High accuracy measuring of volume changes within an aseptic closed system

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Abstract—**Single-use systems have become a prevalent method for storing and transporting drug substances in todays biopharmaceutical industry. Owing to the minimal chances of substance loss and contamination, the storage of small volumes is increasingly appealing. Drug substances are being filled using sterilised single-use assemblies. How precisely the drug substance is being dispensed in the small bag is thereby of utmost importance. Current measurement methods however encounter obstacles that pose significant challenges to achieving optimal accuracy. To meet increasing demand for high-precision filling, a novel measurement technique in this field is employed which uses capacitance measures to detect the filled volume. As there is no equivalent alternative at present, this paper aims to provide a comprehensive overview of the components comprising the error budget. The filling process of a bag is monitored and the water distribution is quantified using image processing at given intervals. This enables the creation of well-fitting equivalent circuit models which are employed for Monte Carlo simulations. Additionally, the image processing pipeline is utilised to generate 3D models that depict the morphology of water inside the bag during the filling process. After validating the model, the water distribution within the capacitor is modified in order to uncover its impact on the measurement, alongside multiple simulations that explore the potential presence and quantity of errors. Finally, measurements were conducted using a plate capacitor and an automated filling process to determine their accuracy.**

Index Terms—**IEEEtran, single-use assembly, biopharma, capacitance, image processing, EM-simulations.**

List of Acronyms

CI	Confidence Interval
CDC	Capacitance Digital Converter
CI	Confident Interval
EVA	Ethylen-Vinylacetat-Copolymer
HSV	Hue,Saturation,Value
IC	Integrated Circuit
RGB	Red,Green,Blue
ROI	Region of Interest

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I. Introduction

WHEN packaging valuable drug substances into Single-Use bags, filling accuracy and speed are two crucial factors to be considered. There is a growing demand for machines that can quickly fill over a hundred bags, making it imperative to identify an affordable measurement solution that brings robust and accuracy.

Traditional measurement techniques, including scales and flow meters, have limitations when used with disposable equipment. A common problem hereby is that the bag which weight is to be determined is attached to a tubing of the manifold during the filling process.

Single-use Coriolis flow meters are achieving high accuracies[1] but are also paired with high costs per manifold.

Ultrasonic flow meters only clamp-on options meet cost expectations in most cases. In addition, they have drawbacks when it comes to measurement robustness. Air bubbles, inhomogeneities, and chaotic flows are leading to a decrease in measurement accuracy. Changing temperatures are also introducing errors as the change the speed of sound[2]. These effects are particularly prominent in filling systems with peristaltic pumps, which are often utilised. These pumps are appealing as they operate from the outside of the tubing, but also creating high shear forces on proteins in processed liquids[3]. As a result, the flow can be affected by spikes in flow rate and system pressure. Furthermore, are clamp-on sensors reliant on the diameter of the tubing in which the flow is measured, which introduces a large possible error into the measurement.

To devise an innovative solution that could potentially overcome the current difficulties, the measurement of bag volume can be reduced to simply calculating a change in mass or volume within a controlled region. A field-based technique is proposed, using a plate capacitor in which the capacitance is heavily dependent on the matter present between the plates. If a bag is placed between the plates and filled, significant changes in capacitance occur due to a water-based drug substance with an 80 times higher dielectric constant compared to the air that was initially between the plates. Capacitance measurements are common in modern touchscreen devices, and there exists a wide variety of Capacitance Digital Converter (CDC) chips that are often available as an easy to use Integrated Circuit (IC). A promising point of departure for a potential use of these cost-effective solutions also in the pharmaceutical industry.

