6). However, PFSs can have internal diameter variations of ±0.1 mm, resulting in delivered volume variations of up to ±2.2 µL. The process used to apply the external dose mark on the syringe can yield a dose mark tolerance of ±0.25 mm, which can result in variations of up to ±4.3 µL in the delivered volume (7). These two factors introduce the potential for inaccurate delivered doses before considering any operator-related inaccuracies or imprecisions. A human factor study revealed that the average dose administered when aiming for a 50 µL target dose by dosing from a 500 µL PFS was 56.2 µL (12% error) (7). Because the ratio between the syringe volume and the intended application volume plays an important role in determining both accuracy and precision, smaller syringe sizes and dosing systems capable of filling small volumes are necessary (8–10).

Filling Systems

Various filling systems are currently available on the market and are currently in use during pharmaceutical production, and each system has advantages and disadvantages. The following sections will provide an overview of the most commonly used filling systems in the pharmaceutical industry and highlight a novel and innovative filling technology, capable of overcoming the challenges and restrictions associated with filling volumes <1 mL.

Table I provides an overview of the four filling systems discussed in this article. Each filling system has strengths and weaknesses that must be considered when choosing a filling system to perform an intended application.

Piston Pump

The rotary piston pump (Figure 3A) consists of a cylinder and a "truefit" stainless-steel or ceramic piston. The filling process can be divided into three different steps. First, the uplifting of the piston in the first position creates a pocket of reduced pressure in the filling system, resulting in fluid being drawn into the

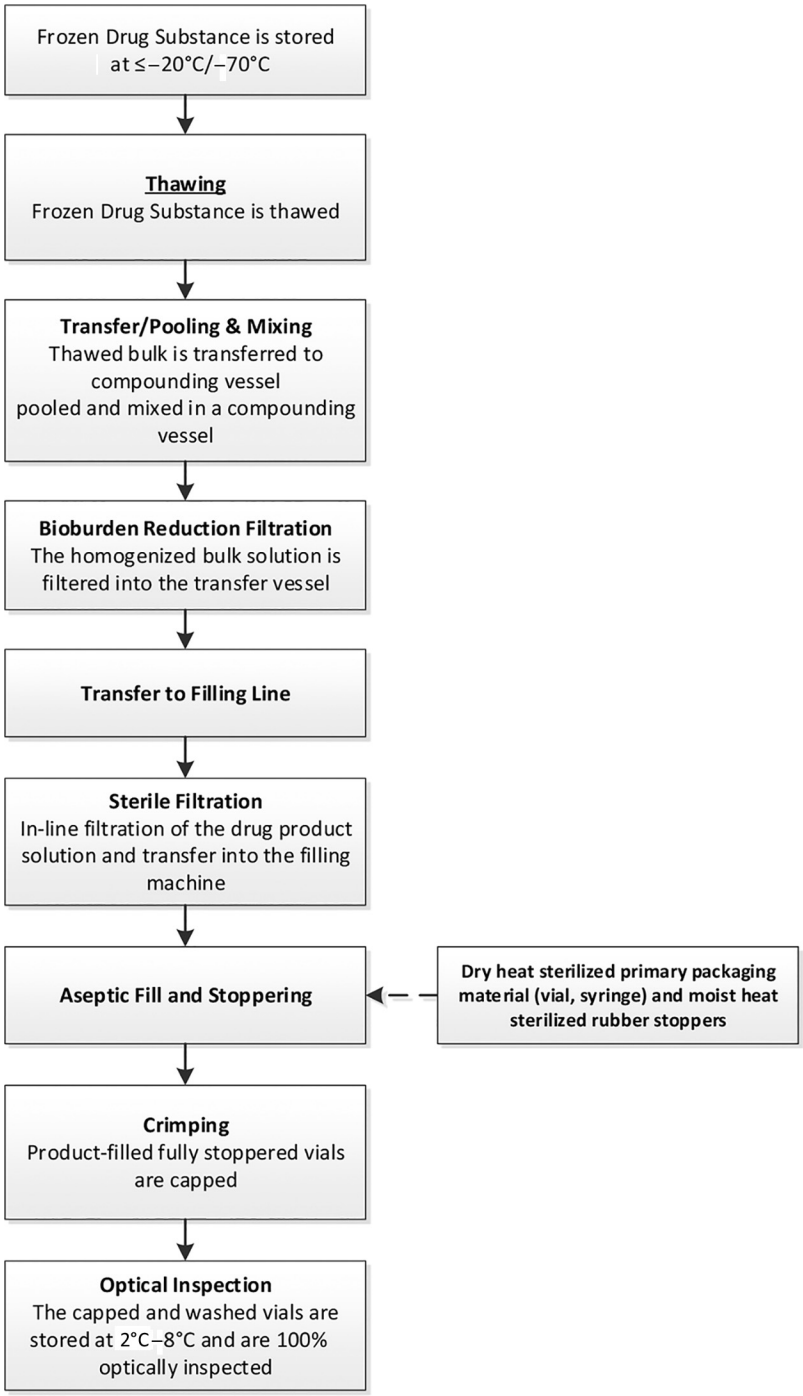

Figure 2

Example of an aseptic manufacturing process. Flowchart showing the steps followed during a representative aseptic manufacturing process.

reservoir. The piston has a one-sided notch in the direction of fluid entry. Second, the piston rotates 180˚, placing the notch in the direction of the cylinder outlet. In the final step, the piston moves downwards, delivering the fluid from the cylinder into the filling needle.

The piston pump is often used specifically for high-precision, aseptic filling because the fill volume can be adjusted in a very precise manner. Every piston pump is limited to a designated fill volume range; therefore, different pump sizes are necessary for different ranges of fill volumes.

TABLE I
Overview of the Different Dosing System Characteristics

Criteria	Piston Pump	Time-Pressure System	Radial Peristaltic	Linear Peristaltic
Filling Accuracy < 100 µL	++[a]	−−	−	++
Fill Volumes < 100 µL	+	−−	−	++
Fill Volume Range	−−	+	++	++
CIP/SIP Complexity[b]	−−	+	++	++
Single-Use Compatible	−−	++	++	++
Product Stress	−−	+	+	++
Interfacial Stress	−−	−−	++	++
Maintenance	−	+	+	+

[a]+ is favorable, − is unfavorable.
[b]CIP is clean-in-place; SIP is sterilize-in-place.

The piston pump has a high degree of filling accuracy and precision, particularly for low fill volumes (≤1 mL) (11). However, multiple studies have reported elevated subvisible particle counts and a few visible particles in protein formulations filled using piston pumps, leading some to suggest that piston pumps may cause the denaturation and aggregation of delicate biopharmaceutical products sensitive to related product stress (12–16).

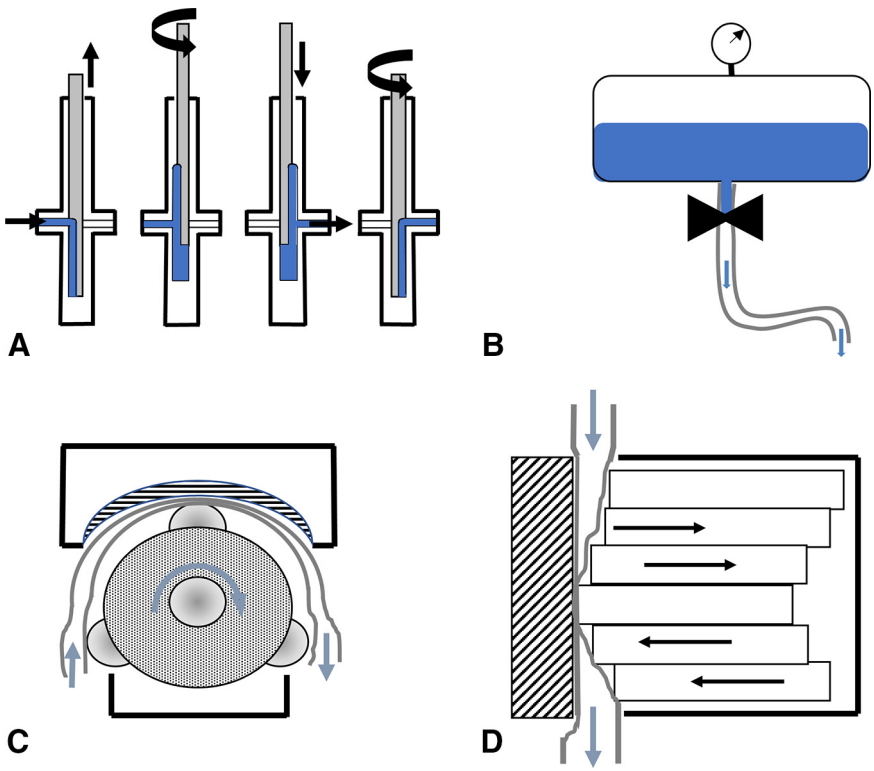

Figure 3

Schematic presentations of the investigated pump types. (A) Rotary piston pump. (B) Time-pressure filling system. (C) Radial peristaltic pump. (D) Linear peristaltic pump. Blue arrows indicate the direction of liquid drug product movement. Black arrows indicate the directions of system component movements.

Time-Pressure System

In a time-pressure filling system (TPS), the fluid is stored in a surge tank, overlaid with an inert gas (usually nitrogen) that is maintained at constant pressure (Figure 3B). The fluid is distributed by a manifold, through the tubing, to the filling needles. The fill volume is controlled by valves, which are opened for a defined time, the overlaid pressure, and the diameter of the tubing. Product temperature affects filling accuracy (indirectly, via changes in the density and viscosity of the fluid), and, therefore, must be controlled. The TPS is more prone to bubble formation than other systems because of the pressurized surge tank. The overlaid gas pressure increases the concentration of soluble gas in the cooled liquid, and these gases may expand when the liquid moves out of the surge tank, negatively influencing the filling accuracy. This would be especially relevant for small fill volumes such as <1 mL. The machine setup and environmental variabilities, such as pressure fluctuations in the surge tank and temperature changes in the bulk solution, can also influence the accuracy. The TPS requires complicated computer systems and fast actuators to ensure reliable process control and is the most inaccurate of the described filling systems.

Radial Peristaltic Pump

Radial peristaltic pumps (RPPs, Figure 3C) are composed of a rotor, a stator (counterpressure plate), and tubing. The pump acts as the stator, and the three rollers (which are driven by a servomotor mounted on the pump head) act as the rotor. The counterpressure plate is adjustable and can be adapted to the thickness of the tubing. Fluid delivery, in the direction of the filling needle, is achieved by the positive displacement of a liquid column, which moves from a reservoir toward the filling needle. The tubing is squeezed between the rollers and the counterpressure plate by the radial movement of the rollers. After squeezing, the tubing relaxes to its original shape, creating a low-pressure pocket that draws additional fluid into the tubing.

The fill volume is determined by the inner diameter (ID) of the tubing, the tubing elasticity, the number of rollers, the counterpressure, the rotation angle, and the size of the pump head. One advantage associated with RPPs is that the biopharmaceutical solution is only in direct contact with the disposable and single-use tubing, not with the pump itself, which can minimize the required cleaning and sterilization efforts. Multiuse components with direct product contact can be limited to the surge tank and filling needles. Therefore, the tubing material must be suitable for aseptic fill-finish processes, able to be cleaned and sterilized under conditions of 121°C and 2 bar pressure. Additionally, leachables, extractables, and particle shedding into the fluid must be carefully controlled. Recent studies have indicated that the radial movement of the pump can cause particle shedding of the tubing material during aseptic fill-finish processes (17, 18).

Linear Peristaltic Pump

The linear peristaltic pump (Figure 3D) relies on the same physical principle as the RPP (positive displacement) (19). Because of the subsequent squeezing of the tubing, the forward movement of a fluid column is achieved. The linear peristaltic pump consists of multiple piezo actuators that act in line. The actuators squeeze the tubing through synchronized orthogonal displacement, resulting in a sine wave. Initially, each filling cycle actuator is displaced by 100%, resulting in a phase shift of 90° relative to the base sine wave, which prevents dripping and air entry. Each piezo can be displaced between 0 and 1000 µm. The phase shift of each actuator is fixed 60° relative to the phase of the previous actuator, and the displacement of all six piezos is defined as one cycle. The fill volume is directly controlled by the number of cycles, the number of displaced actuators, the actuator sizes, the precompression of the tubing (by the counterpressure plate), and the elasticity of the tubing. Because of the orthogonal movement and the lack of tangential force vectors, the tubing inner surfaces could potentially experience reduced movement relative to the tubing in the RPP. This would result in reduced material strain and reduced particle shedding.

In addition to the linear peristaltic fill mode, the filling system can also be operated in a time-pressure mode. Within this mode, two piezo actuators in the filling system act as a valve, and the fill volume is controlled comparable to a "standard" TPS, as described previously. In this study, the time-pressure mode was not used; however, it may play an important role in future development, particularly when extending the fill volume from the microliter range to the higher milliliter range.

Aim of the Study

Aseptic fill-finish facilities traditionally use the piston pump–mostly for small molecule and compatible large molecule products–because of its high degree of filling

accuracy and precision. However, the trend has shifted in recent years toward RPPs. RPPs generate less product stress for biopharmaceutical drug products and limit the number of product-contacting surfaces (14, 20). The major concern for RPPs is the filling accuracy, particularly when using very small fill volumes <200 µL (11).

To close the gap between the highly accurate but product-straining piston pump and the less accurate but gentler RPP, a linear peristaltic pump prototype was constructed, and the feasibility of its application during the low-volume filling of parenterals was examined in preliminary experiments. Our work focused on the further development of the optimal control parameters for accurate and precise filling. Therefore, we designed a study to monitor a target fill volume of 50 µL, for up to 8 h, which was representative of a typical manufacturing shift. Additionally, we investigated the fill volume range, using two different types of platinum-cured silicone tubing, to frame the scope of the application. As a final step, we investigated whether a potential reduction in the sizes of the piezo actuators would affect the accuracy of the system, to lay the groundwork for future prototype evolutions.

Materials and Methods

All filling accuracy experiments were performed using water or a highly concentrated glycerol-water solution (glycerol-water 60% v/v and 0.02% polysorbate 20). The glycerol-water solution had a measured viscosity of 16.49 cP, which was considered to be the worst-case scenario for standard protein formulation viscosities. For all data analyses, the mass density of the distilled water (δ_{H2O}) was assumed to be 1 $gscm^{-3}$, and the mass density of the glycerol-water solution was measured as 1.1538 $gscm^{-3}$ at 20 °C. The gravimetric measurement results were directly converted from mg into µl, according to density.

Filling System

The linear peristaltic pump is a patent-granted technology. The pump consists of multiple piezo actuators (P-602 PiezoMove, PI, Karlsruhe, Germany) in a stainless-steel housing and was operated together with platinum-cured silicone tubing (Flexicon Accusil), with IDs of 0.8 mm and 1.6 mm. The wall-strength values of both tubings were 1.6 mm, and the tubings were chosen after a preliminary study that examined the effects of compression and restoration forces on the tubing shape. The tubing was connected to a surge tank on one end and to a low-volume filling needle on the other end. The filling needle had an ID of 0.6 mm and a length of 150 mm. Both the surge tank and the filling needle were made of 316 L stainless steel. For drug products that are sensitive to oxidation, the filling needle has the ability for simultaneously inert gassing with nitrogen. The working principle underlying the pump and the volume control is described, in detail, in the patent (19).

Filling Accuracy Target

The strategy for this technology development was to minimize the filling process variability. We therefore abstained from defining a fill volume acceptance criterion. The overarching aim was to create a drug product presentation in the form of a PFS that does not need dosing based on a dose mark. It would be a great patient benefit if the dose variability introduced by the operator and additional handling steps could be eliminated. For comparison with typical performance data for filling accuracy in the small-volume range, we present the data with a typically process consistency window of ± 10% in the figures.

Filling Accuracy Readout

The filling accuracy and precision were determined by gravimetric measurements. Therefore, a Mettler Toledo balance (XPE105) was used with gravimetric software (GraviDrop, BiofluidiX GmbH, Freiburg, Germany) during all dispensing experiments.

Results

Our results provide an overview about the construction of the linear peristaltic pump as well as its applicability for the aseptic filling of low fill volumes.

Linear Peristaltic Pump: Construction

The development and construction of the linear peristaltic pump were described, in detail, in the patent (19). Figure 4 shows an overview of the pump. The pump was constructed from stainless steel and consists of 6 piezo actuators and a counterpressure plate. The fluid was delivered toward the filling needle by positive displacement through a silicone tube, which was fixed between the actuators and the counterpressure plate.

Linear Peristaltic Pump: Parameter Settings

The fill volume control was determined by multiple variables, using both fill modes (**A:** linear peristaltic and

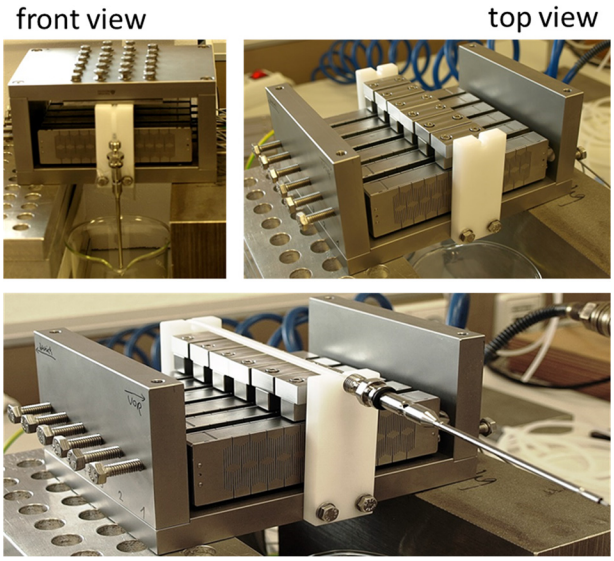

Figure 4

Overview of the linear peristaltic pump construction. The upper left image shows a front view of the pump, with the assembled counterpressure plate, whereas the upper right image shows a top view of the pump, without the counterpressure plate. The bottom image shows how the tubing (black arrow) is placed on top of the actuators and connected to the filling needle.

B: time-pressure). For the linear peristaltic mode, the fill volume was determined by the following variables:

- The wavelength of the sine wave
- The amplitude of the sine wave
- The frequency of the sine wave
- The phase shift of the sine wave that controlled the different actuators
- The number of actuators being controlled
- The tubing geometries
- The precompression (offset) of the tubing
- The elasticity of the tubing

The wavelength of the sine wave and the amplitude represented the preferred control mechanisms, as the tubing properties, the distance between each actuator, and the number of actuators remained constant. Figure 5 shows

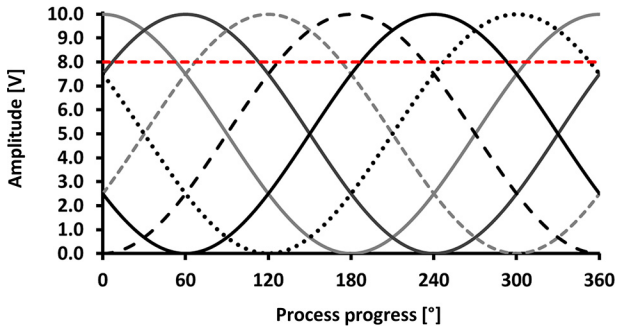

Figure 5

Visualization of the parameter settings used for a 50 µL target fill volume. The process progress is measured in degrees, with 360° representing a full filling cycle. The amplitude is measured in volts, with 10 V representing the full amplitude of positive displacement. The red dotted line indicates the actuator position at which the tubing will be sealed completely.

an overview of the positive displacement for all six actuators. The position 0° was the starting point of the filling process. Prior to any filling step, the tubing must be manually adjusted and controlled, especially if it becomes tight after reaching a certain displacement of the actuators. The chosen displacement for a full closure of the tubing was set to an offset of 8 V (80% displacement). This setting was necessary because the counterpressure plate was slightly variable, and the tubing must be able to be sealed at any of the six actuator positions to prevent uncontrolled liquid flow. Figure 5 shows that at least one actuator is sufficiently displaced to seal the tubing at any given time during the filling. In our model, actuator 1 entered the sine cycle at full amplitude, which prevents air entrainment in the system or the leakage of the level tank. Actuators 2–6 were shifted by 60° relative to the phase of the previous actuator.

Linear Peristaltic Pump: Filling Accuracy

During preliminary tests, the volume range of the linear peristaltic pump was tested using two different silicone tubing diameters (ID = 0.8 mm and ID = 1.6 mm). Figure 6 shows the volume coverage of the pump for each tubing diameter, with increasing numbers of sine wave cycles.

The increase in volume was proportional to the increase in the number of sine wave cycles, as expected, and a volume range from 12 to 430 µL was achievable when using

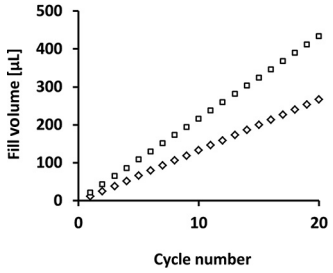

Figure 6

Fill volume range of the linear peristaltic pump. Diamonds indicate fill volumes when using silicone tubing with an inner diameter of 0.8 mm with a maximum deviation of 0.64%. White squares reflect the fill volumes when using silicone tubing with an inner diameter of 1.6 mm with a maximum deviation of 0.43%. The data points shown reflect the means of three independent experiments.

as many as 20 sine wave cycles. Although higher fill volumes are possible when using the linear peristaltic mode, the time required for a single fill when using >20 sine wave cycles would be too long for commercial filling processes. Therefore, the time-pressure mode should be used for fill volumes >500 µL.

To achieve a target fill volume of 50 µL, a platinum-cured, silicone tubing with an ID of 0.8 mm and a wall strength of 1.6 mm was used, and 24 actuators were displaced, in total, equal to 4 complete sine wave cycles for each of the six actuators. The frequency was set to 30 Hz, resulting in a total fill time of 133 ms. Although various frequencies were examined during preliminary experiments (data not shown), no improvements for the process were observed when using different frequencies. When the frequency was <15 Hz, no fluid transport was possible, and this effect was especially noticeable for high-viscosity solutions because of the increased mass inertia. At frequencies >40 Hz, the tubing began to vibrate, and no fluid transport was possible in a controlled manner. Figure 7 shows an overview of the filling accuracy of 3×50 µL single-filling steps (A: water; B: glycerol-water solution, cP 16.49). The highly viscous model solution was chosen to investigate the effects of higher viscosities on the filling accuracy and consistency of the linear peristaltic pump.

After each set of 50 fills, a 10 min process interruption was simulated. The fill volume was collected in a plastic beaker on a high-precision gravimetric balance (Mettler Toledo XPE105), and the volume was calculated based on the mass and density of the solution.

Both tests showed that the linear peristaltic pump was capable of filling fluids within a viscosity range of 1–16.49 cP at targeted volumes of 50 µL. To further investigate the filling accuracy and consistency over time, a long-term filling test was performed for >8 h, which represents the length of a typical shift during commercial production, during which 1500 single filling steps were performed (Figure 8). The filling speed was consciously reduced to allow for the optimal settling time during gravimetric measurements.