II. METHODS

A. Overview

In order to perform a comparison of the calculated, simulated data and the real setup, it is necessary to define constrains of the system:

- The electrodes of the capacitor have the following dimensions: $110 \times 90 \times 1\,\mathrm{mm}$.
- The Bag between the plates is out of Ethylen-Vinylacetat-Copolymer (EVA) with dimensions of: $68.1 \times 91.8\,\mathrm{mm}$. Such a bag is shown in Fig. 1.
- The filling is performed with Water at $21\,^{\circ}\mathrm{C}$ for which a relative permittivity of $\varepsilon_r = 81$ is used in the calculations
- The surrounding air is calculated with a relative permittivity of $\varepsilon_r = 1.0006$
- The distance between the plates is $d = 5.7$mm

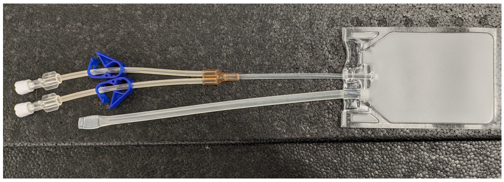

Fig. 1. Image of the EVA bag on which the tests and considerations are based.

B. Water distribution in the Bag

In order to model the filling process accurately, image processing is used to monitor the water distribution in a bag during filling.

The system has been configured with a camera placed perpendicular to the bag, which is positioned to occupy most of the space in the image. To ensure uniform exposure in each frame, the camera is enclosed in a box and fitted with a ring light. To view water through the bag, blue food dye is used. The dosing system (see Sec. II-F) fills the bag at a rate of $100\,\mathrm{ml/min}$. The detection of water distribution is easy to humans, but measurement can be a challenge. However, image processing algorithms can overcome these challenges. The subsequent section outlines the methodology of utilizing 2D data sourced from an image during the filling to finally generate a 3D model that can be used in simulations. Along with the 3D model, examination of the bag provides valuable information that can assist in the overall modelling process, including the distribution of water within the bag.

As for the image, these areas with more water can be found by evaluating the saturation as well as the lightness of the blue. Together, with the shape and the size of areas where blue is present. With a normal image coming in the Red,Green,Blue (RGB) color space[1] it is helpful for the development of this task

[1]This common known color space describes a color by the ratio of red, green and blue being present.

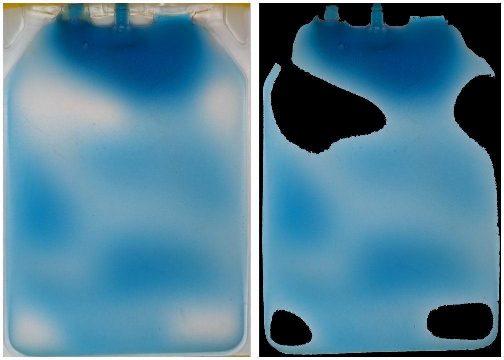

Fig. 2. Demonstration of different filter values for the [Hue, Saturation, lightness], performed on the same basis-image

to transform it into the Hue,Saturation,Value (HSV) color space[2]. As it is more intuitive to find a more saturated blue, than one with more green or red.

In that image acquired, the Region of Interest (ROI) is the bag itself. In that ROI water can be found by searching pixels, being blue, with saturation and lightness within a certain threshold. Using a thresholding algorithm a binary mask can be created in which every point within the thresholds will be a 1 and if not a 0. 2.

The image is then sectioned in smaller clusters (n = 100^2) of which the mean intensities of the pixels within the cluster is calculated. Now it is possible to determine two important values for the modeling of the filling process:

1) The area of the bag, which is covered by water, depending on the filled volume
2) How the Water is distributed (here called density)

These two values are depicted in Fig. 3. The area coverage is then used for building the equivalent circuit model, as described in Sec. II-C.

[2]This color space expresses the value of a pixel using the hue, saturation and value of the present color. Where hue represents the basic color such as blue[4].

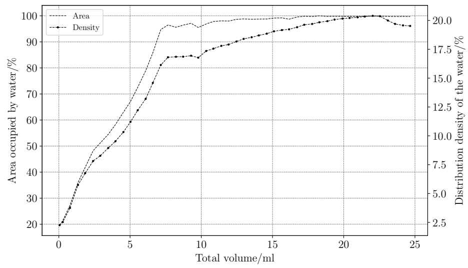

Fig. 3. Graph illustrating the proportion of the area covered by water during different stages of the filling process. Additionally, the graph displays the distribution of water across this area, quantified by the density measure. This density measure represents the percentage of the area in which the top 25 % of intensities are concentrated.