The long-term filling test confirmed the preliminary accuracy and consistency data, over a time period of >8 h, without any feedback loop (industry standards for aseptic filling). Additionally, no tubing fatigue was

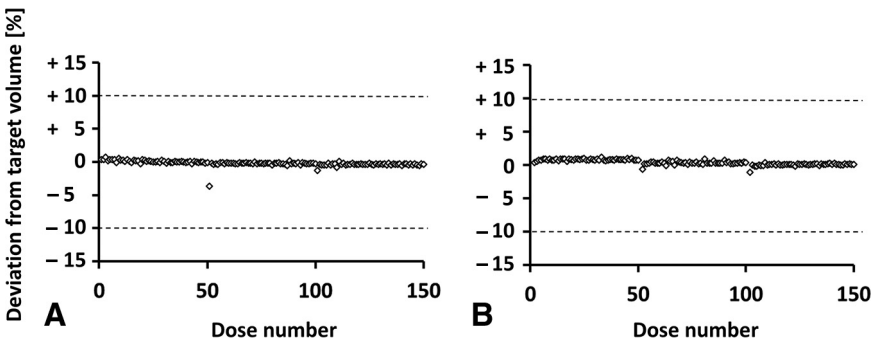

Figure 7

Filling accuracy for 3×50 single filling events. (A) The filling accuracy for purified water (viscosity = 1 cP). (B) The filling accuracy for a high-viscosity glycerol–water solution (16.49 cP). Dotted lines indicate the accepted deviation range of ±10%.

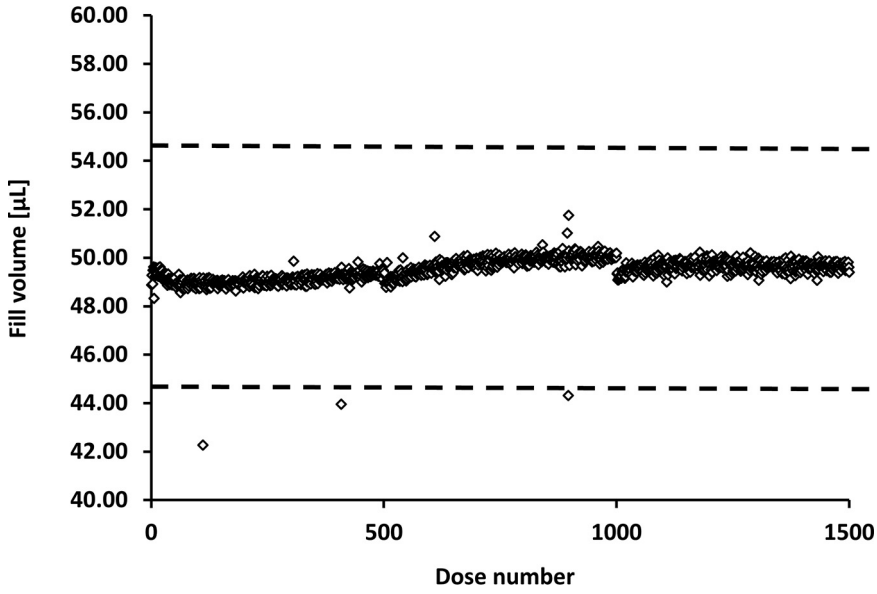

Figure 8

Long-term filling accuracy for water. Repeated single fills, with targeted fill volumes of 50 µL, were performed for >8 h (representing 1500 individual filling events). The dotted lines indicate the acceptable upper and lower 10% deviation limits for low fill volumes. A total of three individual filling events were identified as being outside of the acceptable range because of insufficient control of the environmental surroundings (e.g., air-pressure changes because of door openings).

observed, which would typically be observed with an RPP system.

Linear Peristaltic Pump: Filling Accuracy After Actuator Reduction

The linear peristaltic pump remains in the prototype stage and requires further improvements before the full system can be implemented on a commercial filling line. To achieve the optimal filling accuracy, the dosing element should be co-located with the filling needle to prevent any pressure drops over the tubing length, which could lead to worse filling accuracy. Currently, the pump weighs approximately 20 kg, which is not suitable for fitting directly onto the filling needle. Therefore, additional tests to investigate potential methods for size and weight reductions in the pump prototype were performed (Figure 9). The most promising improvement for the future commercial implementation of this pump would be the reduction of the piezo actuators, which would minimize both the size and the weight of the pump.

A test was performed using only three operational actuators, to investigate whether the filling accuracy and consistency were affected by a reduction in the number of actuators. Three represents the minimum number of actuators required for fluid transport, as two would merely move the fluid back and forth. The testing parameters used were identical to those used in the previously described filling accuracy tests. The amplitude for the state during which the tubing remained fully sealed was lowered to 7 V to ensure that at least one closed position was recorded for each state of the fill.

Discussion

The filling accuracy of the linear peristaltic pump showed a maximum relative standard deviation of 0.65% within a fill volume range of 12–450 µL. The filling accuracy studies were performed with set parameters, which are shown in Figure 5. The piezo actuators were displaced by continuous movement, following the shape of a sine wave. Therefore, the fill volume of the pump was not limited to one fill cycle (displacement of piezos 1–6) because the wave continued at actuator 1 after reaching the last actuator (in this case, actuator 6). Because of this continuous movement, the fill volume could be adjusted in a very precise and accurate manner. Figure 4 shows the fill volume ranges for both tubing sizes used in the study.

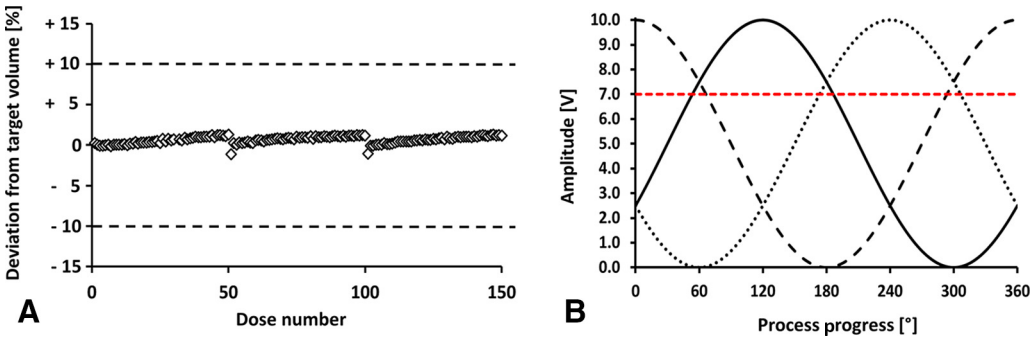

Figure 9

Filling accuracy after reducing the actuators by 50%. (A) The filling accuracy of 3 × 50 single filling events at a target fill volume of 48 µL, when only actuators 4–6 were in operation, using 13 sine wave cycles. The dotted lines indicate the acceptable deviation range of ±10%. (B) Visualization of the parameter settings. A full filling cycle is represented as 360°, and the amplitude indicates the displacement of the actuators. The tubing was sealed after an actuator displacement of 7 V (70%, red line). The filling speed was set to 20 s/filling event, at 30 Hz (433 ms).

We successfully demonstrated that linear peristalsis could cover a fill volume range from 12 to 420 µL, with a linear relationship between the number of sine wave cycles and the total fill volume. This linear behavior confirmed that the fill volume was directly dependent on the number of piezo movement cycles. The linear peristaltic pump requires further improvements to prepare it for use in a good manufacturing practice (GMP) environment. Currently, the tubing seal must be adjusted by hand, depending on the piezo offset, and this process was necessary to prevent any deviations caused by small disparities in the tubing material or the piezo mounting, particularly because the prototype was manually built. Future processes should be fully automated, such as by measuring the airflow through the needle at constant pressure and exactly adjusting the distance required for full tubing closure.

Another advantage of the linear peristaltic pump is that the whole system can be established before the regular clean-in-place (CIP)/sterilize-in-place (SIP) processes, which occurs before aseptic filling. Usually, components must be placed after CIP/SIP, through manual manipulation, by gloved workers in a sterile environment. Therefore, the linear peristaltic pump could potentially minimize human interference with the aseptic system.

To enhance the applicability of the linear peristaltic pump for an even broader fill volume range, the fill volume range can be divided into submilliliter and milliliter ranges. For drug products requiring submilliliter fills, the pump should be operated in linear peristaltic mode. For fill volumes in the milliliter-range, the system can be switched from linear peristaltic mode to time-pressure filling, without requiring major adjustments to the pump system. The only difference would be the pressure control of the surge tank. This two-mode operation system would enable pharmaceutical companies to cover the complete product portfolio of drug fill requirements with a single filling system. The present study focused on the accuracy and precision of the linear peristaltic mode for targeted fill volumes of 50 µL, which is the typical application dose for intravitreal injections.

As shown in Figure 6, the fill volumes of three sets of 50 fills, using 50-µL target volumes, remained stable and met the process consistency window of ±10%. Figure 7 shows that the first fill volume after the 10 min process interruption was slightly lower, before reaching the initial volume within the next 2–3 fills.

This effect may be caused by the following issues. (1) During the 10 min break, the tubing may be compressed at one position, resulting in the tubing being flattened and regaining its shape after the filling process started again. This effect has been demonstrated in RPP studies, which showed that some tubing did not regain its original shape, even after one week (11). (2) Depending on the fluid properties and the needle material, liquid level variations may occur at the end of the filling process because of the rapid suck-back of liquid into the needle tip. Shieu et al. showed that reductions

in liquid pressure, using custom adjustments, could minimize this effect (21). (3) A slight amount of liquid may evaporate from the needle during the 10 min break, resulting in this volume being absent from the first fill following process interruption. In another experiment, the filling accuracy was tested for 8 h, which was equivalent to a standard shift during commercial production (Figure 8). The filling accuracy remained stable for the entire 8 h filling period without any major interruptions. The only process interruptions occurred after every 500 filling events, when the reservoir on the gravimetric balance was emptied. The maximum deviation measured over 8 h of continuous dosing was 4.4%, which was well-below the 10% limit. Three clear outliers were identified, for which the volume was underfilled.

A follow-up experiment, in which only the background was monitored for 8 h, revealed a comparable number of outliers over time (data not shown). The gravimetric balance was not installed on a massive weighing table, which is normally decoupled from the environment. Therefore, some background noise, caused by air turbulence and vibrations, may have influenced the gravimetric measurement. This effect is known to occur during gravimetric in-process control during commercial manufacturing and has been investigated, in detail, in another study (22).

Another effect that could negatively influence filling accuracy is the use of connective tubing between the pump and the filling needle, which is a common practice during commercial manufacturing. Previous studies demonstrated decreased filling accuracy over the length of the tubing used to connect the filling system with the filling needle. Poor filling accuracy occurred because of fluctuating fluid column pressures between the pump and the filling needle. The Hagen–Poiseuille law describes the dependency of pressure loss on the tubing length. For smaller fill volumes, this effect can become especially relevant because any small deviations can lead to under- or overfilled drug products that would be rejected by the in-process control system. Therefore, the filling needle was connected directly to the pump for all filling accuracy studies. To achieve a degree of co-localization between the filling system (point of dose = PoD) and the filling needle (point of fill = PoF) that would be suitable for commercial manufacturing, the pump must be revised and miniaturized. Currently, the actual size and weight of the pump make it impossible to mount a needle-attached pump on a movable filling bar.

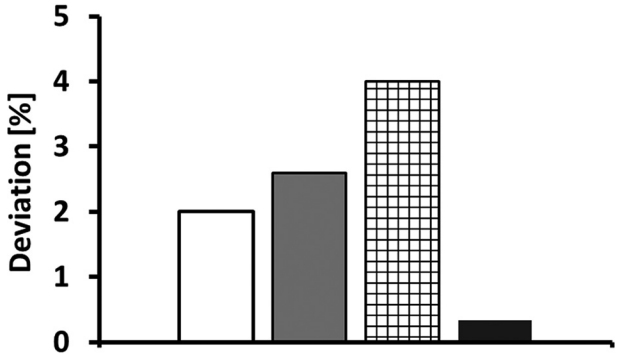

Figure 10

Filling accuracy of the linear peristaltic pump and three commercially available filling systems. The commercially available filling systems (White column: Rotating piston pump; Gray column: Time-pressure filler; Black mesh: Radial peristaltic) showed a deviation from 2% to 4% to a target fill volume of 70 µL. The linear peristaltic pump showed a deviation of only 0.34% to an even lower target fill volume of 46 µL. Data for the commercial filling systems was extracted from: Peterson et. al., Figure 11 (11).

All filling accuracy experiments were performed without any applied suck-back mechanism. As discussed previously, a weak suck-back effect may have occurred, triggered by fluid dynamics and material properties, but not actively controlled by the filling system. During suck-back, a defined volume of the fluid is withdrawn from the needle tip, to prevent the tip from clogging. The suck-back mechanism of a filling system becomes increasingly important with highly concentrated and low-volume protein formulations. Because of the high proportion of solid fractions in liquid protein formulations, they tend to dry faster than standard formulations (21, 23–25). In addition to the prevention of potential needle clogging, the suck-back mechanism also minimizes contact between the fluid and air, which protects the product from exposure to interfaces that can lead to protein particle formation (20, 26, 27). For future implementations, a controllable suck-back mechanism should be developed.

In a final experiment, the possibility of reducing the sizes of the piezo actuators was investigated to determine the potential for the future miniaturization of the pump. The number of active piezo actuators was reduced by 50%, from six to three (Figure 9), and accuracy was examined, using similar procedures as the other accuracy experiments described. The reduction

from six actuators to three had no negative influences on filling accuracy or precision.

This finding suggests the potential for future pump optimization and miniaturization improvements that may increase the applicability of the pump for commercial manufacturing processes. Finally, the linear peristaltic pump was compared to three state-of-the-art filling systems investigated by Peterson et. al in the fill volume range between 0.03 and 1 mL. Figure 10 shows the deviation from the target fill volume in percentage. It is obvious that among all four filling systems, the linear peristaltic pump performed with the lowest deviation from the target fill volume and can therefore be considered as a valid alternative for aseptic filling, especially in the low volume range <1 mL.

Conclusion

Our study showed that our linear peristaltic pump prototype was able to fill volumes as low as 12 µL, with a maximum fill volume of up to 420 µL. The target fill volume of approximately 50 µL was achieved with a maximum deviation of 0.34% and was maintained within the process consistency window of ±10% during repeated filling for up to 8 h. Because of the two-operation mode potential, the linear peristaltic pump could represent a valid alternative to the state-of-the-art filling systems that are currently in use, by covering the full range of fill volumes required for typical injectables. However, before any routine applications, the pump must be further improved and adapted (e.g., reduction in size and weight).

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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Chapter II

Low Volume Aseptic Filling: Impact of Pump Systems on Shear Stress

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Highlights: The study summarizes the development of a novel liposomal model for the prediction of shear stress levels during aseptic filling. A more detailed and profound understanding of the filling process itself and possible mechanisms, which play a role in protein denaturation and particle generation, was gained by the use of a novel developed shear sensitive liposomal model, computational fluid dynamics and a monoclonal antibody study. By combining all three approaches a toolbox can be generated which helps to identify ideal process parameters and limit the use of precious drug-product during development studies.

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Research paper

Low volume aseptic filling: Impact of pump systems on shear stress

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ABSTRACT

Low volume aseptic filling of parenterals, particularly monoclonal antibodies is becoming increasingly important with the development of more and more intravitreal drugs and high concentrated formulations. Especially monoclonal antibodies are very delicate products to fill and the use of the right fill finish equipment plays an important role during process development. Protein aggregation can occur under conditions described in literature and can be influenced by the fill finish processing. The mechanism of product stress inside the filling systems is yet not fully understood. This study evaluated three different dosing systems to assess protein degradation caused by the shear rate during low volume filling of monoclonal antibodies. The newly developed quantitative liposomal shear stress model revealed the highest shear rate in the radial peristaltic pump, followed by the rotary piston pump and the linear peristaltic pump. In contrast to that, we found the highest sub-visible particle counts (> 2 µm) in the rotary piston pump. We used computational fluid dynamics for a better and deeper understanding of filling processes inside the different dosing systems. Our results document that the rotary piston pump creates a recirculation zone inside the cylinder, where the protein formulation could be trapped and be exposed to the shear stress multiple times resulting in a cumulative shearing. This finding could serve as an explanation for the highest sub-particle counts in low volume filling using a rotary piston pump.

1. Introduction

Intravitreal ocular treatments for diseases, such as wet age-related macular degeneration (AMD) or diabetic macular edema, require a suitable filling system which is capable of accurate and precise filling of the drug product formulation. However, small volume filling of parenterals, in particular monoclonal antibody formulations such as for example Lucentis® or Eylea®, are a challenge with respect to industrial processing. Consequently, all formulations are currently filled with more volume than required. This facilitates filling and ensures that enough drug product can be administered to the patient. Although overfilling is a common practice, it also has multiple disadvantages. Problems include medication errors due to wrong dosing, misuse of leftover product, or waste of drug [1,2]. For these reasons, pre-filled syringes, filled with the desired fill volume in the range of 50–100 µL, are used in addition to vials. To ensure the seamless supply of the market with low volume drug products, there is a need to identify and characterize a suitable dosing system for low volume aseptic filling of parenterals. To date there is no suitable filling system available on the market, which fulfills all desired requirements for accurate low volume aseptic filling [3]. Besides requirements such as filling accuracy, cleaning-in-place/sterility-in-place (CIP/SIP) compatibility and materials with a low extractable and leachable profile, there is shear stress during the entire filling process, which plays a pivotal role in discussions about the most suitable filling system [4].

Shear stress is present in almost all bioprocesses and is known to play a role in protein aggregation and denaturation of proteins which may be susceptible to shear stress [5–7]. Looking at the extensive portfolio within the pharmaceutical industry, protein-based products are a significant part of the pipeline, both in the present and the future. Direct administration of medication into the human eye (intravitreal) requires the control and analysis of key parameters during aseptic filling and poses daily challenges to ensure a safe product supply to the patients. These parameters include the correct filling accuracy and monitoring, control of mechanical and surface stresses during filling, and the assessment of the subsequent cleaning. Furthermore, the outside sterilization of ophthalmologic syringes is another challenge for intravitreal drug products. To ensure patients safety, there are increased requirements for particles in ophthalmological preparations defined in USP ⟨789⟩ compared to classical parenteral preparations.

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Nomenclature

CFD	Computational Fluid Dynamics
RT	room temperature
d	days
h	hours
CIP	Cleaning in place
SIP	Sterilization in place
NTU	nephelometric turbidity unit
PDI	Polydispersity index

Due to the fact that every pump type operates differently, there are controversial discussions ongoing regarding the most suitable pump type. It is important to understand how the pumps filling mechanism may affect the stability of a drug product formulation. Currently, the compatibility of pump types (with the desired drug product) is chosen with stability studies over a period of multiple months to several years. The stability of the drug product formulation is then analyzed using different surrogate parameters (i.e. particle counts, turbidimetry, particle morphology). A suitable model, which allows the quantification of the applied shear stress during high precision aseptic filling of parenterals (in connection with the pump type or correlations with fluid dynamics models which would allow to draw conclusions to potential drug product damage) is still missing.

1.1. Working principles of the different dosing systems

1.1.1. Piston pump

The rotary piston pump (Fig. 1A) consists of a cylinder and a 'truefit' stainless steel or ceramic piston. The filling process can be divided into four different steps. The uplifting of the piston in the first position results in an under pressure in the dosing system and the fluid is "sucked" into the reservoir. The piston has a one-sided notch in the direction of the fluid's entry. Secondly, the piston rotates by 180° and the notch is placed in the direction of the outlet of the cylinder. In the final step the piston moves downwards and delivers the fluid from the cylinder to the filling needle. The piston pump is used specifically for high precision aseptic filling since the fill volume can be adjusted in a very precise manner. Every piston pump is limited to a designated range of dosage, therefore different pump sizes are needed if a certain range of fill volumes should be covered. The Piston Pump is often used because of its high filling accuracy and precision, particularly for low fill volumes (≤0.3 mL) [3,8]. Multiple studies show elevated sub-visible particle counts for protein formulations filled with piston pumps which lead to the hypothesis that piston pumps may lead to denaturation and aggregation of delicate biopharmaceutical products [4,5,9].

1.1.2. Radial peristaltic pump

The radial peristaltic pumps (Fig. 1B) are composed of a rotor, a stator (counterpressure plate) and tubing. In this case, the pump acts as the stator and the three rollers (which are driven by a servomotor -mounted on the pump head) act as the rotor. The counterpressure plate is adjustable and can be adapted to the thickness of the tubing. The delivering of the fluid (in the direction of the filling needle) is achieved by positive displacement of a liquid column from a reservoir towards the filling needle. The tubing is squeezed between rollers and counterpressure plate with radial movement of the rollers. After squeezing, the tubing relaxes to its original shape which results in an underpressure what subsequently leads to the fluid being sucked back into the tubing. The amount of fluid dose is determined by the inner tubing diameter, tubing elasticity, number of rollers, counterpressure, rotation angle and size of the pumphead. One of the advantages of the radial peristaltic pump is the fact that the biopharmaceutical solution is only in direct contact with the tubing and not with the pump itself, which would minimize potential extractables, leachables and presence of particulates. Therefore, it is of importance to use tubing material which is suitable for aseptic fill finish processes and hence, have the capability of being cleaned and sterilized under 121 °C and 2 bar pressure. Additionally, there are restrictions concerning leachables, extractables and particle shedding into the fluid [10,11]. Recent studies indicate that due to the radial movement of the pump particle shedding of the tubing material during aseptic fill finish processes could be observed [12].

1.1.3. Linear peristaltic pump

The linear peristaltic pump (Fig. 1C) [13] is based on the same physical principle as the radial peristaltic pump (positive displacement). Due to the subsequent squeezing of the tubing a forward movement of the fluid column is achieved. The linear peristaltic pump consists of multiple piezo-actuators acting in line. The actuators squeeze the tubing by orthogonal displacement by acting together, resulting in a sine-wave. Initially, each filling cycle actuator 1 is displaced by 100% which results in a phase shift of 90° to the base sine wave. This prevents the system from dripping or entry of air. Each piezo can be displaced between 0 µm and 1000 µm. The phase shift of the second actuator is fixed by 60° to the phase of the first actuator. These phase shifts, by 60°, are effective for all six actuators in the pump. The displacement of every piezo is defined as one cycle. The fill volume is controlled directly by the amount of cycles, the number of the displaced actuators, the actuator size, the pre-compression of the tubing (by the counterpressure plate) and the elasticity of the tubing. Due to the orthogonal movement and the lack of tangential force vectors, compared to radial peristaltic pumps, there is less relative movement of the tubing inner surfaces resulting in less material strain and less particle shedding.

Based on these considerations, the aim of the present study was defined as an evaluation of different types of filling systems (piston pump, radial peristaltic pump and linear peristaltic pump) in the range of 100 µL with respect to shear stress levels. Since shear stress is a complex term, we used this term for all shear related events which occur during aseptic processing (including mechanical shear, liquid-gas and liquid-solid interfaces). Therefore, we classify the stress which the mAb is exposed to as product-stress.

Initially, a study with determination of particle numbers using actual protein material was conducted to analyze the direct impact of

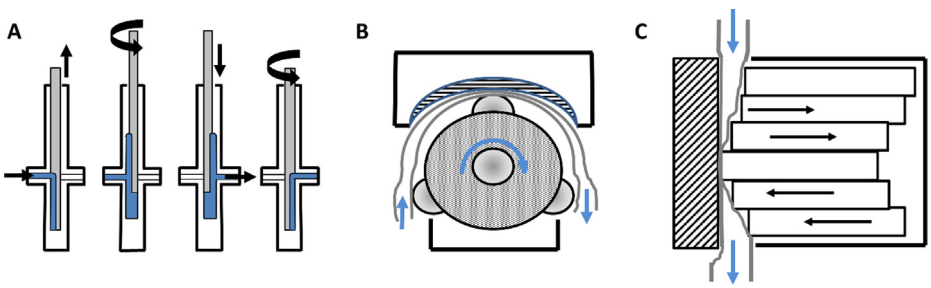

Fig. 1. Schematic presentation of investigated pump types. (A) Rotary Piston Pump (B) Radial Peristaltic Pump (C) Linear peristaltic Pump.

product-stress on two different mAb formulations. Therefore, two mAb formulations were initially filled by all three types of pumps and then recycled up to 15 times. Particle counts, particle morphology and particle identity were analyzed to see if there is a correlation between shear rate in different aseptic filling systems and particle formation.

In addition, a mechanical shear stress sensitive liposomal model was established and used as a novel tool for a direct quantification of mechanical shear stress during low volume aseptic filling. Liposomes are well-established drug delivery systems. They are vesicular in shape with an aqueous inner cavity, formed spontaneously when amphiphatic phospholipids contact water and align into mechanically stable bilayers [14]. It is possible to encapsulate both, (I) hydrophilic substances in the inner cavity and (II) hydrophobic in the lipid bilayer. One way to trigger the release of substances (i.e. dyes or drugs) from liposomes is to engineer liposomes that are sensitive to mechanical stress. Recent research has shown that lenticular shaped liposomes could be used for shear stress dependent targeted drug delivery particularly in the application for atheriosclerosis [15,16]. The idea behind this approach leads to multiple fields of application besides medical usage. For this study, carboxyfluorescein (CF), a well-known fluorescence marker, is encapsulated in the inner cavity of liposomes consisting of 16:0 Dipalmitoylphosphatidylcholine (DPPC) (AvantiLipids) in a self-quenching concentration of 60 mM. When encapsulated in the inner cavity of the liposomes, the dye quenches and no fluorescence signal is detected. Fig. 2 shows a schematic diagram of the liposomal system. After membrane-deformation (i.e. due to exposure to mechanical shear) the dye is released to the outer phase and becomes detectable [17]. The idea is to use this model as a novel tool which allows a quantification of shear stress during different process operations (e.g. filling, mixing). Since the measurement of product-stress is done by the measurement of subvisible particles, the methods show variability when using micro methods. It is common use to pour multiple drug-product container together for particle analysis. Here, the innovative liposomal model could contribute to considerable material savings.

Computational Fluid Dynamics (CFD) was used to gain deeper insight into flow patterns and the related shear stress levels during low volume aseptic filling processes. Based on the exact geometries of different pumping systems CFD data provided a mapping of shear stress and the distribution of shear rate classes during low volume aseptic filling. In this study CFD was used to verify the newly established quantitative liposomal shear stress model and to investigate, further, the different process conditions which may trigger particle formation during low volume high precision aseptic filling. A key part of the studies was to investigate, if there is a relation between observed protein damage and different shear rates depending on the pump type.

2. Materials and methods

2.1. Material

A monoclonal antibody was used as a model protein for these studies. The protein solution containing 20 mM His/His-HCl buffer (pH 5.3) was provided by F. Hoffmann-La Roche Ltd. (Basel, Switzerland). The material used for this study was either formulated at 63.2 mg/mL (formulated without surfactant) and at 150 mg/mL. Formulations were filtered using a 0.22 µm SteriTop filter and stored at 4 °C.

2.2. Liposomal preparation and calibration curve

All Lipids were purchased from Avanti Polar Lipids and used directly without any prior purification. All other compounds were purchased from Sigma Aldrich. Liposomes were prepared as follows: Lipid (10 mmol) was weighed into a 20 mL glass vial and dissolved in 1 mL Chloroform (Sigma Aldrich). Any other excipients such as 5 mol% Cholesterol and 5 mol% DSPE-PEG2000 were added at this point. The composition was chosen based on literature research to find a storage-stable formulation without any false positive dye release deriving from storage instabilities.

The solution was evaporated to dryness overnight under a continuous stream of nitrogen. Carboxyfluorescein-Buffer solution (60 mM 5(6)-carboxyfluorescein, 1X PBS buffer, pH 7.4). The suspension was hydrated in a water bath (T = Tm(Lipid) + 10 °C). The mixture was then extruded 11 times using a barrel extruder (Lipex) and a polycarbonate membrane (200 nm, Whatman). The size of the vesicles was reduced by changing to a smaller polycarbonate membrane (100 nm, Whatman) for a further 20 extrusion steps. The sample was finally purified by size exclusion chromatography (30 mL Sephadex G-50 super fine) in 1X PBS buffer, pH 7.4 and ensured pure 5-(6)-carboxyfluorescein-loaded vesicles.

The liposomal calibration curve was generated by using a plate-cone rheometer (MCR 301 Rheometer, with the cone CP50-0.5 d = 50 mm, angle 0.5°, Anton Paar GmbH, 8054 Graz, Austria). 200 µL of the liposomal preparation was pipetted on the plate and subjected to shear rates from 10 to 2000 1/s for 30 s at 20 °C. The shearing time was experimentally determined. After shearing 50 µL were transferred into a 96 well plate (Nunc) and measured for fluorescence intensity. After measurement, liposomes were solubilized with Triton X to obtain 100% fluorescence values and to rule out any errors from dye loading differences.

All experiments with loaded vesicles were performed within 48 h after preparation.

2.3. Computational Fluid Dynamic (CFD) simulations

Numerical modeling is a proven method to solve theoretical fluid properties which allows investigation into shear rates during a variety

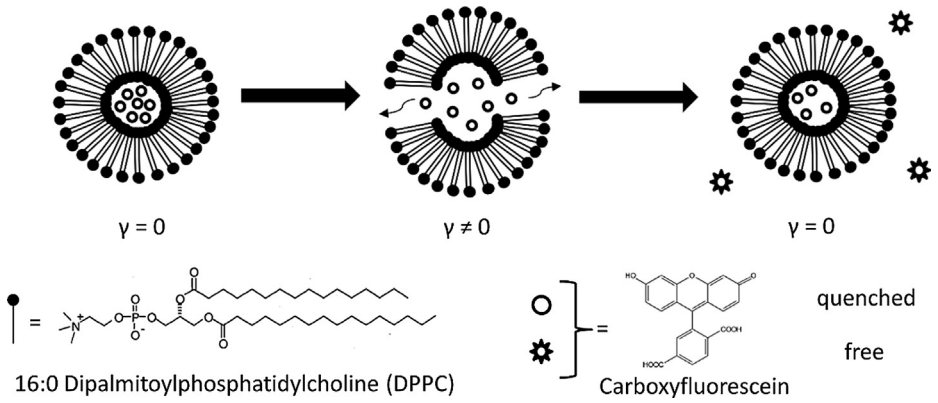

Fig. 2. Release of a fluorescent dye from shear stress sensitive liposomes. The hydrophilic dye carboxyfluorescein is encapsulated in the inner cavity of DPPC liposomes at a self-quenching concentration of 60 mM. When at rest (left) no fluorescence signal is detected. Under shear exposure (middle) the lipid-bilayer membrane is deformed, and the dye is released and a fluorescence signal can be detected. After shear exposure, the liposomal membrane regenerates (right).

of different processes [18,19]. In this study flow patterns during low volume aseptic filling and the corresponding shear rates were calculated and modeled by the means of CFD. The calculations are based on the Navier-Stokes equation and were solved using the commercial Flow-3D v. 11.2 (FlowScience Inc., Santa Fe, USA) CFD software. The chosen pressure solver option is the GMRES (generalized minimum residual method) with enhanced constant subspace size of 25.

The geometries of the three different pump types were modeled based on technical drawings of each pump using primitives (S5). The moving parts of the model are defined as General Moving Objects (GMO). The discretization consists in a structured rectangular grid, a cartesian mesh with cubic cells over the whole considered volume. The cell size was chosen to mesh the thinnest gap between solids with a minimum of 5 cells (typically 50 μm) but not larger than 250 μm.

The drug product liquid is defined as an incompressible fluid with constant viscosity of 1 cP and density of 1 g/cm^3. The simulation resulted in Reynolds numbers from 29 - 3375 implying laminar flow [20,21]. The flow can be considered as laminar after consideration of the Reynolds numbers in the cylindrical parts of the pumps. No additional turbulence model was implemented. A first order approximation for the fluid advection was chosen which proved accurate enough for our purpose.

In all pumps utilizing silicone tubing (radial peristaltic and linear peristaltic), the tubing was defined as a second elastic fluid with high viscosity in addition to the drug product solution. The sharp interface calculation (using the specific Volume of Fluid method of FLOW-3D with Split-Lagrangian-Method) allows the investigator to treat this Fluid-Solid-Interaction problem (with large deformation) with an adequate accuracy for the quantification of the shear rate in the drug product formulation (S6).

The three different processes (for the three pump types) are defined as displacement over time functions of the GMOs: (1) for the piston pump this equates to the displacement of the piston; (2) for the linear peristaltic pump, the maximum displacement of the piezo actuators and (3) for the radial peristaltic pump, the advancement of the rollers along the counter pressure-plate of the pump. For the three models, the boundary conditions are the same: two pressure boundary conditions with atmospheric pressure are chosen for both the fluid inlet and the fluid outlet. Furthermore, a fluid entrance is allowed. The initial conditions of all three models were a complete filling of the calculation domain with fluid at atmospheric pressure and velocity equal to zero. The dispensed volume during the process was compared with the experimental results and allows us to verify the simulation against experimental data. After simulation of the liquid flows, a post-processing analysis of the shear rate field over time was performed for the delivered fluid volume.

Additionally, to investigate further into fluid mechanics during piston pump operation, a tracer was modeled using a so-called marker scalar. This is an advectable scalar field following a general transport equation without specific diffusion. To verify the theoretical modeling, 1.15 mL fluorescein-Na solution (which corresponds to the dead volume of the piston pump) was filled inside the pump cylinder. This volume was replaced by pump operation with purified water. The pump operated with the same parameters (0.15 mL/stroke) as used for the filling study. Every stroke was collected in a 96-well plate and analyzed with a fluorescence plate reader.

2.4. Filling study

For the filling study three different filling systems (Rotary Piston Pump, Bausch & Stroebel; Radial Peristaltic Pump, BOSCH; Linear Peristaltic Pump Prototype [13] were used. The studies were performed following appropriate aseptic preparations (autoclaved tubing and glass material, sterile filtered product formulation and pharmaceutical grade components). For all filling systems, the protein formulation was recirculated up to 15 times. Within the filling study, one cycle represented the required number of strokes (one stroke was set at the lowest possible volume of 0.15 mL and the highest possible speed = 6.67 S/mL) to transfer the whole formulation (100 mL) from one reservoir (bottle) into another. After 0; 1; 5; 10 and 15 recirculation cycles of the whole formulation, 10 mL samples were taken out of the reservoir and collected in formazin-turbidity-unit (FTU) tubes and 6 mL vials. The contents of the FTU tubes were analyzed for turbidity and sub-visible particles by light obscuration directly after filling whereas the samples collected in the vials were crimped and later analyzed for particle morphology and identification.

2.5. Micro flow imaging (MFI)

A DPA 4200 instrument from Protein Simple (San Jose, CA) was used. This Flow Microscope System uses a 5-fold magnification and was equipped with a 100 μm flow cell. Cleanliness and the prevention of sample cross contamination was achieved by rinsing each day with fresh 1% Tergazyme (Alconox Inc., White Plains, NY) solution for 2 min at maximum speed, soaking for 30 min and rinsing with particle-free water overnight. Method parameters were set to: 1 mL sample volume, 0.2 mL as purge volume. The light optimization step was performed with the corresponding filtrated sample matrix. Blank measurements were performed at the beginning and after every 3rd sample using fresh particle-free water. Acceptance criteria, for blanks, was less than 100 particles per mL ≥ 1 μm. The suitability test, each day, consisted of a of 5 μm standard count measurement (COUNT-CAL Count Precision Standards; ThermoScientific) with acceptance limits of ± 10% reported concentration for particles ≥3 μm. Furthermore, the measurement of 5 μm size standard with acceptance limits of the spherical circular diameter mean of ± 25% reported size and standard deviation ≤0.4. MFI View Analysis Suite (MVAS) 1.3 software was used to analyze the particle images and counts.

2.6. Turbidimetry

Samples were prepared by transferring 7 mL of the stressed protein solution under unidirectional airflow (UDAF) into washed and sterilized, particle-free 11 mm glass tubes (Hach Lange GmbH, Düsseldorf Germany). Measurements were performed accordingly to Ph. Eur 2.2.1 using the HACH 2100AN turbidimeter (Hach Company, Loveland, CO). The instrument was calibrated against 3 different reference suspensions.

2.7. Particle identification

The separation of the particles from the stressed samples of the filling study was performed by filtering the samples through a gold-coated filter (pore size 0.8 μm, rap.ID Particle Systems GmbH, Berlin, Germany). Each filter was analyzed under a microscope (Digital Microscope VHX-6000, Keyence Corporation) before and after the filtration step. Fourier-transformation infrared spectroscopy (FTIR) was conducted using a Nicolet 6700 FT-IR spectrometer with a Continuum IR microscope unit (Bruker Optics, Karlsruhe, Germany) using 64 scans and a resolution of 4 cm^{-1}. Data was analyzed with the Omnic 8.3 software (Therma Scientific, Waltham, MA) and compared against a 300.000 entity reference spectra database as well as against reference spectra recorded from tubing material and filter membranes from the filling study.

2.8. Light obscuration HIAC

A ROYCO System 9703, sensor HRLD400HC (Pacific Scientific Co., Washington, DC) was used. The sensor used 780 nm wavelength and was calibrated with polystyrene beads to measure particles in a size range from 1.3 to 300 μm. The sensor limit was set to 18,000 particles/mL. The rinsing volume was set to 0.2 mL and 4 runs, each of 0.4 mL

were performed. The flow rate was set to 10 mL/min. The first run was discarded and the average ± standard deviation of the last 3 runs was reported. Blank measurements (with fresh particle free water) were performed at the beginning of the analysis and repeated after every third sample. The acceptance criteria for blank measurements were set to ≤5 particles ≥1 µm. The integrity of the instrument was tested by means of a 3000 particle count standard (5 µm particle size) (COUNT-CAL Count Precision Standards; ThermoScientific) with acceptance limits of ± 10% of the reported concentration for particles bigger than (5 µm), respectively. PharmaSpec version 3 (HACH Ultra Analytics, Oregon) was the software utilized to collect and analyze the raw data from the sensor.

3. Results

3.1. mAb formulation filling study

During all currently known aseptic filling processes, the drug product is exposed to shear stress. To investigate the influence of shear stress on potential protein degradation, two different mAb formulations were investigated after filling. Both mAb formulations were re-circulated up to 15 times as a worst-case condition to amplify any particle formation deriving from protein degradation or material shedding during the filling process. Recirculated samples (1×, 5×, 10×, 15×) were collected and compared to the control (starting material after sterile filtration) which did not undergo the filling step. The results of the recirculation study are shown in Figs. 3 and 4. There is an increasing trend in subvisible particle formation with an increasing number of re-circulations. The increase in subvisible particle formation is reflected in increasing turbidity. Comparing the three different pump types in terms of subvisible particle formation results in highest particle counts deriving from recirculation in the piston pump, followed - with a substantial difference - by the radial peristaltic and the linear peristaltic.

In order to elucidate subvisible particle formation from the three different pumps, follow up studies were conducted to analyze particle identity and morphology. In order to prove the particles identities are proteinaceous and not exogenous impurities, particle identity analysis was performed using FT-IR. The surface of the gold filter shows a homogenous layer evenly distributed over the whole filter after piston pump recirculation. The majority of observed thin and fine particles clogged all the filter pores resulting in a particle-layer. Three specific spots on the filter prove that the layer is proteinaceous. Another three spots (with larger particles) were analyzed and found to be proteinaceous. The larger particles were found on top of the protein layer after all pores were clogged by the smaller particles. The filter surface (after radial peristaltic recirculation) was different as larger particles could be identified purely by visual inspection. The spectra confirm the proteinaceous character of all particles found on the filter surface. The third filter surface also proved proteinaceous particle identity after linear peristaltic recirculation. In addition to the analysis for the particle identity, the particles' morphology was analyzed. Most of the particle pictures (taken with the MFI software) visually confirm the observed particles as being proteinaceous in origin due to their translucency. Examination of the particle morphology (after recirculation in the different pumps) showed that there were some clear differences in n the size and shape of particles. The selected pictures in Fig. 4 highlight the most representative particle morphology. Piston pump filling operations generate mostly elongated, fine and thin particles. Both peristaltic pumps showed comparable particle morphology: The particles morphology was found to appear clustered with a flake-like structure and occasional a elongated particle structure. The size range distribution of all particles is comparable. For a broader overview of the particle morphology see supplementary data S7.

3.2. Quantitative liposomal shear stress model

To further elucidate the correlation between shear stress and protein

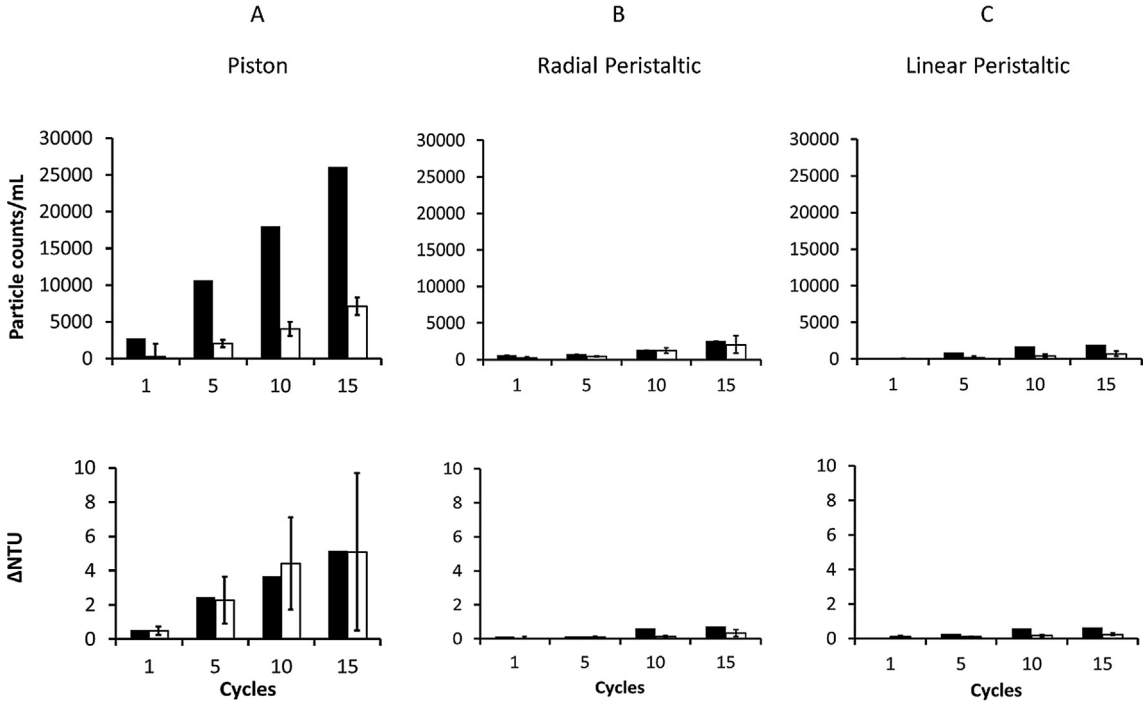

Fig. 3. Effect of pump recirculation on subvisible particle formation and turbidity for two different mAb formulations. Black columns show the mAb formulation without surfactant (65 mg/mL) and therefore more prone to degradation. White columns show the market mAb formulation (150 mg/mL). Upper row: Subvisible particle counts for all particles ≥2 µm. Lower row: Increase in turbidity (nephelometric turbidity unit, NTU) as a measure of aggregation. Differences are shown compared to untreated solution. Due to material restrictions, the experiment with the formulation without surfactant could only be executed once but confirms the trend from the results with market formulation. Results are in the expected order and range based on literature data on other monoclonal antibodies.

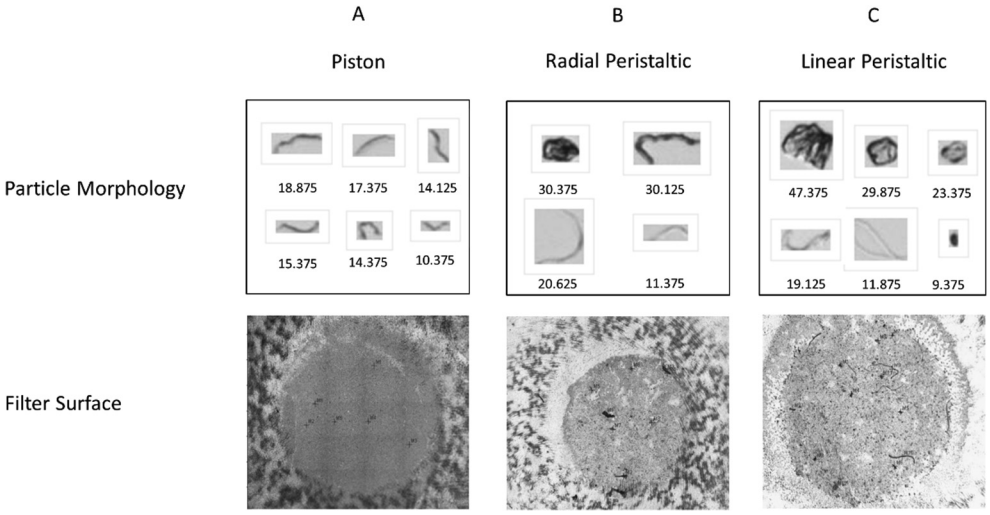

Fig. 4. Particle morphology after recirculation of a monoclonal antibody formulation. Solutions were recirculated 15 times using the indicated pump types. Morphology of protein particles was analyzed by microflow imaging (MFI). Identity of particles collected after by filtration (0.8 µm, rap.ID Particle Systems GmbH, Berlin, Germany) were confirmed by fourier-transformation infrared spectroscopy (Supplement Fig. S2–4).

degradation, a novel model was developed to gain deeper insight into quantitative shear rate levels from the different pumps. The liposomal formulation consisting of 10 mmol DPPC + 5 mol% Cholesterol and DSPE-PEG2000 was selected because it showed very good storage stability at 5 °C. This sample was most ideal in order to obtain a better signal to noise ratio. Recent data suggests that the liposomal formulation is stable for at least 10 h at RT and up to 5d at 4 °C [15]. Prior to any shear stress related experiments, there were preliminary tests performed to examine the background release of the dye from the liposomal carrier over time (data not shown). Fig. 5A shows the calibration curve for the shear-stress sensitive liposomal formulation. It covers shear-rates up to 2000 s^{-1} and includes shear-rate levels in the aseptic fill finish environment [6,7].