C. Equivalent circuit model

As described, the filling process uses a 2D disposable bag made of EVA, a pliable polymer that allows the bag to bend to about 90° during port handling. A simple model of filling such a bag can be obtained by taking the area of the bag and assuming that the water is filled into a rectangular shape.

This can be converted into a model, using a plate capacitor and placing a cuboid of water at its center. The cuboid's area will then reflect the bag's area, and its height will represent the filling process' current state. To create a capacitance model, the capacitor is split into two parallel capacitors, as illustrated by Eqn. 2. One having a distance of $d_1 = d$ and an area of $A_1 = A - A_{Bag}$, represents this process. This capacitor signifies the capacitance of the plates' air dielectric area. The second one consists of a pair of capacitors representing a set of dielectrics showcased in Fig. 4 as C_{Air} and C_{Water}. To determine the capacitance of the series of two capacitors, refer to Eqn. 1.

$$C_{surr} = \frac{\varepsilon_1 \varepsilon_2 A}{\varepsilon_2 d_1 + \varepsilon_1 d_2} \tag{1}$$

$$C_{bag} = \frac{\varepsilon_1 A_1}{d} + \frac{\varepsilon_2 A_2}{d} \tag{2}$$

Where d_1 is the height of the water and $d_2 = d - d_1$ which is the height of the air gap between the plate and the water. The total capacity is then given by

$$C_{total} = C_{surr} + C_{bag} \tag{3}$$

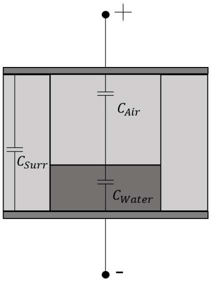

Fig. 4. Schematic of the cuboid-model, which is represented by three capacitors

As can be seen from figure 3, the area of the water is not constant. To take this into account, the area of the water is calculated using only a percentage of the original area. Form the graph, several points were selected as anchor points with and area values between points are linearly interpolated. This results in a model that follows the fill curve very well (Fig. 5). However, a 70 % gain is applied to the model to achieve this fit.

D. 3D Modeling

Taking the modelling a step ahead, the average value of the cluster, created from the image can be interpreted as the z-value of a point within a point cloud wherein the corresponding x and y values represent the middle of the cluster. There exist numerous well-elaborated algorithms to triangulate a surface from a point cloud, with the Delaunay triangulation being a commonly employed algorithm[5].

To obtain a 3D body from a 2D surface, the upper surface is mirrored on the XY-plane, and then it is extruded in the z-direction using the lower plane as a clipping plane. This results in a watertight body with triangles that form sharp edges. A model alongside with the image it has been created from is shown in Fig. 6.

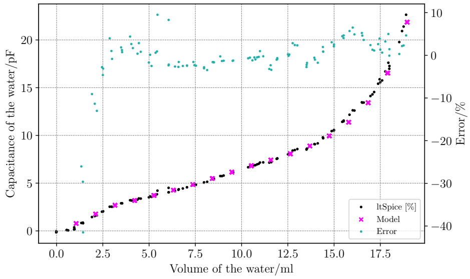

Fig. 5. Graph of the fitted 4-Phase Cuboid-Model in magenta, compared with the measurements in black which it has been fitted to. Errors of the measurement data are excluded. See II-F for reference.

Nonetheless, the external boundaries and volume of the 3D-model require adjustment to fit the physical bag. It is preferable to scale the point cloud rather than the mesh. However, volume calculation can only be performed on the finished and filtered mesh. Due to the unpredictable output of the filters, the scaling requires iterative adjustment. An PI-controller is utilized to control the volume error to zero by adjusting the scaling factor. The controller is set up to reach its target in 5-10 iterations. In addition to incorporating a volume controller, the imposed physical limits are also upheld. Specifically, the bag's maximum height which is restricted by the two plates of the capacitor. Should any points exceed the allowable limit during point scaling, they are restrained, resulting in the surrounding points being scaled up till the target volume is reached.