The calibration allows a quantification of shear rate levels during low volume aseptic filling. Therefore, the liposomal formulation was filled and re-circulated with the same experimental setup as compared to the mAb formulation filling study (see Section 3.1). After fluorescence analysis, the liposomes were characterized by size and PDI in their formulation to gain further information on aggregation or degradation of the liposomes. PDI and size remain stable during the shear studies (data not shown). The radial peristaltic pump triggers the highest release of the fluorescence dye followed by the piston and the linear peristaltic pump. Plotting the data into the calibration curve enabled the quantification of shear rate levels during aseptic low volume filling. Experimental data showed that shear rate levels (for the radial peristaltic pump) appear to be a magnitude of ~20 times higher compared to the piston pump we used during the study. The linear peristaltic pump showed only a shear rate of 5.48 s^{-1} which is significantly less (Fig. 5B).

3.3. Computational Fluid Dynamic (CFD) simulations

Technically, it is very difficult to obtain information about the fluid dynamics inside the dosing systems. In order to determine fluid dynamics in this area, CFD simulation can become a promising and supportive tool. CFD provides data about aspects or phenomena which cannot be directly measured and makes them accessible for further investigation and interpretation. However, it was important that the studies verified the results with experimental data to avoid misinterpretation of the numeric modeling data. Since the results of the novel quantitative liposomal model revealed that despite highest particle counts in the piston pump, the shear rate was not the highest of all three pumps tested. To investigate further into the possible root cause for protein damage, during low volume aseptic filling, there was a follow up study by means of CFD.

Based on the technical drawings of each pump, the shear rate and velocity (during low volume aseptic filling) was simulated and compared to the experimental results. Fig. 6 shows the shear rate and the corresponding fluid velocities for two consecutive states of motion in the different filling systems. This analysis allows an estimation regarding shear rate levels during low volume filling depending on the pump type.

The shear rate in the radial peristaltic pump is approximately 10–20× higher compared to the piston pump. In contrast to previous findings, CFD analysis shows the highest shear rates from the linear

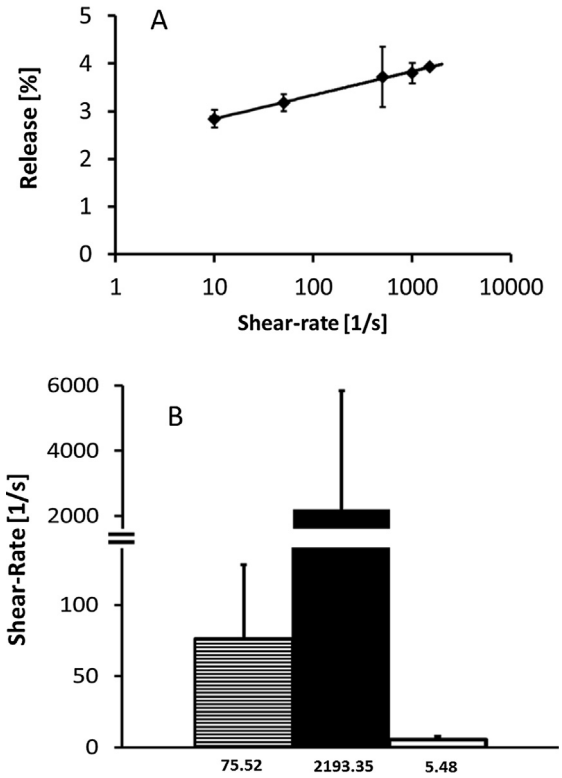

Fig. 5. (A) Release of a fluorescence dye from shear stress sensitive liposomes at different shear rates. Liposomes (10 mmol DPPC + 5 mol% Cholesterol and DSPE-PEG2000) were subjected to shear rates from 10 to 2000 1/s (depicted at logarithmic scale). 100% release is defined as signal obtained after Triton X triggered solubilization. Representative experiment is shown (R^2 = 0.9972) as n = 3. (B) Quantitative shear rate results after 1 filling cycle. Showing from left to right: Piston Pump, Radial Peristaltic, Linear Peristaltic. Results are displayed as means (n = 3).

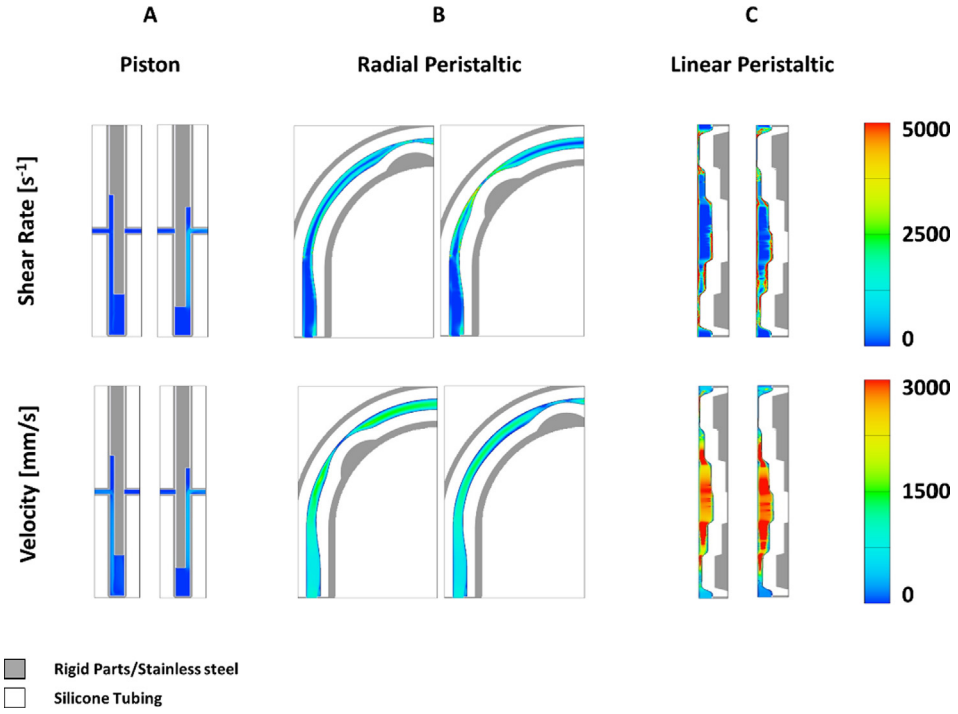

Fig. 6. Computational fluid dynamics (CFD) analysis for the tested pump types. Upper row: Shear rates during typical phases of operation (Color scale: 0–5000 1/s). Lower row: fluid velocity during operation (Color scale: 0–3000x/x). Color bars: values increase with increasing color temperature.

peristaltic pump prototype. This observation confirms that the high fluid velocities are being induced by the rapid movement of the six piezo actuators inside the pump. Since the mAb formulation study showed the highest subvisible particle counts after piston pump operation it was decided that the dosing process itself required analysis in more detail. The small interspace between the piston and cylinder and its contribution to protein damage is heavily discussed within the pharmaceutical industry [5,6,22].Therefore, a detailed model was developed to investigate this knowledge gap further. It was considered that the liquid flow is mainly defined by the rotation and the vertical movement of the piston in the cylinder resulting in a Taylor-Couette-like flow. The consideration of this interspace, creating a high shear zone could not be confirmed by this studies CFD analysis. The impact of the relative movement (translation) of the piston along the inner surface of the cylinder was analyzed. In addition, the rotational movement of the piston inside the cylinder (180°) was investigated. The data could not reveal any shear-stress levels which would explain the high subvisible particle counts. We could show that the rotation of the piston contributes to the higher shear rates up to 325 1/s, whereas the translational movement contributes to shear rates down to 200 1/s (S8). In a further experiment a “tracer” was simulated by the means of a marker scalar which allows the tracking of the fluid through the entire dosing process. Fig. 7A shows the decrease of the marker-scalar (light-grey) with increased strokes of the piston pump. This simulation was then repeated experimentally with Fluorescein-Na-Solution (0.0003 mg/mL) (Fig. 7A). Both graphs show a comparable dynamic and furthermore reveal that the piston pump generates a “recirculation-zone” inside the cylinder where the protein formulation is exposed multiple times to a constant but lower shear rate compared to the two other pumps. Even after more than 20 pump hubs there is still 22% protein solution inside the cylinder. To compare the different shear rate levels in the three pump types we introduce a new parameter called “mean volume fatigue”. Since fluid dynamics remain constant during filling, this parameter allows us to estimate the stress level a protein solution is exposed to at an arbitrary timepoint during the aseptic fill for 30 pump cycles. Fatigue is a well know parameter in material science, describing the weakening of a material after repeatedly applied

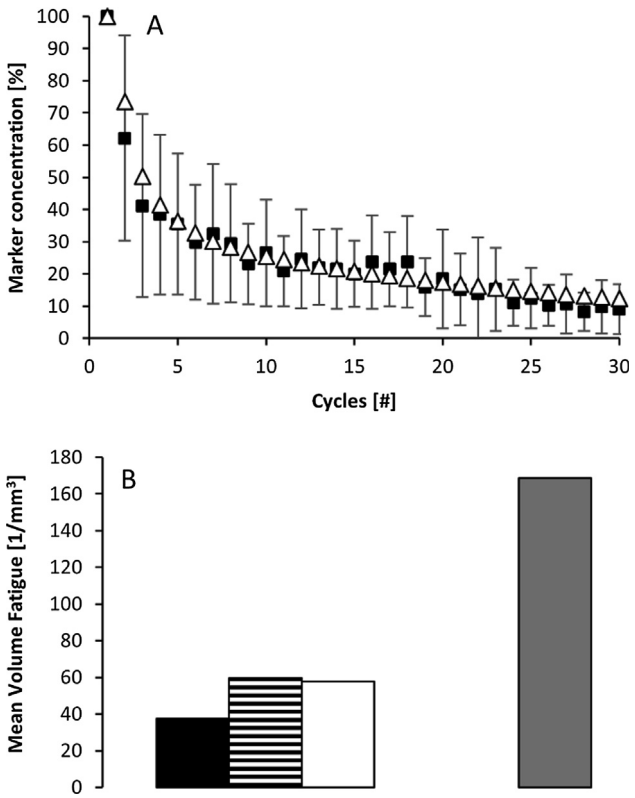

Fig. 7. (A) Recirculation of fluorescein-Na solution (0.0003 mg/mL) (black) showing the recirculation zone in the piston pump compared to simulation data using a marker scalar (white). Measurement was performed as n = 3 showing means with ± SD. (B) Mean Volume Fatigue of the three pump systems tested. Showing from left to right the results of the piston pump, the radial peristaltic pump, the linear peristaltic pump and the piston pump with the recirculation over 30 circles included.

use or load. We analyzed each filling process by measuring the volume fraction of the total fill volume with their corresponding shear rates. After considering the integral shear of one filling operation we were able to calculate the shear stress after one single filling event. The mean volume fatigue shows that after one fill cycle the shear rate levels are comparable in all three pump types (Fig. 7B). By taking into account our previous findings of the recirculation zone inside the piston pump we included the repeatedly sheared volume fractions in the calculation and could show a cumulative mean volume fatigue for the piston pump, which will end up with the highest particle counts as reported.

4. Discussion

Based on the drug product filling study (Fig. 3) we can conclude that the piston pump generates the highest sub-visible particle count for all particles ≥2 µm, whereas both peristaltic pumps had significantly less particle counts. Each pump type, tested, revealed specific zones with increased shear rate levels. During piston pump operation, the interface between piston and cylinder could be linked to higher shear rates during 180° rotation of the piston compared to the vertical movement of the piston alongside the cylinder wall. Furthermore, a recirculation zone at the bottom of the cylinder could be identified, where the fluid exchange is very poor and leads to multiple shear stress exposure of the fluid. Both peristaltic pumps show elevated shear rates where the tubing material is compressed by the rollers (radial peristaltic) or piezo actuators (linear peristaltic) (see Fig. 6). The elevated shear rates occur close to the tubing wall, whereas the fluid, which is in the center of the tubing, shows reduced shear stress exposure due to less exposure to friction. These individual “risk-zones” are reflected by the different particle counts and particle morphology deriving from each filling system. The highest subvisible particle counts were found after piston pump filling resulting in counts up to 25,000 particles ≥2 µm/mL. This was somehow expected and reflects the information within the literature. Compared to both peristaltic pumps, the counts for the piston pump were 20 times higher (see Fig. 3). The trend in particle formation is also reflected in turbidity analysis which shows equivalent results. Regarding particle morphology, MFI data shows that piston pump filling generates rather small fibre-like particles. The analysis of the particle shape and identity using FT-IR (Fig. 4B) confirms the MFI morphology data. A homogeneous particulate film can be seen on the filter surface which has clogged all filter pores. On this particle film, individual larger particles could attach themselves. All particles found consist of protein and were cross referenced against a database. (Supplement 2–4). The morphology of particles from both peristaltic pumps shows rather clustered and flake-like structured particles with occasional elongated particles. These are sporadically visible on the filter surface and can be identified as protein particles. The particle size distribution was found to be comparable in each investigated pump type. No tubing particles or other extrinsic particles could be found (4B). In addition to the protein particles, a protein-silicone particle was found and identified on each filter. However, this is expected to originate from siliconized rubber stoppers, which were used for sampling in 6 mL vials and therefore has no significance for the interpretation of the data. The reason for the different particle morphology is assumed to be linked to the different construction and operation of the pumps and must therefore, be confirmed by further investigations and studies. Regarding the influence of the shear-rate during aseptic filling there is an ongoing controversial discussion.

Mechanical shear stress alone does not lead to protein degradation or unfolding [23]. An unfolding event is required which can be due to different multiple effects like charge-charge, liquid-air or liquid-solid interfaces. These effects which are different for all three pumps investigated in this study are different.

Another important factor for subvisible particle formation is the presence of dissolved air bubbles in protein solutions. Fuller et al. investigated the impact of air bubble dilatation which is directly translatable to situations in the aseptic manufacturing or transportation of protein solutions when thermal or pressure fluctuations lead to volume changes of air bubbles [24]. During our experiments we also investigated about the impact of possible cavitation effects. At 23 °C, the cavitation pressure of water is about −7000 Pa relative pressure and the lowest relative pressure calculated is not below than 70 Pa. Therefore, cavitation (as well as local volume and pressure fluctuations) should be a neglectable factor during our study. We can conclude that each pump will generate protein degradation to an extent which depends on their operation mode and the number of re-circulations. The piston pump is suspected of producing a high shear zone in the gap between the piston and cylinder, which leads to increased drug product degradation [5,22]. The positive displacement of the piston leads to the creation of reconstruction of liquid-solid interfaces. It is known from literature, that forces exerted on proteins adsorbed at the liquid interface are within the range needed for protein degradation [25]. The repeatedly created liquid-solid interfaces may play an important role for the particle formation in this study [9,26]. The radial peristaltic pump may produce tubing abrasion (as material stress) as well as protein degradation due to the friction of tubing material when squeezed by the rollers and the counterpressure-plate [12]. The linear peristaltic pump has the advantage of reducing protein damage and tubing abrasion, as the fluid is conveyed only by vertical squeezing of the tubing (see Fig. 3). The novel and innovative approach of the experimental determination of shear stress using shear-stress sensitive liposomes could reveal that despite higher particle counts, the shear rate in the piston pump was not the highest of all three pumps. The shear rate in the radial peristaltic pump was 10–20 times higher compared to the piston pump, whereas the shear rate in the linear peristaltic pump can be neglected. The root cause for protein degradation and particle formation during low volume filling (with a rotary piston pump) was identified by a recirculation zone with a poor fluid exchange inside the cylinder of the piston pump, by the means of CFD simulation (Fig. 7). Therefore, the protein solution is exposed multiple times to a shear rate which is lower compared to the radial peristaltic pump but overall higher considering the multiple shear cycles of the same fraction of volume (cumulative shear stress). On top of that, the piston pump creates additional protein degradation effects caused by e.g. liquid-solid interfaces, which are enhanced by the recirculation zone. This phenomenon of a recirculation zone could not be observed in both peristaltic pumps and remains exclusive for the tested piston pump tested. The introduction of a new parameter called “mean volume fatigue” could help to deepen the understanding of mechanisms during low volume aseptic filling taking into account different volume fractions exposed to their individual shear rates. The CFD data also confirmed the 10–20 times higher shear rate of the radial peristaltic pump compared to the rotary piston pump. The only deviation between CFD data and shear sensitive liposomal model was the shear rate observed for the linear peristaltic pump. The low shear stress value observed with the liposomal model can be explained by the liposome’s exposure time to the shear stress. The linear peristaltic pump operates at a frequency of 30 Hz which is not comparable to state-of-the-art filling systems. Therefore, the time under shear is not sufficient to deform the liposomal membrane and allow a release of the fluorescence dye. Additionally, we can conclude that the liposomal shear stress model works well under mechanical shear conditions. When it comes to possible protein degradation, multiple factors have to be taken into account and cannot be reduced to mechanical shear. Therefore, the liposomal model has its limitations, but showed its potential as a predictive tool for mechanical shear determined processes.

Considering all the information gained during the studies it could be shown that CFD simulation is able to predict for example shear rate levels during aseptic filling of parenterals. This could serve as an effective future tool in order to understand the mechanics of filling processes and predict the impact on drug product formulations. The combination of the different tools such as mechanical shear stress

sensitive liposomes, CFD simulations and drug product stability data allows a characterization of the process impact on drug product formulations: The drug product formulation could be stressed with increasing shear rates until an elevated amount of drug product degradation is observed. The shear stress sensitive liposomal model in combination with the CFD simulation could serve as a prediction tool for shear rates and shear stress time in various filling systems and could therefore help to identify the most suitable filling system for the desired drug product formulation.

5. Conclusion

Low volume filling of parenterals is an essential unit operation of the manufacturing process in the pharmaceutical industry which is receiving continually increasing attention. During aseptic fill finish processes, different pump types are considered state of the art regarding fill volumes ≤100 µL. Therefore, care should be taken to ensure the most appropriate choice of pump in terms of dosing accuracy and potential product stress.

Using the proposed three-tiered test-strategy, it can be stated that shear stress for different types of pumps could be characterized and reported. The initial study shows that protein degradation depends, to a large extent, on the type of pump used. Low volume filling (using a rotary piston pump) resulted in the most notable protein degradation compared to all three pumps tested. In a further investigation an experimental liposomal model was established which is sensitive to shear stress and thus allows a direct quantification of the shear rate. This model reveals that the radial peristaltic pump operates at higher shear rates than the rotary piston pump. This was to a certain extent unexpected because available literature clearly states that there is a tendency for rotary piston pumps to damage biologic drug product formulations [5,7]. One of the study intentions was to resolve the conflict between high shear rates, implying drug product damage (to be expected during the use of a radial peristaltic pump), but also confirming literature information stating that more product damage is created by a rotary piston pump. The explanation for this inconsistency between model and reality could be found by using a CFD model.

The CFD model reliably simulated the impact of mechanical shear during the filling process of the three different pump types (representing parts of the overall product stress). Using this approach, we could show that the piston pump generates a re-circulation zone, which promotes further protein degradation by a repetitive exposure to surfaces creating shear stress. The results were verified with additional experimental data using a fluorescence marker. This data revealed an incomplete fluid exchange in the cylinder of the piston pump, leading to repetitive product stress exposure.

In combination of all three studies (product filling study, CFD simulation, liposomal model) a correlation between protein damage, experimental shear stress measurements and CFD data could be established. Finally, we can state that the combination of all three models can be used to optimize aseptic filling processes in particular regarding fill volumes ≤100 µL.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.12.006.

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Chapter III

Assessment of sensor concepts for a 100 % in-process control of low volume aseptic fill finish processes

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Highlights: The gravimetric in-process control is the current state-of-the-art method to determine the fill volume during aseptic filling. Nevertheless, this method reaches the limits of accuracy and robustness when it comes to fill volumes < 200 µL. This study evaluates and tests novel sensors, which allow an accurate and precise 100 % contact-free measurement of drug-product formulation with respect to fill volumes. Finally, a promising sensor was identified as serious alternative for the in-process control of fill volumes < 200 µL.

RESEARCH

Assessment of Sensor Concepts for 100% In-Process Control of Low-Volume Aseptic Fill-Finish Processes

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ABSTRACT: The pharmaceutical industry is currently being confronted with new and complex challenges regarding the aseptic filling of parenterals, especially monoclonal antibodies, particularly for fill volumes <200 µL, which have become increasingly important with the increasing and continued development of intravitreal drugs and highly concentrated formulations. Not only does low-volume filling pose challenges to aseptic manufacturing, but the development of suitable in-process control to ensure reliable and robust filling processes for low-volume conditions has also been difficult. In particular, fill volumes <200 µL exceed limits of accuracy and robustness for the well-established method of gravimetric fill-volume control. Therefore, the present study aimed to evaluate and test novel sensors, which may allow the accurate and precise 100% contact-free measurement of drug-product formulations, with respect to filling volumes. These sensors were designed to be less influenced by inevitable noise factors, such as unidirectional airflow and vibrations. We designed the study using five different sensor concepts, to screen and identify suitable alternatives to gravimetric fill-volume control. The examined sensor concepts were based on airflow, capacitive pressure, light obscuration. and capacitive measurements. Our results demonstrated that all of the tested sensor types worked in the desired low-volume range of 10–150 µL and showed remarkable results, in terms of accuracy and precision, when compared with a high-precision gravimetric balance. A sensor based on capacitance measurement was identified as the most promising candidate for future sensor implementation into an aseptic filling line. This sensor design proved to be superior in terms of both sensitivity and precision compared with the other tested sensors. We concluded that this technology may allow the pharmaceutical industry to overcome existing challenges with respect to the reliable measurement of aseptic fill volumes <200 µL. This technology has the potential to fundamentally change how the pharmaceutical industry verifies fill volumes by facilitating 100% in-process control, even at high machine speeds.

KEYWORDS: Low-volume filling, Microdosing, In-process control, IPC, gravimetric IPC, sensor concepts, capacitance.

1. Introduction

The low-volume filling of parenterals, including monoclonal antibody formulations such as Lucentis and Eylea, is of increasing future importance within the pharmaceutical industry (1, 2). Because of the high demand for intravitreal ocular treatments for diseases including age-related macular degeneration and diabetic macular edema, the development of suitable aseptic filling processes is of extraordinary importance (3). The typical administration volumes used for intravitreal applications range between 50 and 100 µL (4), and the typical fill volume used for intravitreal drug products is 150–250 µL because existing filling systems currently struggle to deliver lower fill volumes with acceptable accuracy and precision (5). The therapist who delivers the treatment is expected to downdose the higher fill volume to the recommended therapeutic dose.

A complete filling line consists of multiple compartments, which each serve a distinct purpose within the entire aseptic manufacturing process (i.e., washing machine, heat tunnel, and crimping station) (6). In

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addition to the necessity of a suitable filling system that is capable of handling fill volumes <200 µL, the specification and assurance of the in-process fill-volume control must also be assessed (7). Various methods are currently available that have been designed specifically for liquid handling, and these methods differ in terms of accuracy, speed, hygiene, and costs.

1.1. Mass Flow Measurement

Mass flow measurement is based on the Coriolis force principle (8). The liquid is conveyed forward, through two vibrating pipes, and the Coriolis force acts on the pair of pipes, generating a phase shift in the vibrations. This phase shift allows the mass of the liquid passing through the pipes to be calculated, facilitating fill-volume determination. Coriolis meters are very accurate (9); however, because the flow meter must be installed before the point of fill and is, therefore, susceptible to the variabilities that occur after the control point established by the flow meter, the use of this method has not been broadly established in the industry. In addition, the effort required to maintain and clean a flow sensor is much higher than that for other methods. Because the flow meter comes into direct contact with the drug product, it must be subjected to a validated cleaning and sterilization process. During maintenance, the flow meter must be fully removed from the filling line and then reintegrated when maintenance has been completed.

1.2. Gravimetric Measurement

The current state-of-the-art method is the gravimetric measurement, which represents the most precise method available and is suitable for virtually any liquid. Here, the fill volume is controlled by a gravimetric balance, typically a load cell (8). During aseptic vial filling, the vials are weighed when empty, and the tare weight is measured. Then the vial is transported beneath a filling needle, filled, and transported to a second balance, where the gross weight is measured. To determine the fill volume, the net weight is multiplied by the density of the drug product formulation. This fill-volume analysis process represents a well-established and common practice in the pharmaceutical industry that is applied to the aseptic filling of volumes >200 µL. Unfortunately, gravimetric in-process control (IPC) poses multiple new challenges when applied to fill volumes <200 µL, which are common for highly concentrated drug product formulations and formulations designed for intravitreal administrations.

Challenge 1: Unidirectional Airflow

In an aseptic fill-finish environment, the drug product formulations are usually filled in isolators to protect both the drug product and humans from direct contact and potential contamination. Isolators are supplied with air quality regulators, according to ISO 5 specifications, that facilitate the uncompromised, continuous isolation of the isolator interior from the external environment. To minimize airborne particles in the supplied air, high efficiency particulate air filters are installed and the particle load in the isolator is minimized by the use of unidirectional airflow (UDAF) (0.45 m/s ± 20%) (10). The UDAF and its variability directly influence the gravimetric measurement of the fill volume by creating a vertical variable force vector on the load cell.

Challenge 2: Influences by Vibrations

As filling equipment improved and container sizes increased, the production rate of the systems also increased. Some large filling lines that are currently in operation consist of different manufacturing modules with a length of several meters. As the sizes of the filling lines and the numbers of moving parts (i.e., robotic arms and roller bands) increase, the frequency of influencing factors that may affect the process, such as vibrations, also increases. Vibration can be a major problem during aseptic filling; therefore, isolators are bolted to the floor to secure them more permanently. To minimize the influence of vibration on gravimetric IPCs to the greatest extent possible, the load cells are decoupled from the machine as strictly as possible (11). However, filling lines are known to transmit vibrations to gravimetric IPC systems. Some suppliers of IPC systems, such as Wipotec, have developed and offered a system that attempts to compensate for vibrations during the operation of the filling line. Active vibration compensation (AVC) technology uses intelligent algorithms to ensure precise gravimetric measurements. AVC technology focuses on vibrations lower than 10 Hz, which are the primary vibrations that affect the measurement signal. Vibration frequencies above 10 Hz are compensated by a low-pass filter (12). When attempting fill volumes <200 µL, the vibrations from the filling line, together with the laminar airflow, play pivotal roles during gravimetric IPC processes. These

effects have reduced impacts for fill volumes ≥1 mL because the signal-to-noise ratio is large enough for these effects to be negligible. However, for fill volumes ≤200 µL, these influences cannot be neglected.

To guarantee label claims regarding volumes and efficacy, pharmaceutical manufacturers must increase vial fill volumes to ensure that the recommended dose can be administered to the patient and the loss of drug product that occurs during administration (e.g., hold-up volume and dead volume in the syringe) is properly compensated. Additionally, variabilities that may occur during the filling process must also be accounted for. Because of the existence of various influencing factors, including UDAF and vibrations, that can directly influence gravimetric IPC systems during aseptic filling processes, the delivery of a robust process for the IPC of fill volumes <200 µL remains a challenge for the pharmaceutical industry. Deviations caused by UDAF and vibrations often exceed the acceptance criteria for fill-volume accuracy, which requires the submission of appropriate justifications to health and regulatory authorities. USP General Chapter <1151> Pharmaceutical Dosage Forms provides excess volume recommendations for mobile and viscous liquids for a wide range of fill volumes, noting that the excess volumes recommended are usually sufficient to permit the withdrawal and administration of the labeled drug product volumes (13).

This study aimed to assess different sensor technologies for the continuous nongravimetric determination of fill volumes for application with a very accurate, low-volume filling system (linear peristaltic pump) (14). The target volume range was set to 15–100 µL, which reflects a typical range for an average single intravitreal injection dose (1, 2).

2. Materials and Methods

For each sensor type, a prototype installation was constructed and added to an existing peristaltic dispensing system. The performances of the different sensors were investigated individually. Each sensor signal was analog/digital (A/D)-converted and stored. An XPE105 balance (Mettler Toledo) combined with gravimetric software (GraviDrop, BioFluidix GmbH, Freiburg, Germany) was used during all dispensing experiments to facilitate comparisons between the sensor signals and the gravimetric reference measurements. All dispensing experiments were performed using distilled water as a model solution. Distilled water was found to reflect most viscosities for therapeutic monoclonal antibody formulations designed for intravenous use at protein concentrations <100 mg/mL. The formulation viscosity is dependent on the protein concentration and was found to be <3 cP for protein concentration <100 mg/mL (15). For all data analyses, the mass density of the distilled water was assumed to be $\delta_{H20} = 1\ g\ cm^{-3}$; thus, 1 μl_{H20} = 1 mg. The gravimetric measurement results were, thus, directly converted from milligrams into microliters.

2.1. Experimental Setup

The experimental setup used for this study and the location of each investigated sensor is described in Figure 1.

2.2. Sensor Principles

The working principle of each sensor is described below.

2.2.1. Airflow Sensor: The airflow sensor uses thermal mass flow to measure air displaced because of to the venting of the reservoir during the dispensing process. A central heating element warms the adjacent air, which is monitored by two neighboring thermocouples (Table I, Airflow, a). As shown in Table I, Airflow b, the gas flow shifts the thermal distribution in the direction of the airflow, resulting in a decreasing temperature on the upwind side and an increasing temperature on the downwind side. This change in temperature difference can be measured and transformed into an electrical signal, which corresponds to the airflow.

The sensor housing contains an inlet, a dampener, the sensor itself, and an outlet to the reservoir. To assess the dispensing volume, the airflow sensor is connected to the reservoir. Each dispensing event causes the fluid level to drop within the reservoir, causing air to flow into the reservoir. The volume of the air that flows into the reservoir is identical to the dispensed fluidic volume. During postprocessing, the integration of the airflow over time can be used to retrieve the air volume. Air flows through the inlet, the dampener, and the thermal mass flow sensor and flows out through the outlet into the reservoir (and vice versa). The dampener is used to reduce the influence of turbulence. The electronics convert the measured temperatures and the

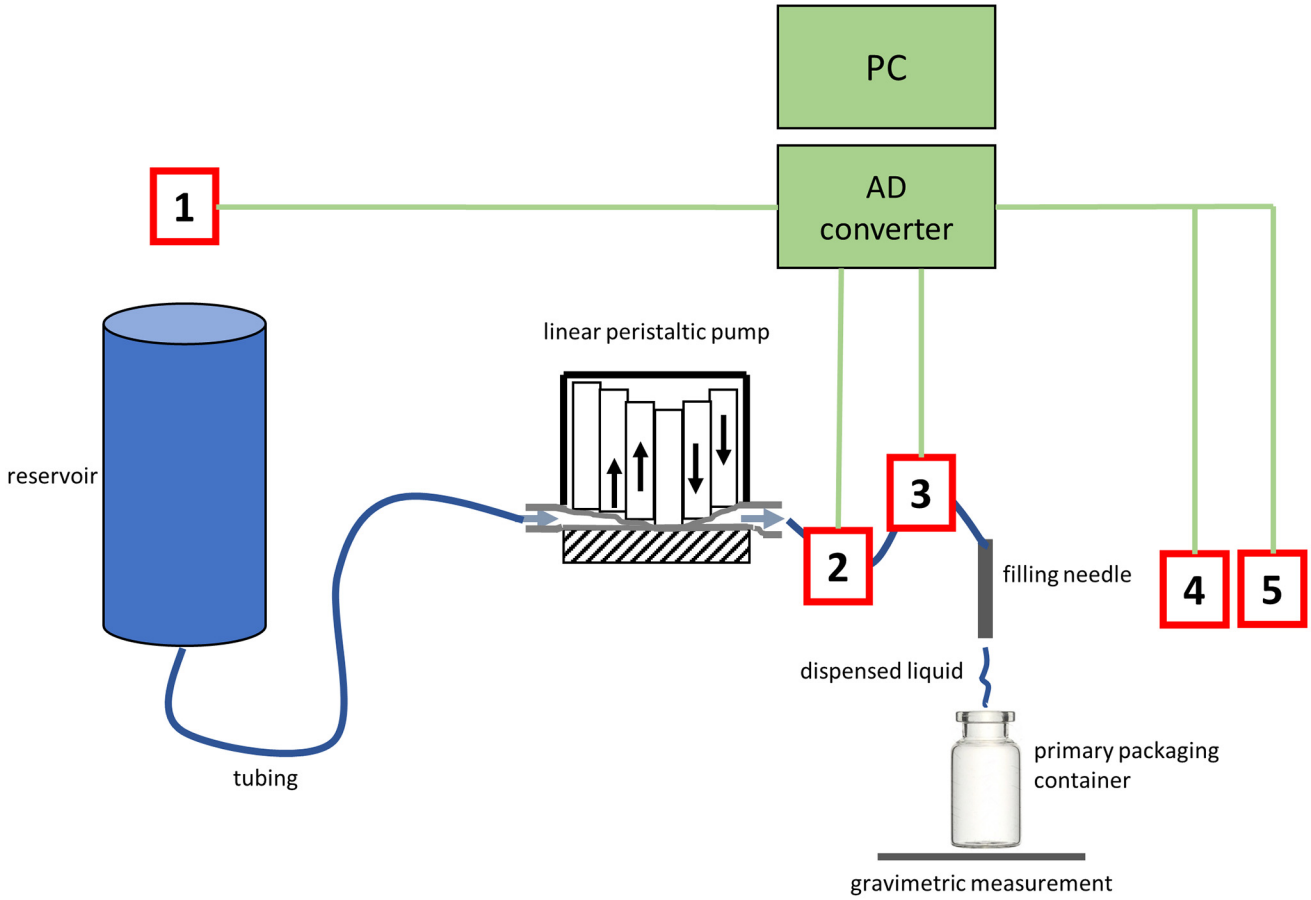


Figure 1

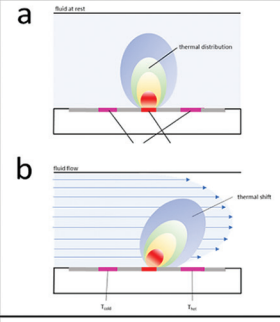
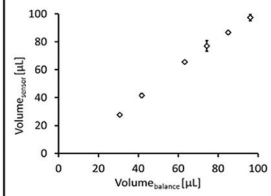
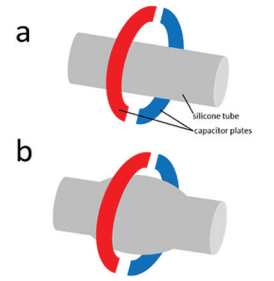
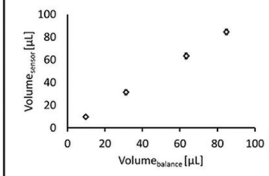
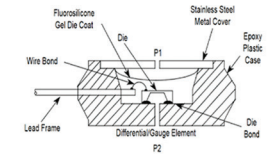
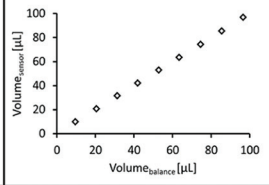
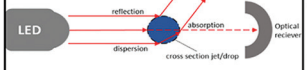
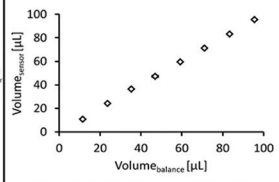
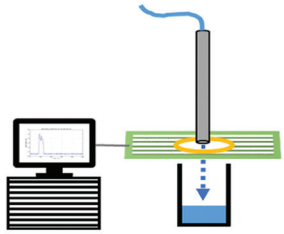
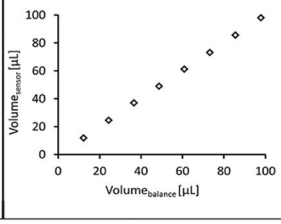
Schematic overview of the experimental setup. The system electronics are displayed in green, the tested sensors are shown in red, and the filling pathway is shown in blue. Five different sensors were tested: (1) airflow into the reservoir, (2) capacitive pressure sensor (differential and relative), (3) MEMS sensor (differential and relative), (4) optical sensor, and (5) capacitive sensor. The positions of the sensors indicate their positions relative to the filling system. The airflow sensor (1) was at the reservoir, outside of the filling line. The pressure sensors (2 and 3) were in-line with the filling system, connected to the tubing. The optical and capacitive sensors (4 and 5) were outside of the filling system, at the filling needle orifice.

temperature difference into electrical potential, which is recorded by the A/D converter board for analyses.

2.2.2. Capacitive Pressure Sensor: The sensor principle of the BioFluidix' capacitive pressure sensor makes use of a specifically designed silicone tube, which is placed inside of two semicircular metal plates, forming a capacitor (see Table I, capacitive pressure, a). This sensor is installed between the dispensing system and the filling needle, as a part of the fluid path. Increases in pressure within the silicone tube cause the tube to expand, resulting in an increase in the liquid within the expanded volume (b). This increase in liquid, which represents an increase in permittivity, changes the capacitance of the measuring cell, which subsequently causes a pressure increase. Specifically designed electronics allow for the highly sensitive detection of small changes in capacitance and can transfer these capacitance values into readable electrical voltage levels (16, 17).

To measure the dispensed volume, two different measurement setups can be applied (Figure 2). The first setup takes advantage of the pressure increase relative to the ambient pressure, using a single pressure sensor that is placed between the peristaltic pump and the dispensing needle (Figure 2A). In this setup, the dispensing needle itself provides the fluidic resistance ($r_{needle} \ll r_{tubing}$), which causes the pressure to increase during filling. This pressure increase is proportional to

TABLE I
Matrix Overview of All Investigated Sensors[a]

Sensor Type	Working Principle	Sensor Performance	Accuracy at 60 µL target fill	Precision at 60 µL target fill
Airflow[b]			3.4%	+/- 1.1%
Capacitive pressure[c]			0.0%	+/- 2.9%
MEMS[c]			0.0%	+/- 0.6%
Optical[d]			-0.1%	+/- 1.1%
Capacitance[d]			-0.3%	+/- 0.3%

[a]Column 1 indicates the sensor type, column 2 shows the underlying working principle of each sensor, column 3 presents the sensor performance tested against a high-precision gravimetric balance (Mettler Toledo XPE105), and columns 4 and 5 show the accuracy and precision, respectively, for each sensor at a representative target fill volume of 60 µL.
[b]Indirect measurement sensor.
[c]Pressure-based sensors.
[d]Sensors located at the orifice of the filling needle.

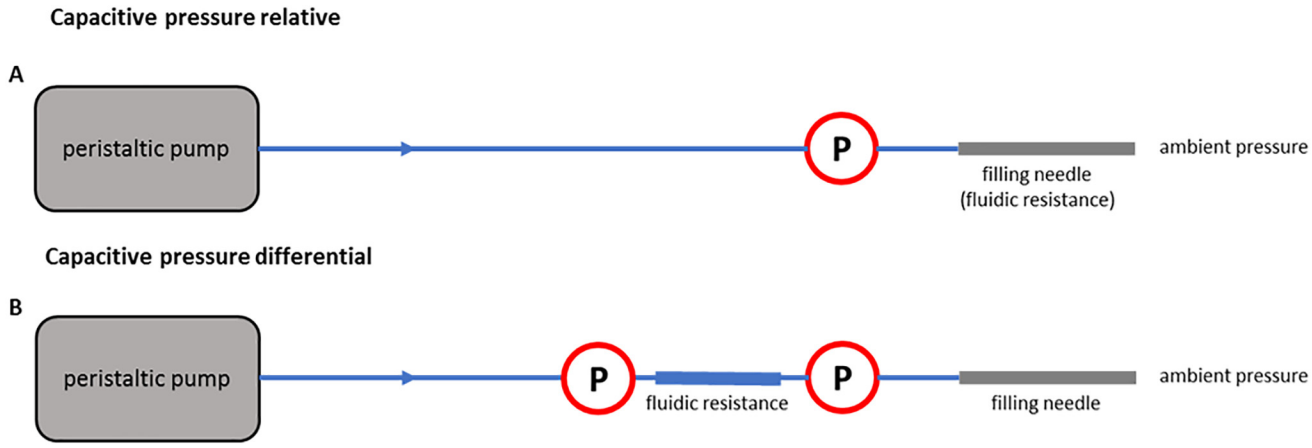


Figure 2

Experimental Setup for the capacitive pressure sensor. Schematic drawing showing the setup installation of one and two capacitive pressure sensors, measuring relative (A) and differential (B) pressure increases over a fluidic resistance. The relative system utilizes the filling needle to provide fluidic resistance and compares the increase in pressure in the fluid line with the ambient pressure, and the pressure change can be used to determine the fluidic volume based on the Hagen-Poiseuille formula. The differential system uses the differential pressures measured by two different sensors placed on either side of an additional fluidic resistance element.

the volumetric flow through the dispensing needle, according to the Hagen-Poiseuille law, which describes the pressure increase that occurs when a Newtonian fluid flows through a cylinder (premise: laminar flow). To select a suitable pressure sensor, the expected pressure increase over the dispensing needle can be estimated using the Hagen-Poiseuille formula. The inner diameter, r, of the dispensing needle was measured as 300 µm. The length, L, of the needle was 145 mm. The average flow rate of the pump was roughly 50 µl per 200 ms; thus the flow value (Q) = 250 µl/s. The dynamic viscosity, η, of water is 1.0 mPa·s. According to the Hagen-Poiseuille equation, the expected pressure increase can be calculated as:

$$\Delta P = \frac{8\mu L Q}{\pi R^4} = 11.4\ kPa = 114\ mbar \qquad (1)$$

Because the peristaltic pump used for this experiment does not provide a perfect, constant, fluidic flow, pressure peaks beyond and below this estimated pressure value were also considered for the selection of a suitable pressure sensor type and pressure range. The pressure signal was analyzed by a base level calculation of the sensor signal, followed by an integration of the measurement sensor signal after base level subtraction.

The second setup (Figure 2B) relies on the differential pressure measured between two pressure sensors, one before and one after an element that applies additional fluidic resistance (a stainless-steel pipe) between the peristaltic pump and the dispensing needle. The additional fluidic resistance was chosen to be comparable to the fluidic resistance of the dispensing needle. The differential pressure between the two sensors was derived by subtracting the pressure values and is proportional to the fluidic flow through the added fluidic resistance element. The dispensed volume can be derived from an integration of the flow over time, which is comparable to the relative pressure setup.

2.2.3. MEMS Sensor: The MPXV pressure sensors from Freescale Semiconductor use the piezoresistive effect and are made of a monolithic silicon material. The sensor die is protected by a synthetic rubber coat to avoid condensation. The sensors are designed for a wide range of applications. The output signal is an analog voltage, which is proportional to the applied pressure, in the range of 0 to 5 V. Table I, MEMS shows a sectional view of the MPXV5050 sensor with a pressure range from 0 to 500 mbar (18). Similar to the capacitive pressure sensor, two possible setups can be used to measure either the relative pressure (Figure 3A) over the dispensing needle or the differential pressure (Figure 3B) on either end of an additional fluidic resistance element. In contrast with the capacitive pressure sensor, the pressure sensors cannot be placed inline but, instead, require the installation of a T-

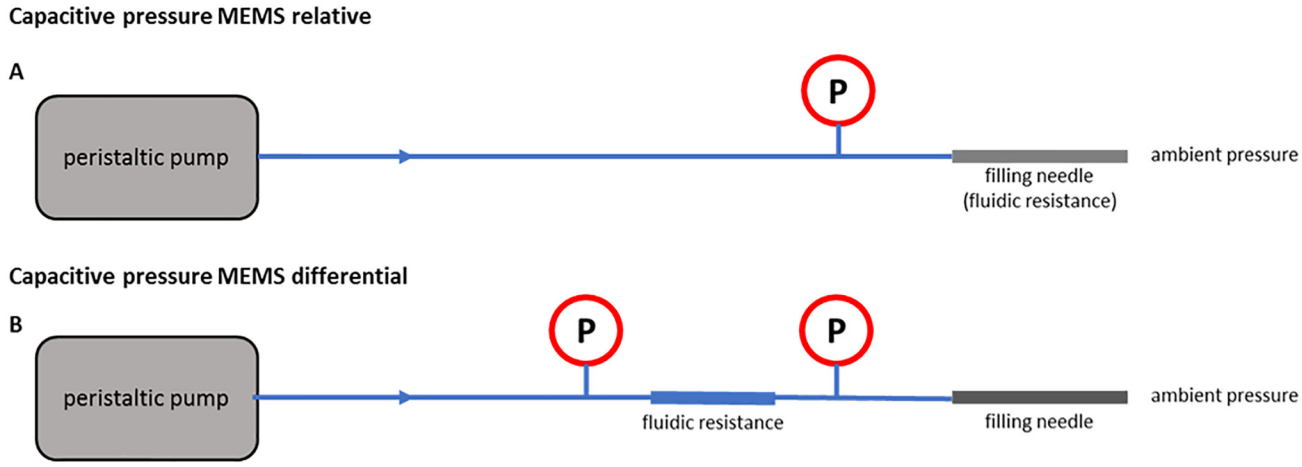


Figure 3

Experimental Setup for the MEMS Sensor. Schematic drawing of the relative (A) and differential (B) pressure setups using MEMS pressure sensors. The relative system utilizes the filling needle to provide fluidic resistance and compares the increase in pressure in the fluid line with the ambient pressure, and the pressure change can be used to determine the fluidic volume based on the Hagen-Poiseuille formula. The differential system uses the differential pressures measured by two different sensors placed on either side of an additional fluidic resistance element.

junction within the fluidic path. The introduction of a T-junction causes a minimal volume fraction of the dispensed volume to deviate from the direct path to the filling needle and instead flow into the T-junction, compressing a tiny air bubble located in front of the pressure sensor. The deformation energy in the compressed air bubble is released back into the liquid at the end of the fill, resulting in a completely relaxed system.