E. Simulation

There were multiple simulations performed, which are investigating the impact, of the measurement setup itself on the measurement. This Section primar-

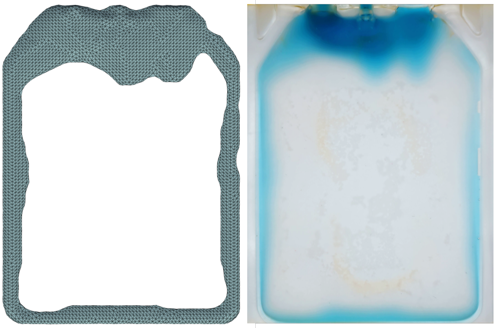

Fig. 6. Side by side comparison with an image of a bag, filled with $2.5\,\mathrm{ml}$ and a model with $2.4\,\mathrm{ml}$

ily emphasizes the impact of the bag on the measurement, including its placement and the distribution of water. The simulations are conducted, using models of 3D water structures for different volumes which are created using the method outlined in Sec. II-B.

A mesh convergence study was performed on different volumes in order to ensure the independence of the simulation result and the mesh size. After performing a gain on the simulation, the results of the simulation, which shows the normal filling process fits the real measurement data very well. The error of the fit was evaluated, by linear interpolating between available simulation points and calculating then an offset towards available measurement data. After the gain this error then just showed the error present in the measurement itself.

To investigate the impact of a bag, which does not fully open, the lower $30\,\mathrm{mm}$ of the bag is set to zero, leading the controller to scale the rest of the model till the target volume is reached again.

F. Measurements

In order to produce measurements, a two plate capacitor is build out of 3D printed parts which are capable of fixating the measurement plates. The capacitance is then measured using a calibrated LCR-meter. Namely, a UT622E from UNI-T®. Three measurements where conducted on three days in a row, of which a single measurement is performed within $2\,\mathrm{h}$.

Fig. 7. Showing the UT622E, connected to the plate capacitor setup.

III. Results

A. Monte Carlo Simulation

The developed model provides an opportunity for further exploitation, such as using it to establish constraints through Monte Carlo simulations. As for example, if a constraint for the tolerance of the plate distance needs to be defined, a realistic value could be:

$$d = 5.7\text{mm} \pm 0.1\text{mm} \tag{4}$$

Which indicates a deviation of $\pm 1.75\,\%$. This error is applied to the model where 10k runs are performed in which for every point in the model a random plate distance is drawn with an error, build from a group with a σ of $1.75\,\%$ and a mean of 0. The error is then added to the original assumed $5.7\,\mathrm{mm}$. The results are shown in Fig. 8. The graph shows the simulated values, together with a walking standard deviation being calculated for multiple windows. For each window, together with the point estimate the Confident Interval (CI) of $95\,\%$ certainty is calculated. The

TABLE I
ERROR EVALUATION FOR A TOLERANCE OF 1.75 % IN PLATE DISTANCE

Volume V/ml	s/ml	$CI_{95\%}$ of s in ml
$0 < V \leq 3$	0.05	(0.04, 0.06)
$3 < V \leq 7.5$	0.26	(0.22, 0.29)
$7.5 < V \leq 10$	0.43	(0.38, 0.48)
$10 < V \leq 15$	0.39	(0.33, 0.44)
$15 < V \leq 25$	0.35	(0.3, 0.4)

result are again compressed into Tab. I. Here, values are indicating that a tolerance of 1.75 % of the plate distance conveys to a deviation of the measurement data of around 5 % for 10 ml.