The relative pressure setup (Figure 3A) takes advantage of the pressure increase over the dispensing needle relative to the ambient pressure, caused by the fluidic flow through the needle. The differential pressure setup (Figure 3B) measures the pressure before and after an additional fluidic resistance element. As described in detail for the capacitive pressure sensor, the resulting pressure difference is directly related to fluidic flow, according to the Hagen-Poiseuille law. (eq 1).

2.2.4. Optical Sensor: The optical sensor principle tested in this study uses an infrared light-emitting diode (LED) and an infrared-sensitive optical receiver (Table I, optical sensor) (19). The photons sent by the LED are detected by the receiver. Droplets or jets that pass between the LED and the receiver typically reduce the grade of optical transmission; thus, the light intensity on the receiver side is reduced by absorption, reflection, and dispersion effects, and the light intensity measured at the receiver can then be transformed into an electrical signal. Absorption occurs when a fluid, such as a semitransparent ink, absorbs or partially absorbs the light radiation that passes through the medium, increasing its temperature but reducing transmission. The absorption effects on a dark ink are stronger than those on distilled water. Reflection occurs on surfaces with different refraction indices. The reflected component of the light will typically be redirected and will not reach the receiver, resulting in reduced transmission. Dispersion scatters the light, following the laws of optical lenses. All three mechanisms reduce the intensity of light that reaches the receiver (20). For transparent fluids, such as water, the primary light intensity reduction mechanisms occur because of reflection and dispersion on the side of the fluid column. A wide fluid column with a large cross section results in a relatively large area of reflected and dispersed light, which results in a stronger reduction in light transmission compared with a thinner fluid column with a smaller cross section. After calibration against orthogonal methods (e.g., weighing), the intensity of the received light and the resulting sensor signal can be correlated with the dispensed volume, especially when integrated over time. To use this sensor in an industrial environment, stable background illumination levels are required.

2.2.5. Capacitive Sensor: The capacitive sensor makes use of a single capacitor, with the same design as

described earlier for the capacitive pressure sensors, as shown in Table I (17). However, this sensor does not measure pressure, and no silicone tube is placed within the capacitor plates. Instead, the fluid column moves through the capacitor, causing changes in capacitance by altering the dielectric properties of the air space between the capacitor plates (21, 22). The change in capacitance is, therefore, related to the cross-sectional size of the fluid column, as larger cross sections will alter the dielectric properties of the air space more strongly than smaller cross sections. To fix the position of the dispensing needle relative to the capacitive sensor, the sensor was attached to a concentric mounting plate, with a guiding hole. The diameter of the guiding hole was carefully fitted to the diameter of the dispensing needle tip and should prevent any liquid from sprinkling onto the capacitor plates. The detection principle of the dispensed volume is based on the detuning of a high-pass filter. When liquid is dispensed through the electric field of the capacitor, its value changes for the time of flight; therefore, the frequency characteristic of the filter shifts depending on the geometry of the dispensed liquid and its material-specific dielectric constant (16, 17).

2.3. Gravimetric Regression Method

The GraviDrop software is designed to achieve a more precise method for measuring liquids in the submicro- and nanoliter range, based on a method that takes the evaporation and fluctuations of a microbalance into account. The software makes use of a novel gravimetric measurement technique, which measures the weight for a certain measurement time, consisting of the dispensing event and a post dispensing settlement time. The dispensed volume is derived from the difference in weight before and after the dispensing event, taking into account the evaporation effects that occur during dispensing. Additionally, an uncertainty factor delivers information regarding the accuracy and equivalency of evaporation effects and other influences. This novel method relies on the linear regression analysis of continuously monitored gravimetric results and, therefore, is referred to as the "gravimetric regression method (GRM)" (23).

2.4. Measurement Process

The measurement process was started by the GraviDrop software. When the software was ready, it triggered a fill by the filling system. The trigger electronic board was a customized microelectronic board that receives serial commands from the GraviDrop software, via USB, and sends a trigger signal to the filling system. The fill controller software was customized to detect incoming triggers from the trigger electronics, to wait for a delay time of approximately 50 ms, and then to begin the filling event, which caused the peristaltic pump to actively dispense liquid into the measuring cylinder on the balance. The liquid flow in the system caused the investigated sensor to generate a corresponding sensor signal, which was simultaneously A/D converted, at sampling frequencies between 1 and 5 kHz. The sensor signal was stored in a CSV file by Meilhouse Reader software. After a programmed time interval, the gravimetric result was recorded by the GraviDrop software, and the gravimetric data were added to the CSV file containing the sensor signal. Each dispensing process created an individual CSV file. Typically, one run contained 24 dispensing repetitions with stable parameter settings (24). The amount of dispensing repetitions was chosen to account for small deviations in the measurement setup as well as temperature fluctuations during measurement.

3. Results

We investigated the applicability of different sensor types for the aseptic filling of volumes $<200\mu L$ as well as the influence of unidirectional air flow on gravimetric fill weight accuracy.

3.1. The Influence of Unidirectional Airflow

Figure 4 illustrates the influence of UDAF on the gravimetric measurements, after a calibration weight of 50 mg was placed onto fifty 2-mL vials to guarantee a continuous weight by eliminating influencing factors, such as the possible evaporation of a drug product solution. The dotted lines indicate the upper and lower limits of $\pm 7\%$ which is used as an accuracy limit for gravimetric IPC for fill volumes $<100\,\mu L$. With the UDAF in full operation, 12 out of 50 vials were outside of the accuracy range and would be discarded, resulting in a 24% loss. When the UDAF was off, the loss of out-of-range vials was reduced to 4 out of 50 vials (8%), which still represents a significant magnitude. However, this experiment could only reduce the impacts of the UDAF. All other environmental disturbances remained constant (e.g., microvibrations), which could introduce variations. This was found to be

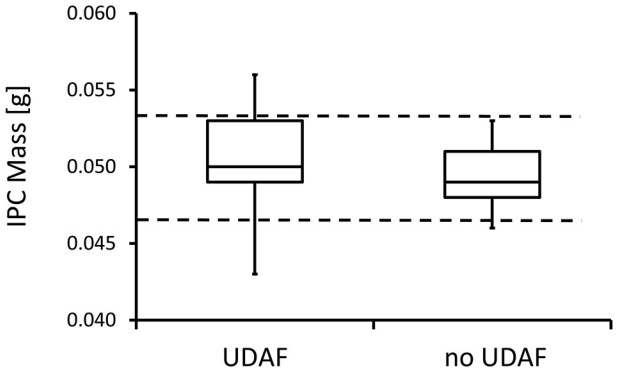


Figure 4

Influence of laminar airflow on a gravimetric scale (Mettler Toledo WM124-W22) for a commercial filling unit. Fifty individual 2 mL vials were tested, with and without unidirectional airflow (UDAF). The vial passed a tare scale, a 50 mg calibration weight was placed onto the vial, and the vial was further transported to the gross scale, where the weight difference was measured. The scales were calibrated using a 20 g stainless-steel weight (Mettler Toledo, OIML E2, Item number 158,437) and used in the 2 mL vial format, according to standard production routine. Dotted lines indicate the upper and lower fill accuracy limits (±7%). Data points are shown as box plots, based on the median values.

the root cause as to why the median of the no UDAF data set was not equal to 50 mg of the calibration weight. Overall, the study showed that the influence of UDAF on a gravimetric scale cannot be neglected.

3.2. Sensor Results

The overall aim of the study was to investigate potential technologies that could be applied to industrial filling processes, to enable aseptic filling with a level of precision that would allow the complete elimination of necessary downdosing processes during the administration of treatment. Because the typical administration volume of intravitreal treatments is 50 µL, we strictly focused our study on this volume, accounting for a <10 µL dead space in a prefilled syringe. Therefore, the data point of the highest importance, in terms of therapeutic dosing, was the 60 µL target fill volumes, which is within the range of all tested sensors.

The sensors tested in this study can be grouped into three individual categories, based on application similarities: (1) indirect measurement sensors, (2) pressure-based sensors, and (3) sensors located at the orifice of the filling needle. Pressure based sensors (capacitive pressure and MEMS) are installed in the liquid path, and their signals are based on the liquid flow toward the filling needle. The sensors that are installed at the orifice of the filling needle (direct capacitive and optical) measure the amount of liquid that is actually dispensed through the filling needle. The third measurement technique (airflow) measures the physical effects occurring at the fluid reservoir. All of the sensor results, as well as the working principle of each sensor, are presented in Table I.

For each investigated sensor, a dispensing volume in the range of 10–100 µL was investigated. Each dispensing series consisted of multiple runs, depending on the investigated sensor type. Each run represented 24 dispensing repetitions, with unchanged, consistent filling parameters. Initially, the sensor performance was compared directly to the gravimetric measurement, which represents the current state-of-the-art method. The weight of each filling event was used to determine accuracy and precision. The accuracy of one run was calculated as the deviation between the recorded sensor signal and the gravimetric value. The precision of one run was calculated as the standard deviation of the sensor signals. All sensors showed an individual signal and had to be postprocessed by integration. Thus, after linear regression to the integration results, the outcomes were the calibrated sensor results, in microliters.

Based on this calibration, a comparison between the sensor results and the gravimetric results was feasible. Each graph shows the sensor results, on the *y* axis, plotted against the gravimetric measurements, on the *x* axis. If the sensor data and the gravimetric data matched perfectly, all data points would be located on the biangular section. For more detailed comparisons of the data, the means and standard deviations for all sensor and gravimetric results were calculated. The accuracy and precision data for a dispensed volume of approximately 60 µL (resulting from six peristaltic cycles) were compared for the final evaluation of the different sensors.

Indirect Measurement Sensor

3.2.1. Airflow sensor: The airflow sensor is capable of measuring small amounts of any gas (air in this case) that vents to the reservoir. The results demonstrated that the application of this sensor concept could be

used quantitatively to measure dispensed volumes. After data sampling and data processing, the airflow could be correlated with the dispensed volume, independent of fluid properties. The sensor performance graph in Table I shows the correlation between airflow sensor measurements and gravimetric results, based on nine runs and dispensed volumes ranging from 30 to 100 µL. The accuracy and precision for a representative volume of 60 µL were measured and shown to be +3.4% and ± 1.1%, respectively, which was considered to be a promising performance.

Pressure-Based Sensors

3.2.2. Capacitive Pressure Sensors: Four individual runs were performed using the relative pressure setup based on the capacitive sensor. The dispensed volumes ranged from 10 to 85 µL. The accuracy of the sensor signal was 0%, relative to the gravimetric results for a representative volume of 60 µL. A value of ±2.9% calculated for the precision of this sensor reflects its very high performance. The differential setup, shown in Figure 2B, was also tested but showed no improvement in sensor performance (data not shown).

The test results for the relative pressure setup using the MPXV5050 MEMS sensor covered a dispensing range from 9.7 to 96.7 µL. For a 60 µL volume, the sensor showed a remarkable deviation relative to the gravimetric measurement of 0.0% and a precision of ± 0.6%, which was the best performance for all pressure-based sensor setups. Comparable with the capacitive pressure sensor, the differential pressure setup using two MEMS sensors showed no benefit over the relative setup (Figure 3) (data not shown).

Sensors Located at the Orifice of the Filling Needle

3.2.3. Optical Sensor: The optical sensor was installed underneath the filling needle and measured (100% contact-free) the fluid directly as it was dispensed from the needle. For this measurement, airflow and pressure conditions do not need to be considered. The sensor performance graph in Table I shows the calibrated results of the optical sensor relative to the gravimetrical measurements. Eight different runs were performed, covering a volume range from 11.6 to 95.7 µL. The investigated volume of 60 µL showed a deviation of −0.1% relative to the gravimetric results and a precision of ±1.1%.

3.2.4. Capacitive Sensor: The strengths of the capacitive sensor were comparable to those for the optical sensor approach. The sensor capacitor was installed directly below the filling needle and measured the dispensed liquid by measuring the direct change in the sensor's capacitance, which was caused by the liquid's permittivity. The sensor was tested over a volume range of 12.3–97.9 µL. The results for the representative volume of 60 µL showed an accuracy of −0.3% and a precision of ± 0.3% relative to the gravimetric results.

4. Discussion

To compare the performances among all tested sensor types, the estimated accuracy and precision values for a fill volume of 60 µL were calculated relative to the gravimetric measurements, as shown in Table I. This volume was chosen as the reference value for the desired fill volume during the manufacturing process because intravitreal injections are often administered at 50 µL doses; therefore, a 60 µL fill volume represents the hypothetical manufactured volume used for a prefilled syringe, including the required overfill volume. Each data point was measured with n = 24 repeated dispensing events to account for small deviations (e.g., temperature fluctuations) during the measurement.

The indirect measurement sensor (airflow) showed good accuracy and precision over the whole volume range tested. The measurement of airflow into the reservoir could serve as an effective concept because of the independence from fluid properties and a high degree of accessibility. However, an airflow sensor installed at the reservoir has the greatest distance from the filling needle. Therefore, this method bears the risk of false-positive volume detections because of influences such as leakages and UDAF, which could lead to falsely filled primary packaging containers. The accuracy and precision of the airflow sensor for a 60 µL volume revealed promising sensor performance (Table I). However, the performance of this sensor was less promising compared with the results for the other sensor types. However, the advantages of this noninvasive measurement method and its independence from fluid properties could be of potential interest for further development.

The examination of the pressure-based sensors demonstrated that the relative measurement method, which uses a single pressure sensor, was easier to implement

and performed better than the differential measurement, which requires a second pressure sensor and an additional fluidic resistance element. Similar findings were observed for both the capacitive pressure sensor and the MEMS sensor. The reduced performance observed for the differential method was unsurprising because of the requirement for two sensors, increasing the risk of individual errors associated with each sensor, which could potentially result in increased measurement errors. Thus, the additional installation of the second sensor was unnecessary if the dispensing needle can provide fluidic resistance. When comparing the MEMS sensor with the capacitive pressure sensor, the MEMS sensor revealed the best measurement performance. This sensor is commercially available at a comparably low cost. Additional investigations have shown that the MEMS sensor is capable of detecting errors and failure modes, which could represent an interesting feature for improved process control. However, the implementation of any sensor into the fluidic path remains a key challenge because of the requirements associated with medical and aseptic procedures (i.e., cleaning in place/sterilization in place, temperature, and high pressures), and both pressure-based systems examined in this study are incompatible with industry-standard sterilization procedures. Another potential weakness of this sensor type is its sensitivity, regarding air bubbles in the fluid. This weakness could be overcome by intelligent signal analysis, which could also be used for further failure detection.

The primary advantage of sensors that are installed at the filling needle orifice (optical sensor and direct capacitive sensor) is that the dispensed liquid volume is directly measured. If spilling, dripping, and off-targeting can be avoided, the dispensed volume will be delivered into the primary packaging container and successfully detected by the installed sensors, resulting in a low risk of either false-positive or false-negative results. Both of the sensors tested in this study showed promising results in terms of accuracy and precision and should be explored for further development and future implementation. Both sensors promoted contact-free measurement, with 100% IPC for every single container. The optical sensor may be sensitive to the transparency and color of the dispensed fluid, which must be tested in future investigations. Furthermore, the optical sensor signal can be directly influenced by a variety of fluid properties, including color, turbidity, velocity, shape, and the position of the sensor, which could limit the application of this sensor type. Therefore, a calibration step against orthogonal methods remains necessary.

The capacitive sensor showed the best performance and was, therefore, considered to be the most promising for further development and future integration into an aseptic filling line. The impressive accuracy and precision of the capacitive sensor, in combination with the 100% contact-free measurement of every single fill, was a promising outcome of the study. However, some hurdles must still be cleared during development. The capacitive sensor remains in the development stage and was used as a prototype during this study. The sensor is susceptible to the permittivity of the dispensed liquid, and the velocity of the liquid cannot yet be measured. Both the sensor and the corresponding electronics would require further development before implementation is possible.

An important factor for the successful implementation of a novel IPC sensor is the sensor read time and the alignment of the sensor reads with high-speed filling rates. A typical filling time on high-speed filling machines is approximately 700 ms. During gravimetric IPC, the measurement can only start after the actual filling step is complete. Scales require settling time, measurement time, and time for the final communication with the programmable logic controller. Conceptually, the presented IPC systems work based on the data processing (integration) of sensor signals generated during the filling process. All data is readily available at the end of the fill, and no stabilization or post-fill measurement time is necessary, making the studied technologies comparably fast, which may represent a large advantage during high-speed aseptic filling. The calculations are thought to take approximately 1 ms. Nevertheless, there is the need to implement a feedback loop between the IPC sensor and the filling system to allow corrective actions to account for deviates from the target fill volume.

Because every sensor type has specific advantages and disadvantages (Table II), the combination of several sensor technologies could merge the advantages of the individual sensor types. One possible option for a suitable setup could be to use a capacitive sensor near the filling needle orifice, to detect the dispensed fluid, whereas the MEMS sensor is used to monitor process stability and the identification of possible interferences, such as bubble formation, needle clogging, and leakage/major spill detection Furthermore, the addition of a

TABLE II
Summary of the Advantages and Limitations of All Tested Sensors

Sensor Type	Advantage	Limitation
Airflow	• Contact-free measurement • Liquid independent • Very sensitive to venting airflow	• Slow signal generation (indirect measurement) • Sensitive to environment • Long distance between the reservoir and the dispensing needle
Capacitive Pressure	• Contact-free measurement • High accuracy and precision • Direct measurement of the pressure of the dispensed fluid • Troubleshooting analysis possible by specific pattern recognition	• Susceptible to electromagnetic fields (shielding necessary) • Air bubbles strongly influence the signal • Distance between the point of measurement and the dispensing needle
MEMS	• Well established technology • High accuracy and precision	• Sensor in direct contact with the fluid • Air bubbles influence the measurement signal • Sensor material incompatible with medical standards • Distance between the point of measurement and the dispensing needle
Optical	• Contact-free measurement • High accuracy and precision • Fast, analogue signal generation • Located at the orifice of the filling needle	• Jet velocity information is not measured • Color, turbidity directly influence the signal • Different stray light conditions • Quantification of results rather complex
Capacitive	• Contact-free measurement • High accuracy and precision • Fast, analogue signal generation • Located at the orifice of the filling needle • Independent of fluid properties like viscosity, color, turbidity	• Jet velocity information is not measured • Wetting of the sensor electrodes might imply cross-contamination • Permittivity of the fluid influences the signal

third sensor (e.g., airflow) could provide information regarding the whole filling pathway, ranging from the start (reservoir) to the center (tubing between the pump and the filling needle) to the end (filling needle). The use of additional sensors in different regions of the filling pathway could also assist with troubleshooting situations by automatically recognizing specific patterns based on performance algorithms. Additionally, the influence of different fluid properties remains to be investigated in future studies. Highly concentrated protein formulations designed for subcutaneous applications have shown the potential for non-native aggregation, which contributes to higher viscosities (25–27). Among the sensors tested in this study, both of the sensors placed at the filling needle orifice should be independent of fluid viscosity; however, these sensors do require calibration for fluid velocity. Calibration and recalibration activities may be a finicky and time-consuming step during routine manufacturing. Therefore, additional testing of different protein formulations will be necessary to fully investigate the extent of calibration activities needed.

In summary, the capacitive sensor was the most promising sensor technology, in terms of accuracy and precision, for the determination of fill volumes in the desired volume range of $<200\ \mu L$. The MEMS pressure sensor, using the relative pressure method, and the optical sensor also showed potential for further development.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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Chapter IV: Unpublished Results

Low volume particulate testing using light obscuration method

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Highlights: The light obscuration method is the most used and state of the art method for subvisible particle analysis. Our study investigated the single method parameters and the possibility to reduce the sample volume as much as possible. Additionally we showed that immiscibilities between high concentrated protein formulations and the acqueous phase in the instrument create streaks, which are falsely identified as particles. Our study provides a detailed insight how this overcounting effect can be reduced for stable and reliable results in the future.

Introduction

Subvisible particle analysis is required by health authorities for all parenterally administered drug products. Both, USP <787> and <788> recommend the technique of light obscuration (LO) for sizing and enumeration of subvisible particles. In cases where LO is not possible, microscopy is indicated as alternate method. Particle limits for parenteral formulations in a volume of 100 mL or less is defined as 6000 particles ≥10 µm and 600 particles ≥25 µm [34, 35]. These limits are associated with concerns about particles blocking capillaries (average diameter around 7µm) upon injection and causing other health issues. During aseptic manufacturing particle contamination can be classified in intrinsic and extrinsic particles [36, 37]. To characterize a particle contamination, the source of the particles needs to be known and investigated. Intrinsic particles may derive from the protein formulation itself, whether extrinsic particles may be introduced as silicone oil (from rubber stoppers) or glass particles (from primary packaging material) [38, 39]. As described in **Chapter II**, the chosen dosing system may also influence the quality of the filled drug-product [20, 25]. Particle burden is considered to be a critical quality attribute and needs to be monitored over the whole lifecycle of a product. Therefore, in aseptic manufacturing, random samples are drawn and analyzed for sub-visible particles and represent the quality of the whole batch.

As more and more drug product formulations with a low fill volume enter the biopharmaceutical market (e.g intravitreal injections, high concentrated protein formulations), the minimum sample volume required by USP <788> with 25 mL was not feasible anymore [35]. Since intravitreal drug products have filling volumes of 100-200 µL (with even lower administration volumes of around 50 µL), extensive pooling of single units will be required to achieve one single measurement [13, 40]. Pooling of single units masks container variabilities and is also prone to the introduction of foreign particulates due to longer sample preparation and handling time [41]. Therefore, health authorities provided guidance regarding subvisible particle analysis for this special case by allowing sample volumes down to 1 mL [34]. Additionally, USP <789> “Particulate matter in ophthalmic solutions” describes the acceptance criteria used for intravitreal drug product formulations: Not more than 50 particles/mL ≥ 10 µm, not more than 5 particles/mL ≥ 25 µm and not more than 2 particles/mL ≥ 50 µm are the limits for ophthalmic solutions [31]. These low particle limits are very challenging for the applicability of the light obscuration methods since recent research had shown that the precision of subvisible particle quantification was strongly dependent on the particle numbers in the sample volume [42, 43].

The light obscuration method itself is rather complex and susceptible to disturbances (e.g. high viscosities, settling time of particles in the container, air-bubbles). Also, the LO method tends to undercut translucent particles, such as proteins and polysorbate or the other way around, false positively counts streaks from immiscible phases as particles [44, 45]. Furthermore, the gained information after particle analysis is limited to particle size range and numbers. The particles identity or origin cannot be covered by LO [46]. A schematic view of the light obscuration system is shown in Fig 1.