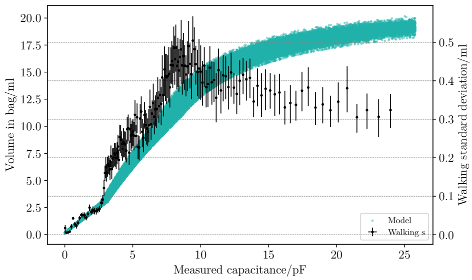

Fig. 8. Monte Carlo simulation of the model with an applied error on the distance of the plates of ±1.75 %. The standard deviation of the data is calculated in a moving window and evaluated using bootstrapping to generate error labels

This method can now be used to investigate impacts different tolerances and errors are making onto the measurement, with simulations being performed within seconds.

B. Simulations

According to the simulation results depicted in Fig. 9, for the bag with the cutoff of 30 mm the error increases to 40 % at 4 ml and remains oscillating between 30 % and 40 % the largest calculated volume of 16 ml. However, higher volumes cannot be analyzed due to excessive bag deformation. For the bag with only 70 % of its length, the error increases to 40 % at 4 ml and remains oscillating between 30 % and 40 %. These results demonstrate that even though the general water distribution is the same, a reduced bag area results in substantially higher capacitance measurements.

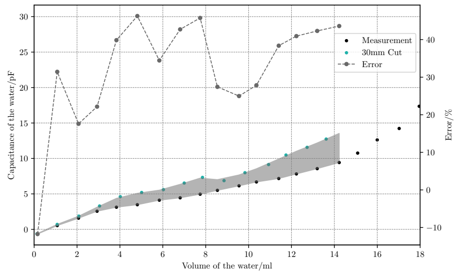

Fig. 9. Simulation of the bag whereby the lower 10 mm in length of the bag not used. The results from this simulation are compared to those from a reference simulation, and the resulting error is plotted

C. Measurements

As an initial stage in assessing the attainable accuracy with the current plate capacitor setup, the X and Y axes of the measurement are swapped. This facilitates a more intuitive interpretation of the accuracy, as now a band of volumes is assigned to a capacitance value. Additionally, the standard deviation of the volume values for a given window of capacitance values is calculated and the confidence interval of this calculation is determined using the bootstrapping method (see Fig. 10). For this, a window of 15 measurement points is selected, which corresponds to a range of approximately 1 pF to 2 pF. For each window of measurement data, a set of 1,000 bootstrapping samples is obtained, and the standard deviation for each of these samples is calculated.

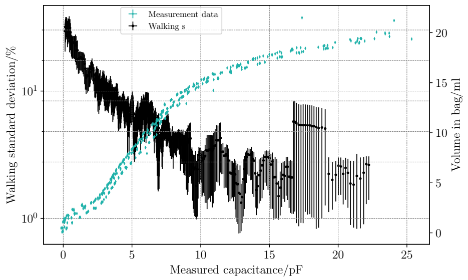

Fig. 10. Graph displaying the statistical analysis of the available measurement data, represented by the black line. The standard deviation of the data is calculated in a moving window and evaluated using bootstrapping to generate error labels.

Additionally, to further analyze the data, intervals of interest are defined as shown in Tab. II, where the mean of the point estimate of the windows within each interval, as well as the mean for the upper and lower confidence intervals, are determined. These values now give a good estimate of the accuracy of this setup over this series of measurements. For volumes less than $5\,\text{ml}$, the standard deviation, ranges from $12\,\%$ to $30\,\%$.

The confidence interval of the standard deviation reveals that some points within these regions have much smaller standard deviations than the initial estimate suggests. This can be verified visually by observing how certain points are clustered, and the distribution is not completely uniform. The standard deviation of these lower parts is particularly high, owing to the measurement M3, with values that have been shifted. However, the standard deviation for values above $5\,\text{ml}$ is already at around $3\,\%$, dropping to $2\,\%$ for values over $12\,\%$. Wit outliers in the measurement being present. If there were no outliers, the standard deviation would drop to under $1\,\%$.