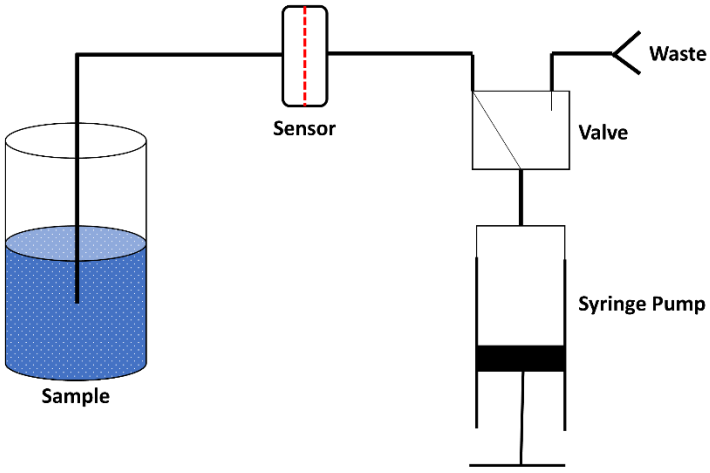

Figure. 1: Light Obscuration instrument for the measurement of subvisible particle counts. The sample is drawed into the measuring chamber with the sensor by a syringe pump. Any particulate contaminant will block a certain amount of light from a laser beam and the resulting shadow is detected by an optical sensor. In a post processing step, the shadow is converted into the equivalent circular diameter. After measurement a valve switches to the waste container and the sample is ejected from the system.

The measuring chamber, the tubings and the syringe have a defined dead volume. This dead volume has to be filled with the sample for analyses to prevent any air to enter the system. Current light obscuration methods need at least 1 mL / sample. Therefore, multiple container has to be unified prior measurement.The aim of our study was to investigate what process parameters of the LO method do influence the counting accuracy the most and which process parameters can be adapted to reduce the sample volume as much as possible. It is important to understand how the light obscuration method is built and which step is critical for the measurement. Fig. 2 gives an overview of the single steps of the light obscuration method.

Figure. 2: Single steps of the light obscuration method for fourfold sample measurement. The tare-volume is defined as the initial volume, drawn from the sample for filling the measurement cell after WFI rinse. The sample volume is the actual volume which is analyzed. The stroke volume is the volume taken between each sample volume (run), to reach the defined flow rate at the time point the sample reaches the sensor. It is therefore very important to understand the different method steps and then to adapt the LO method especially

A deeper understanding of the LO method and a potential reduction of the sample volume results in less sample pooling and less manual handling steps.

Materials and Methods

Materials

Purified water (Milli-Q IQ7000 or Milli-Q Advantage A10, Merck Millipore, Darmstadt, Germany) with a resistivity of >18 MΩ cm and sterile filtered (PVDF membrane with 0.22 µm pore size, Merck Millipak) was used for all experiments. Polystyrene standard particle suspensions (Duke standards 4K-10, 4K-25, and 4K-50) were purchased from Thermo Scientific (Fremont, CA).

Three different monoclonal antibody formulations with different viscosities (1.6 mPas, 12.2 mPas, 17.6 mPas) were used for LO measurements and investigations on viscosity influence. All mAbs were provided by F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

Light Obscuration Measurements

A ROYCO System 9703, sensor HRLD400HC (Pacific Scientific Co., Washington, DC) was used. The sensor used 780nm wavelength and was calibrated with polystyrene beads to measure particles in a size range from 1.3 to 300 µm. The sensor limit was set to 18000 particles/mL. The rinsing volume was set to 0.2 mL and 4 runs, each of 0.4 mL were performed. The flow rate was set to 10 mL/min. The first run was discarded and the average ± standard deviation of the last 3 runs was reported. Blank measurements (with fresh particle free water) were performed at the beginning of the analysis and repeated after every third sample. The acceptance criteria for blank measurements were set to ≤ 5 particles ≥ 1 µm. The integrity of the instrument was tested by means of a 3000 particle

count standard (5µm particle size) (COUNT-CAL Count Precision Standards; ThermoScientific) with acceptance limits of ±10 % of the reported concentration for particles bigger than (5µm), respectively. PharmaSpec version 3 (HACH Ultra Analytics, Oregon) was the software utilized to collect and analyze the raw data from the sensor.

Samples were prepared by transferring the all samples under unidirectional airflow (UDAF) into washed and sterilized, particle-free 11mm glass tubes (Hach Lange GmbH, Düsseldorf Germany). To prevent sedimentation of particles, manual sample homogenization was carried out for 30 s for all samples and prior to any experiment.

Results:

The aim of this study was to investigate further into the different method steps and to evaluate the parameter it needs to adapt or maintain for low volume method development. Fig 3. shows the dependency of stable and reliable particle counts to the viscosity of three different mAb formulations.

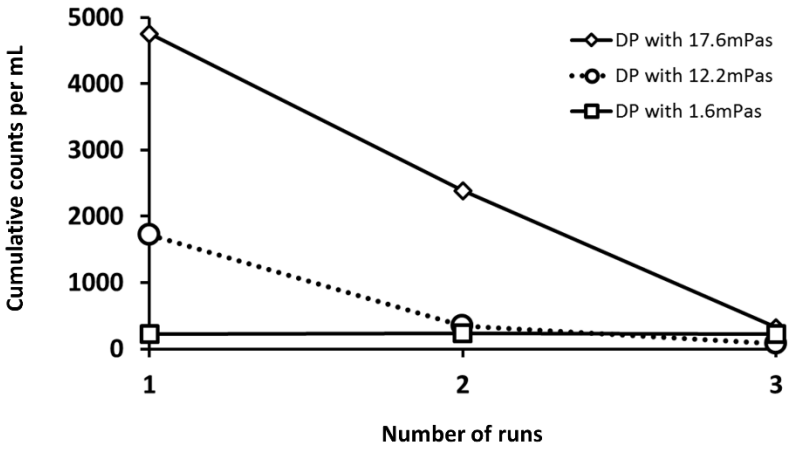

Figure 3. Cumulative particle counts for three drug product formulations with different viscosities. Squares show a mAb formulation with 1.6 mPas. Circles show a mAb formulation with 12.2 mPas. Diamonds show a mAb formulation with 17.6 mPas. Particle counts are shown for all particles > 2 µm after sterile filtration of each drug product formulation.

A drug product formulation with 1.6 mPas reaches stable particle counts already after the first run and therefore needs less sample volume compared to drug product formulations with higher viscosities (stable counts only after the second or third run). In a next experiment, four different light obscuration methods were tested with a marketed high concentrated (150 mg/mL) drug product formulation to investigate into method-to-method

variation and the influence of different method parameters. Currently, we have four different methods for LO in use. Tab. 1 gives an overview for all four methods and the corresponding parameters. We carried out an experiment to investigate on the comparability of the four different methods for a mAb formulation with a high protein concentration (150 mg/mL) spiked with 10 µm polystyrene beads at a concentration of 1000 particles/mL (Fig. 4).

Method	Tare [mL]	Stroke [mL]	Sample [mL]	Total Runs [mL]	Total Volume Needed [mL]
A	0.5	0.1	0.2	4	1.6
B	0.5	0.1	0.4	4	2.4
C	0.1	0.1	0.4	3	1.5
D	1	0	1	4	5.0

Table 1: Overview of the different light obscuration methods currently in use.

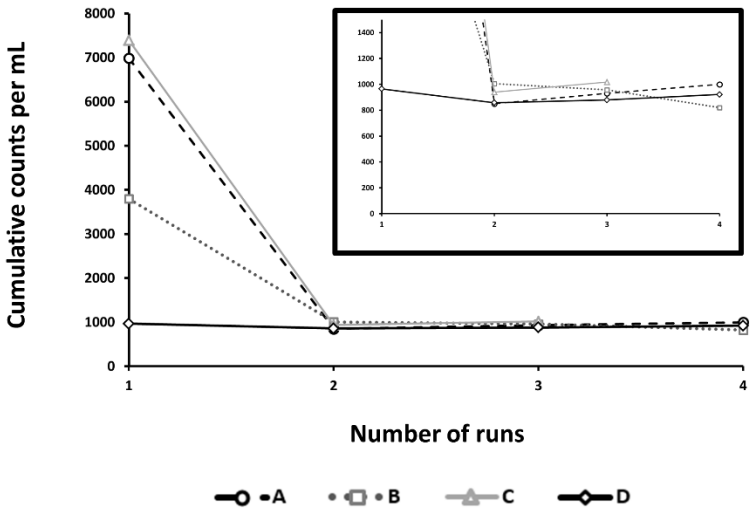

Figure 4. Overview of all four methods currently in use for light obscuration. Cumulative particle counts are shown for a particle size of 10 µm. The mAb formulation has a concentration of 150 mg/mL and was prepared by spiking 10 µm polystyrene beads at a concentration of 1000 particles/mL after sterile filtration.

All four methods showed different sub-visible particle counts for WFI spiked with 10 µm polystyrene beads at a concentration of 1000 particles/mL. Method A & C which had a comparable total volume showed the highest particle counts after the first run. Method B with a higher total volume showed elevated particle counts, but already half of the counts compared to method A and C. Method D was the method with by far the highest total

volume needed, but showed already the expected particle counts after the first run. All four methods reached the equilibrium state after the second run.

To investigated further into the method parameters and their influence on the measurement, there was a study set up with the variation of the single method parameters (tare-, stroke-, and sample-volume). Therefore, a high concentrated drug product formulation (150 mg/mL), was measured after sterile filtration for ten individual runs by varying the three method parameters.

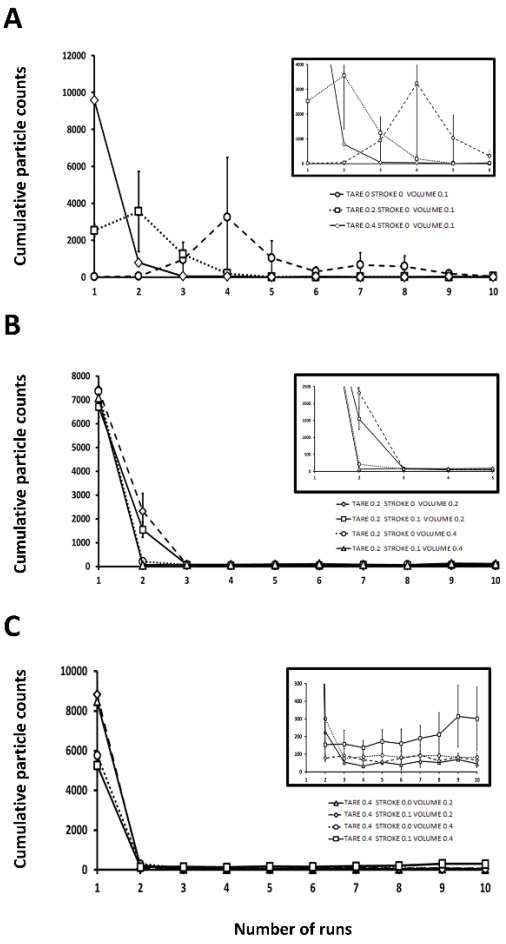

Figure 5. Influence of tare-, stroke- and sample volume of the light obscuration method with a marketed 150 mg/mL protein formulation. A) Influence of the tare-volume. The stroke volume was set to 0 mL and the sample volume was kept constant at 0.1 mL. **B)** Influence of the stroke volume. The purge-volume was kept constant at 0.2 mL and the sample-volume was set to 0.2 and 0.4 mL. The stroke volume was varied at 0 mL and 0.1 mL. **C)** Influence of the sample-volume. The tare-volume was kept constant at 0.4 mL. Stroke-volume was varied between 0 mL and 0.1mL and the sample-volume was varied between 0.2 mL and 0.4 mL. Results are given as average from two different operators and standard deviation of n=2.

The tare-volume (Fig. 5A) shows a direct influence on particle counts. An increase of the tare-volume from 0 mL to 0.2 mL results in a shift at the particle count maximum from run 4 to run 2. Furthermore, a doubling in the tare-volume from 0.2 mL to 0.4 mL results in a decrease of false positive particle counts. The equilibrium state if reached after 6 runs (tare-volume: 0mL), 4 runs (tare-volume: 0.2 mL) or 3 runs (tare-volume: 0.4 mL). When looking at Fig. 5B, we can see that the particle counts decrease when the stroke-volume is increased from 0 to 0.1 mL. With a sample-volume of 0.2 mL the equilibrium state is reached after 3 runs and with a sample-volume of 0.4 mL after 2 runs respectively. The combination of a stroke-volume of 0.1 mL and a higher sample-volume (0.4 mL) showed the best result in terms of particle counts and reaching the equilibrium state. The sample volume itself showed the less influencing effect (Fig. 5C). All four parameter sets reached the equilibrium state after the second run. Only the false positive particle counts were reduced by doubling the sample-volume from 0.2 mL to 0.4 mL. With these results, we can conclude that the tare- volume showed the highest influence on reaching a stable and reliable particle count. An additional experiment was realized to investigate the necessity of the tare-volume (Fig. 6).

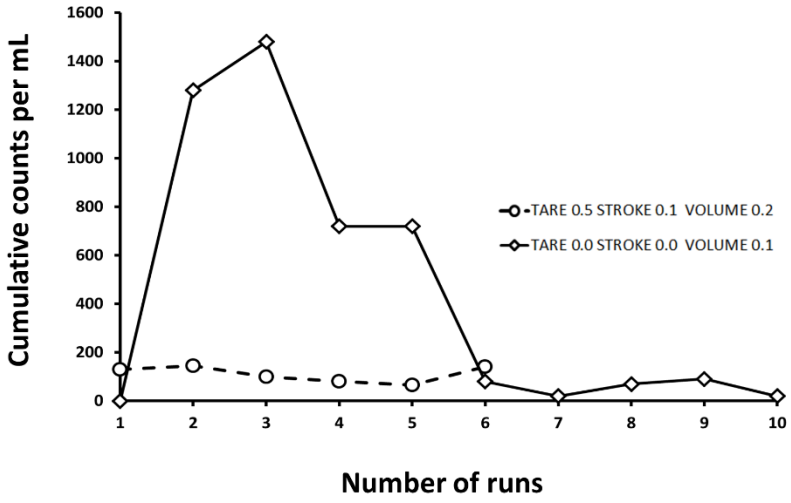

Figure 6. Effect of the tare-volume for light obscuration on reaching stable particle counts. The tare volume was set to 0 and 0.5 mL. The stroke volume was set to 0 and 0.1 mL and the sample volume was set to 0.1 and 0.2 mL.

Fig. 6 shows that a tare-volume is essential for reaching stable results. The equilibration state without any tare-volume is reached only after six runs, whether for a tare-volume of 0.5 mL the equilibrium state is already reached after the first run.

Discussion & Outlook:

With the new USP <789> guideline and the reduced allowed sample volume from USP <787>, the LO method adapts to novel formats and formulations in the biopharmaceutical industry. Recent data showed that it is possible to reduce the sample volume from >20 mL down to 1 mL without compromising the data quality [40]. Nevertheless, the LO method still needs further understanding and improvement to meet the requirements on the low limits presented in the guidelines.

Our study demonstrated that the mAb formulations viscosity shows a direct impact on reliable and stable particle counts. Due to the fact that the LO system has to be cleaned prior to any measurement with particle free, purified water, there is always a residue of water in the system. After drawing the sample volume in the system there will be a mixing process between the aqueous phase and the sample. With viscosities of 7.2 mPas and higher there were high particle counts reportet after the first and the second run of the measurement before reaching the equilibrium state of stable particle counts (Fig. 3). This effect was not seen for a mAb formulation with 1.6 mPas. Therefore, it is our conclusion that higher viscosities lead to immiscible phases between sample and residual water. The resulting streaks are false identified as particles by the system. It is mandatory to know after how many runs the system reaches the equilibrium state and how many runs need to be discarded for representative results. Unfortunately, there is a higher amount of sample volume needed because of the false positive counts at the beginning of the measurement. A small study where we investigated an increase of the settling time before measurement start (more time for mixing) had shown no effect (data not shown).

Not only is the false positive counting of particles a problem for LO measurement but undercounting phenomena were reported in literature too. Due to the slower movement of high viscous mAb formulations, the possibility of air intake into the system results in a lower sample volume drawn into the measurement chamber (citation). USP <787> allow a sample dilution with a low viscosity solvent (e.g.purified water) [34]. This step should be executed when no other possibility obtains acceptable results, because the dilution itself may have an influence on the composition or concentration of the particles and is another manual handling step. Another approach which needs further verification on feasibility is the cleaning or purging of the instrument with a surrogate solution with the same or comparable viscosity of the sample. With that approach the immiscible effects occur only between water and the surrogate and no longer between surrogate and the sample. This

approach can help to reduce the sample volume needed for a measurement to a minimum. The only alternative to the application of LO is the application of microscopy techniques allowed by health authorities and Pharmacopeiae. These techniques are considered even less precise and more complex for operators.

Therefore, we focused on the LO method and the different method parameter itself. The LO method was divided into three different method volumes: A) Tare volume: The volume which is drawed in the system prior to any measurement. B) Stroke Volume: The stroke volume is the volume, which is drawn between the single samples to help the system reach the right flow-rate and C) the actual sample volume. Our data demonstrated that the higher the overall analyzed volume, the lower any false positive particle counts (Fig. 4). This goes along with the fact that a higher overall volume can replace the residual water volume faster than a small volume. For all four methods tested, the equilibrium state was reached after the second run. Since our approach will be a possible sample volume reduction we varied the three method volumes in an additional study (Fig. 5). Our results showed that the tare volume showed by far the most prominent effect on achieving a stable particle count (Fig. 5A). The equilibrium state was reached only after six runs without any tare volume, whether with a tare volume of 0.4 mL it was reached already after half the runs.

The variability of the stroke volume (Fig. 5B) had no clear effect on the results, but we consider the stroke volume necessary to guarantee a constant flow-rate, which becomes more important for higher viscous formulation where the mass inertia increases. The actual sample volume showed a similar effect like the tare volume. Both method parameters have the highest method volume und therefore the highest influence on the results. The influence of the tare volume was verified in an additional experiment (Fig. 6) where it was shown, that without any tare-volume the counting results are only stable after six runs. Therefore, it will be considered for future method development to use a sufficient tare-volume. With the idea of using a purified and particle free surrogate solution the tare-volume may be replaced and the method would only consists of the stroke and the sample-volume. Since our studies showed that at least 0.4 mL are necessary for any improvement on counting stability for the LO method, the reduction of the tare-volume seems to be a promising factor to look at. These preliminary data will provide any scientist with enough background information on the LO method and give some inspiration where to tackle and overcome the challenges in the field of LO especially for high concentrated and low volume mAb formulations.

DISCUSSION AND OUTLOOK

In the presented PhD thesis, a linear peristaltic pump as a novel filling technology for monoclonal antibody formulations has been developed and characterized for its purpose to reliably fill volumes < 200 µL. In combination with innovative IPC sensors and an adapted particle testing method, low volume filling within aseptic manufacturing was investigated in the frame of an end-to-end examination. The following sections summarize all achievements of the thesis and put these in a larger context.

A steady growth in the ocular therapeutic area with intravitreal application, as well as increasing formulation concentration for high dose therapies results in application volumes down to 50 µL. During product and process development of aseptic filling of mAbs, the choice of the right filling system is a key step. The state-of-the-art filling system (i.e radial peristaltic pumps or piston pumps) lack a dose accuracy and consistency when it comes to fill volumes < 200 µL. Consequently, fill volumes have to be elevated to guarantee the lable claim. Due to high material cost and possible patient's safety issues regarding multi-application of drug product these filling systems are not applicable for low fill volumes. Therefore, a linear peristaltic pump prototype was characterized and further optimized for the use in aseptic filling. Due to its unique advantageous working principle, the linear peristaltic pump allows precise and accurate dosing down to 12 µL. As discussed in **Chapter I**, the linear peristaltic pump still needs further improvement on the size and parameter settings to promote reasonable application in aseptic filling of a commercial filling line. Besides the accuracy and precision of the linear peristaltic pump, the impact of the filling system on the mAb stability was investigated and discussed in **Chapter II**. The linear peristaltic pump was found to be the most gentle filling system compared to two other traditional filling systems. By considering the process of aseptic filling in its entirety, the focus continued to be on the area of in-process control of fill volumes < 200 µL (**Chapter III**). The gravimetric in-process control is commonly used in commercial production but has its limits to a lower end of fill volumes about 200 µL. The new sensor prototypes tested in a technical feasibility study showed great potential for in-process control applicability after further optimization and development. With particle analysis being the final process step before the filled drug products are released for patient treatment, we analyzed the state-of-the-art method for subvisible particles and identified

pain points which should undergo improvements for better applicability towards lower fill volumes (**Chapter IV**).

Low Volume Filling of mAbs

In **Chapter I** a linear peristaltic pump prototype (LP) was characterized and further developed to reliably fill biopharmaceutical drug product with fill volumes < 200 µL under aseptic conditions. The construction of the pump out of stainless steel makes the pump robust against decontamination cycles with vapor-phase hydrogen peroxide (VPHP) and higher temperatures during cleaning and sterilization. The work was focused on process parameter development for fill accuracy and precision (i.e. wavelength and amplitude of the sine wave, frequency, number of actuators, tubing geometries), long-term performance, material robustness and possible miniaturization of the system. The LP technology needs at least three increments to produce a directed and backflow-free flow at the outlet. The LP prototype features six piezo actuators responsible for delivering the fluid. The flow is generated by creating a traveling sine wave. One actuator always must act like a valve and fully seal the tubing whether the other actuators load or unload the fluid [47]. With the preference of shifting the displacement of actuator 1 by 90° to the base sine, it was prevented that any air was entrained in the system. The symmetric shift of every actuator of 60° in relation to each other resulted in the most accurate and precise dosing of the LP. The main advantage of the LP is the capability to cover a fill volume range of 12 µL up to several milliliters and to deliver fluids with viscosities of up to 1000 cP. This makes the LP technology eligible for almost all drug product solutions in the portfolio. At the target fill volume of 50 µL the dosing accuracy of the LP was as good as ±3 % which is considered acceptable for commercial production.

One of the main concerns regarding peristaltic pumps in general is the possible abrasion of particles from silicone tubing. A study conducted with a radial peristaltic pump confirmed that particles resulting from tubing material are found to be in the nanometer size range and cannot completely be avoided, but minimized due to material characteristics [48, 49]. Peek et al. studied the time to tubing rupture and the failure pattern of tubes in radial peristaltic pumps. The process of particle shedding in radial peristaltic pump derives from the fact that two force vectors are applied to the tubing material. A) The orthogonal force which is applied by the fixure of the tubing between the counterpressure plate and the rollers and B) the tangential force which is exercised by the radial movement of the rollers

[50]. The linear peristaltic pump prototype exploits the advantage of the piezo actuators to only displace in orthogonal direction, eliminating any tangential force applied to the tubing. Another concern about peristaltic pumps is the possibility of dosing drifts over time caused by tubing shape changes due to distortion [15]. It is necessary to know the potential drift characteristics for the tubing material used during production and to compensate any drift over time with a feedback loop of the filling system. The tubing used in the accuracy and precision testing was the platinum cured Flexicon Accusil with an inner diameter of 0.8 mm and 1.6 mm. The tubing showed no drift during operation and even after 8 hours the dosing volume remained stable. This leads to the conclusion that the restoring force of the tubing plays a pivotal role regarding stable low volume filling.

To achieve the optimal dosing accuracy, the dosing element should be co-located with the filling needle to prevent any pressure drops over the tubing length, which results in poor dosing accuracy. Currently, the pump weighs approximately 20 kg, which is not suitable for fitting directly onto the filling needle. Our study showed that a reduction of the active piezo actuators can be reduced from six to three actuators without compromising the accuracy and precision of the filling system. With that material reduction the size and weight of the filling system can be reduced by 50 %, leading towards better probability of implementation in commercial filling lines. A further reduction of the piezo actuators is not possible, since three actuators is the smallest increment needed for a positive net flow rate [51]. Another pain point is the manual handling of the tubing and the resulting adjustment of the correct sealing of the tubing at the defined actuator displacement. This setup will be automated with a force sensor or an air-flow sensor in the future. Additionally, a feedback loop for an active fill volume control will be added to the setup to reduce manual handling and human error to a minimum. The feedback loop has to be compliant with high machine speeds of typically 700 ms/fill to achieve a sufficient output of drug product which was achieved by the filling system during all the studies. The next steps towards a future implementation of the prototype will be the construction or retrofit of the prototype down to three actuators. It is also worth to screen the market availability on new piezo actuator technology which could also be smaller in size without reduced actuating power.

Shear stress during low volume aseptic filling

In **Chapter II** the impact of the filling system on the stability of a monoclonal antibody formulation was investigated. Peristaltic pumps are known to be a gentle filling system which makes them suitable for the handling of delicate products like mAbs [52, 53]. Bee et al. stated that for a fully protein unfolding a minimum shear rate of 10^7 1/s is needed [54]. The phenomenon of protein unfolding is discussed to be dependent on a combination of several contributing factors (temperature, pH, salt concentrations, gas-liquid interfaces) than on a single mechanical shear event [25, 55]. Besides the traditional product related stress study (served as reference), two different models were developed and verified to investigate shear stress levels during aseptic filling and the possibility to use these models as predictive tools in the future. The first model is based on the concept of shear sensitive liposomes which is used for targeted drug delivery (e.g for the treatment of atheriosclerosis) [56]. The liposomal amphiphatic bilayer structure allows the incorporation of both hydrophilic and lipophilic substances, which are released under mechanostimulation by the deformation of the phospholipid bilayer [57].

Nevertheless, some questions regarding the full predictive power and applicability for shear stress quantification have to be discussed. The used lipid DPPC was chosen because of the formulation stability [58]. The addition of cholesterol was found to show a stabilizing effect to liposome bilayers [59]. Our formulation was found to be stable for 5d at 2-8°C with 5 mol % each of Cholesterol and DSPE-PEG2000. It was shown in literature, that the major stabilization effect of PEG-grafted liposomes is attributed to the steric repulsive force which the polymers confer on liposomes to prevent them from fusion and disruption in vivo [60, 61]. Cholesterol was used since numerous studies showed that the use of cholesterol can reduce permeability of the lipid bilayer and improve the vesicle resistance to aggregation [62, 63]. As this characteristic is a benefit regarding handling and storage it becomes a slightly disadvantage for its predictive power under mechanostimulation. The responsiveness of the liposomal model was found to cover shear rates up to 2000 1/s, which reflects typical shear rates during aseptic filling [23, 25]. On the other hand, the release of 5(6)-carboxyfluorescein as fluorescent dye only covers a total release of 1.5 % following a logarithmic kinetic. This means that even little release variabilities will result in large shear rate deviations which makes it difficult to use the liposomal model for the determination of quantitive shear rate levels. Moreover, 5(6)-carboxyfluorescein was used at a self-quenching concentration during encapsulation

preventing any background noise signal. When the dye was released from the liposome to the medium it was diluted and led to fluorescence dequenching and was therefore detectable at a high sensitivity even at low shear rate level. The liposomal model is only susceptible to the mechanostimulation but not to other types of stresses, a protein may be exposed to during aseptic processing (e.g liquid-air interfaces), which are discussed to affect protein stability in a larger extent [21, 24, 64]. This may shift the applicability of the liposomal model from a quantitative tool towards a qualitative indicator. In the future, the liposomal model needs to undergo further improvement and development to improve its predictive power. Therefore, a reduction in the cholesterol content helps to increase the release of the fluorescent dye under mechanostimulation. Besides the additives like cholesterol and DSPE-PEG2000, the composition of the lipid could help to improve the release of the dye. Needham et. al. eddemonstrated that the addition of MSPC resulted in the release of 80 % encapsulated doxorubicin within 3 min [65]. Therefore, a mixture of MSPC and DPPC should be tested in a future study. Holme et. al. demonstrated that specially designed lenticular Pad-PC-Pad phospholipid vesicles are used to release the incorporated dye at high shear rates. Originally intended for the applicability in atherosclerosis therapy, this formulation may also be used as a model for the quantitative prediction of shear rates during aseptic manufacturing [58]. Then, the model can contribute to considerable material savings during process development, since shear is present in almost every aseptic processing step. In the end, the formulations composition will always be a trade-off between shear sensitivity and storage stability with a low background release profile.

The second model was developed by the means of computational fluid dynamics (CFD). CFD is considered to be a proven method to solve theoretical fluid properties which allows investigation into shear rates during a variety of different processes [66-68]. During all our investigations we considered the mAb solution as newtonian fluid. Since the mAb we used in our studies had a concentration of 180 mg/mL we had to think about the impact of the viscosity on the flow properties. Basically, a newtonian fluid with higher viscosity value will have a very similar flow in all three considered filling systems. As a matter of fact, for all three processes, the flow is defined by a given flow rate over time. Consequently, the velocity field is almost viscosity independent in case of a newtonian fluid (only the pressure level would be higher). Consequently, the strain rate tensor and the shear rate that is purely calculated from the velocity field remains the same. Another phenomenon during aseptic filling which may have an impact on protein degradation is cavitation. Localized

pressure drops along the surfaces in filling systems (i.e. surface of the moving piston and cylinder in the piston pump) or during mixing have the potential to create cavitation [69, 70]. During our CFD simulation the cavitation pressure of water was about -7000 Pa relative pressure and the lowest relative pressure calculated during piston pump operation is not below than 70 Pa. Therefore, cavitation was neglected in our considerations.

Overall, the CFD model reliably simulated the impact of mechanical shear during the filling process of the three different pump types (representing parts of the overall product stress). To compare the different shear rate levels in the three pump types we introduced a new parameter called “mean volume fatigue”. Since fluid dynamics remain constant during filling, this parameter allows us to estimate the stress level a protein solution is exposed to at an arbitrary timepoint during the aseptic fill. As shown in **Chapter-II** the combination of all three studies (product study, CFD simulation, and liposomal model) allowed to demonstrate a correlation between protein damage, experimental shear stress measurements and CFD data. Finally, we can state that the combination of all three models (depending on prior knowledge of the dosing system) can be used to optimize aseptic filling processes in particular regarding fill volumes ≤ 200 µL. Additionally the linear peristaltic pump prototype was found to be the most gentle filling system in terms of mean volume fatigue and showed the lowest particle counts for all three filling systems.

The development of these models will hopefully serve to inspire other scientist for further development and improvement of these models since more and more data will be available during production routine and process monitoring. With more process data the models can be adapted to reflect the aseptic filling process in an even more realistic way.

In-Process control of low volume aseptic filling

As discussed in **Chapter I**, the accurate and precise filling of a monoclonal antibody solution is a key part during the aseptic manufacturing of low volume parenterals. Furthermore, the control mechanism – known as in-process control (IPC) – is of equal importance. The state-of-the-art method for the fill volume control is a gravimetric weight check. Therefore, a weighing mechanism (e.g. load cell) is incorporated into the filling machine and monitors the fill weight at a predefined frequency. The gravimetric IPC allow fast and reliable fill weight control at a reasonable speed of the filling machine. On the other hand, gravimetric fill weight control reaches its limits when it comes to fill volumes

< 200 µL. Within this volume range, which is representative for intravitreal and high concentrated antibody formulations, the gravimetric IPC becomes susceptible to environmental disturbances (e.g microvibrations, uni directional air flow) which negatively impact the accuracy of the gravimetric control system [71]. Another possible IPC to measure the fill volume is with a mass flow sensor based on the principle of the coriolis force. Although the mass flow sensor works for the weight check in the lower milliliter range it is not suitable for a precise and accurate measurement of fill volumes < 0.5 mL [72].

It is therefore necessary to not only provide a suitable filling system for the accurate and precise dosing of liquid parenterals, but to evaluate possible alternative fill volume control mechanisms. In **Chapter III**, five different sensor technologies (air-flow, capacitive pressure, MEMS, optical and capacitive) ranging from the prototype to the commercially available range were evaluated and tested for their applicability in the fill volume range between 10 – 150 µL. The sensors were placed in different parts of the aseptic fluid pathway (Tab. 2):

Table 2. Sensor location in the fluid pathway

Sensor	Surge Tank	Tubing	Needle	Status
Airflow	X			Prototype
Capacitive pressure		X		Prototype
MEMS		X		Commercial
Optical			X	Prototype
Capacitive			X	Prototype

Besides accuracy and precision of the IPC the reading time and the alignment with high-speed filling rates system is an important factor for a successful implementation. A typical filling time on high speed fillers is around 700 ms. During gravimetric IPC the measurement can only start after the actual dosing step is finished. Scales need settling time, measurement time and final communication to the programmable logic controller (PLC). Conceptually, the presented IPC systems work by data processing (integration) of sensor signals generated during the filling process. All data is already available at the end of the

fill. No stabilization and post fill measurement time is needed. This makes the presented technologies comparably fast. This can be considered a big advantage during high speed aseptic filling. Calculation is considered to take 1 ms. All our sensor studies used purified water as model solution. During aseptic manufacturing the IPC system has to deal with a variety of different fluids and its properties. They may differ in viscosity, density, salt- and protein-concentration, turbidity and color which are all factors which may influence the sensor signal and therefore the accuracy and precision of the sensors. Described by Troendle et. al., each dispensed volume and the associated velocity shows a characteristic sensor signal interpreted as “fingerprint”. This fingerprint signal is specific for the dispensing parameters (volume, velocity, liquid type) [73]. Therefore, the sensor needs to be calibrated to the fluid prior to routine manufacturing. The study results on accuracy and precision were compared to a gravimetric measurement which measures the actual weight of the fluid in the primary packaging material (e.g vial or PFS). All sensors used in the study have the advantage to provide a non-destructive fill volume control and showed promising results regarding a future implementation regarding accuracy and precision.

Nevertheless, other requirements and properties have to be discussed for the next steps. Therefore a user-requirement specification was drafted to help to identify the best fit IPC sensor for low volume aseptic filling of parenterals in the volume range of 10 – 200 µL (Table 3). Not all requirements listed below are necessary, but need conscientious consideration.

Table 3. User requirement specification for an IPC sensor

URS Requirements
No influence on the products quality/stability
Representative to the volume at the filling needle (= point of fill)
100 % control (+ data logging/interface to superior system)
CIP/SIP compatible
VPHP compatible
Autoclavable (<126°C, 60 mins pressurized steam)
Disposable (certified materials)
Low extractable/leachable profile
Easy handling
Low Maintenance effort
Easy to calibrate
Robust against environment
Compact housing (to allow needle movement)
Application area: 10 – 200 µL
Reading error < 1 %
Software response time should allow measurement for 50 units/min (= 0.7 s measurement time/unit)
Output signal: Readable for industry standard (e.g. Siemens SPS)
No measurement drift/trending
Built of standard electronic components
Able to widstand acceleration
NIST certification possible

The airflow sensor is very sensitive to environmental disturbances (e.g UDAF). In this case, the sensor has to be shielded from the direct environment to prevent signal disturbances from moving parts or operator activities, which will cause additional airflow.

Additionally, the location of the sensor is the most distant from the filling needle. A major concern to a system which is not placed below the primary packaging material is how the systems comply with off targeting or spilling of the filling system (e.g a fluid drop may pass the sensor but may not be filled in the vial). Even though the capacitive pressure and the MEMS sensor are closer to the filling needle they are more susceptible to air bubbles in the solution and the solution is in direct contact with the sensor. Therefore, the sensor needs to undergo the routine CIP/SIP/DIP process and all sensor components have to be compliant with sterilization conditions (heat, pressurized steam, VPHP) which limits the implementation of these sensors. The sensors located at the orifice of the filling needle were found to be more suitable for an IPC compared to the ones located prior to the filling system. However, the optical sensor is susceptible to different fluid properties such as turbidity and color which are different for each product. For this sensor an extensive calibration step is needed prior to any routine measurement and a broad database with fluid properties has to be established to provide enough information for the signal measurement.

The most promising sensor candidate towards a future implementation is the capacitive sensor. The sensor is relatively thin and allows a placement right above the target vials, so spilling and off target dispensing can be minimized. This sensor performed well during the tests even though the jet velocity information is not measured by the sensor. Ernst et. al. demonstrated that the sensor signal is influenced by changes in the fluid velocity and not only to the volume [74]. Therefore, for an industrial implementation the velocity information of the fluid jet should be known. Two sensors could be used at a defined distance to each other and the velocity information of the fluid jet could be derived.

In a future study the capacitive sensor needs to be tested in a real case environment for aseptic filling. Within this study the focus should be on the influence of different fluid properties (e.g. different salt concentrations and permittivity changes).

Particle Analysis of low volume aseptic filling

After successful aseptic filling and capping, the finished drug product has to undergo visual inspection as a final control step prior to release for patient treatment. This last unit operation serves for the separation of unacceptable units from the batch. Unacceptable defects can range from product related, unintended extrinsic materials or components

derived from the container-closure [26]. Analysis of subvisible particle (SVP) counts is required for final drug product release testing to mitigate the risk associated with particle presence (e.g. blood vessel occlusion) [75]. There is a controversial discussion about a possible immunogenic effect of proteinaceous particles. Artificially generated particles did not provoke any immune response stimulation, but there were observation on oxidized protein aggregates. The oxidation level was found to be at higher levels than typically observed during routine analysis [76-78]. Nevertheless, protein aggregation and particle formation remain a critical quality attribute because there is a concern on product efficacy caused by different biologic activity of protein aggregates [19]. Several analytical methods are available for the quantification of SVP. The Pharmacopoeias allow the use of light obscuration (LO) and microscopy. With the emerging number of drug product formulations for ivt. use which goes along with a reduced fill volume, the analysis of SVP with LO is very challenging to the pharmaceutical industry.

In **Chapter IV** we investigated the LO method in detail and sequenced the analytical method in its single method parameters and their impact on the measurement to potentially identify possibilities to reduce sample volume as much as possible. Our results showed that a minimum volume is required to replace the residual volume in the measurement cell from previous measurements or cleaning steps. This volume was classified as tare volume in our studies. Nevertheless, the volume reduction of the LO method is finally limited by the geometry of the system itself. Shorter needles, tubing, and measurement cell may be an alternative to further reduce the volume needed for LO measurements. Besides any technical adjustments to the LO instrument, the instrument set up with a particle-free surrogate solution, mimicking the fluid properties of of the protein formulation, could prevent the instrument from false positive particle counts due to the formation of immiscible phases. This idea needs further studies to evaluate this approach and an extensive surrogate library will be the result to deal with all the different protein formulation compositions. Overall, our studies showed that the LO method is reaching its boundaries by approaching sample volumes < 1 mL. Besides the need of sample volumes in the milliliter range, the LO method has reported several issues regarding error rate and being susceptible to different solution properties (refractive index, high viscosities, color and turbidity). Whether color and turbidity showed only small effects on SVP counting and seizing analysis, viscosity and refractive index have a more pronounced effect [30]. In terms of the refractive index it is recommended to extent the analytical methods from LO to light independent measurement like resonant mass measurement [45]. The solutions

concentration is also influencing the analytical results from LO measurement and it was found that concentrations as low as 10 mg/mL already impair the correct analysis of SVP [79]. High concentrations and viscosities showed the tendency to result in an undercounting effect during LO measurements. This effect is based on the intake of air into the measurement system and therefore a reduced sample volume in the system. This problem was described in literature and was solved by pressurizing the LO system [44]. In contrast to this effect, we experienced an undercounting effect for viscosities > 12 mPa·s. This effect was explained by the formation of immiscible phases (streaks) between the aqueous phase in the system (from the cleaning and rinsing step) and the sample of protein formulation. Therefore, new methods for sub-visible particle analysis should be investigated. A recently developed high throughput technology is background membrane imaging (BMI). Samples for BMI measurement are isolated on a filter surface for counting analysis by a microscope comparable to the microscope particle count test. Compared to the LO method with sample volumes around 1 mL and long measurement times, BMI only needs 60 µL of sample volume and could therefore be an alternative for the analysis of low volume protein formulations since the required sample amount is close to the administration volume of intravitreals. Recent research has been shown that BMI can be a useful tool in the measurement of protein aggregates, but still there is more data and information needed on the analytics of critical fluid properties (e.g. high viscosity) [80, 81].

CONCLUSION

During this PhD thesis, four major work-packages initiated to tackle low volume aseptic filling of monoclonal antibodies at large.

First, the newly designed and patented technical innovation of the linear peristaltic pump has demonstrated the feasibility of reliably filling fill volumes down to 12 µL at different fluid viscosities. The dosing accuracy of this pump prototype was found to be ±3 % for fill volumes < 100 µL, stable for an investigated time up to 8 hours of continuous operation (equal to one shift in commercial production). In another study, it was shown successfully that the reduction down to three piezo actuators in operation is enough to achieve comparable results regarding accuracy and precision of the targeted fill volume. This paves the way towards further development of the pump prototype as regards weight and size reduction. Consequently, by combining two modes of operation (liner peristaltic and time-pressure) into one single device, the pump prototype is able to cover the filling volumes from lower microlitres up to several mililitres which allows highest flexibility for meeting the requirements on future protein formulations **(Chapter I)**.

Second, the linear peristaltic pump was compared to two state-of-the art filling systems with regards to shear stress. Therefore, three different studies were set up and executed to characterize product stress during low volume filling. The development of a liposomal model allowed a qualitative comparison of shear stress levels in the different pump types. The combination of the liposomal model with computational fluid dynamics and a classic protein formulation shear stress study helped to deepen the understanding of shear related processes during aseptic filling. It was shown that the piston pump creates a recirculation zone where drug product formulations can be trapped and exposed multiple times to product stress. Therefore, the applicability of these quantitative models to other aseptic operations (i.e thawing, mixing etc.) can improve process understanding and reduce the need of extensive product studies during process development. Most importantly, the findings showed that the linear peristaltic pump is superior to both state-of-the-art filling systems regarding particle formation. **(Chapter II)**

Third, the concept of a new in-process control was investigated to provide a reliable technology for low fill volume control. A detailed study was set up and performed, where five different sensor concepts were tested and compared to a highly precise and accurate

analytical gravimetric balance. A capacitive sensor was found to be the most suitable sensor for the needs of low volume aseptic filling. The sensor provides a contact free 100 % measurement of every single container which is filled. The sensors location under the filling needle ensures a measurement of the total volume in the container. In a next step, the testing of the sensor with different fluid properties and velocities will further improve the applicability of this technology and dramatically change the way of in-process control during aseptic filling **(Chapter III)**.

Fourth, subvisible particle analysis was investigated. Light obscuration serves as the state-of-the-art method for subvisible particle analysis. Every single method parameter was analyzed and varied detailly for a possible sample volume reduction. the influence of the different method parameters in combination with different protein formulation properties were elucidated in this study.d. Unfortunately, the sample volume reduction is restricted to the geometry and therefore the size/volume of the measurement cell for reliable measurements. In further studies, the development of smaller measurement cells with less dead volume is necessary and should be considered for future improvement for low volume particle analysis. Another improvement of the analysis method could be the use of particle free surrogate for washing and rinsing of the device to reduce false positive particle measurement rooting from different physic-chemical properties of the drug product formulation and the particle-free water **(Chapter IV)**.

In conclusion, the findings in this PhD thesis will hopefully increase the understanding of low volume aseptic filling of monoclonal antibodies and future newly developed protein formulations. This will foster the discussion in the field of aseptic filling and lead to more innovative technology to better supply patients with precious medicine whenever needed.

ABBREVIATIONS

API	Active pharmaceutical ingredient
CIP	Cleaning in place
DIP	Drying in place
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSPE-PEG2000	1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]
Fab	Antigen-binding fragment
FDA	Food and Drug Administration
i.m.	Intramuscular
i.v.	Intravenous
i.v.t.	Intravitreal
LO	Light obscuration
LP	Linear peristaltic
MFI	Micro Flow Imaging
mAb	Monoclonal antibody
Ph. Eur.	European Pharmacopeia
RP	Radial peristaltic
RPP	Rotary piston pump
s.c.	Subcutaneous
SIP	Sterilization in place
SVP	Subvisible particle
URS	User requirement specification
USP	United States Pharmacopeia
Pad-PC-Pad	1,3-dipalmitamidopropan-2-yl-2-(trimethylammonio)ethylphosphate
VPHP	Vapor phase hydrogen peroxide

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- Fundamental understanding of current Good Manufacturing Processes
- Driver of the MSAT culture initiative with focus on new agile ways of working
- Reputation for quick comprehension and time-management
- Strong inter-personal skills and experience to build good working relationships with key stakeholders and network partners
- Encouraging personality with the ability to empower colleagues with trust, reliability and honesty

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- Validation of drug product launches & transfers
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- Project management of the PhD-thesis (3 peer reviewed publications and 2 patent applications)
- Management of timelines and coordination of multiple projects
- Development of end-to-end low volume filling processes
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05/2015 - 10/2015 **Karl-Apotheke, Karlsruhe, Germany**

- Manufacturing of personalized formulations and quality control
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Siegwerk Druckfarben AG & Co Siegburg, Germany

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Upon request

Publications

04/2020	Patent: WO2020/079236 A1 - “Microdosing”
04/2020	Dreckmann, Tim, et al. "Low volume aseptic filling using a Linear Peristaltic Pump." *PDA Journal of Pharmaceutical Science & Technology* (2020)
04/2020	Dreckmann, Tim, et al. "Assessment of sensor concepts for a 100% in-process control of low volume aseptic fill finish processes." *PDA Journal of Pharmaceutical Science & Technology* (2020)
02/2020	Dreckmann, Tim, et al. "Low volume aseptic filling: Impact of pump systems on shear stress." *European Journal of Pharmaceutics and Biopharmaceutics* 147 (2020): 10-18.
11/2018	AAPS PharmSci360, Washington DC, USA • Talk: “Low volume filling of mAbs – using a linear peristaltic pump” • Talk: “New sensor concepts for the IPC of low volume filling” • Poster: “Low volume filling of mAbs – an end-to-end examination”
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