TABLE II
ESTIMATE OF THE STANDARD DEVIATION, TOGETHER WITH THE $CI_{95\%}$

Volume V/ml	s/%	$CI_{95\%}$ of s/%
$0 < \text{V} \leq 3$	29.02	(34.2, 20.07)
$3 < \text{V} \leq 5$	11.99	(14.5, 7.63)
$5 < \text{V} \leq 12$	3.08	(3.82, 1.95)
$12 < \text{V} \leq 15$	2.03	(2.53, 1.28)

IV. SUMMARY

The simulations demonstrate that there is significant sensitivity in capacitance readings to changes in water volume and distribution. It was revealed that errors of up to $50\,\%$ were observed when simulating a bag with only $10\,\%$ of its original area missing.

Further simulations were conducted to investigate the impact of alterations to the set-up of the capacitor's plates. As with water distribution, even minor adjustments to the plates' position or rotation can result in substantial variations in capacitance measurements. In particular, non-parallel plates have been found to induce errors, which can swiftly accumulate to $5\,\%$ of the measurement. A deviation of the plates' position by just $0.3\,\text{mm}$ has been demonstrated to cause errors of up to $25\,\%$.

With the high sensitivity present, it must now be taken as advantageous, and it is up for future developments to exploit it. Initial measurements were taken using a two-plate capacitor and an automated dosing system to obtain measurement data on the different volumes. These measurements were analyzed to gain a basic understanding of the achievable accuracies. Despite the simple setup without any precautions towards bag fixation, the measurement showed high repeatability with volume reading errors dropping below $1\,\%$ for higher volumes. However, additional measurements using a modified bag are emphasizing the need for further precautions. The model creation pipeline can now be utilized for future development and preliminary studies of new designs. To achieve independence in water distribution, initial simulations using a 12-plate capacitor were conducted. Results are indicating that such a design must be carried out carefully, with the potential errors known from previous simulations now applicable to each of the 12 pairs of plates. Furthermore, the total capacitance reading is now also split into multiple smaller readings making it more sensitive to noise and measurement error.

V. Outlook

This paper lays the groundwork and provides the necessary tools for future advancements towards achieving desired levels of accuracy when it comes to high accuracy measurements. The benefit of the significant impact that minor adjustments in water distribution and bag placement have on the measurement should be exploited.

In future versions of the device, it is recommended that bag placement is fixed to eliminate errors in the baseline. One possibility to achieve this is by using foam to secure the bag in place. Nevertheless, previous research has revealed that this could result in unwanted water distribution and plate bending caused by the foam's force.

Hence, it is advisable to use several plates for measuring capacitance in clusters in future designs. However, it has been shown that these smaller plates produce considerably lower readings, requiring advanced algorithms to perform cross-evaluation of the present capacitances in the system. Future designs should not only be able to measure capacitances of direct partners, but cross-evaluations could also enhance robustness. In systems with multiple capacitors, it is ideal for them to be produced as a single unit to reduce the impact of parallelism and placement errors on measurements.

Before deploying future designs, design constraints can be assessed prior to prototyping via a combination of Equivalent Circuit models and Monte Carlo simulations. These methods provide an effective approach for conducting sensitive analyses on the system. The circuit used can be further developed to incorporate future measurement designs.

Measurements and sensitivity analyzes demonstrate a trade-off between high measurement accuracy in larger filled volumes, which are, however, more susceptible to error. Existing pipelines can then be utilized to assess whether a more precise measurement circuit, combined with smaller volumes or fixed constraints with less costly circuits, yields the desired accuracies.

Further studies should not only focus on improving the design of the plates themselves, but also on replacing the costly LCR-meter with a more cost-effective capacitance measurement circuit. However, preliminary studies have shown that this change introduces significant challenges in terms of value drift and repeatability. The significant impact of temperature changes on measurements can be mitigated by tightly controlled environmental conditions such as those provided by a clean room.

Acknowledgment

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References

[1] Martin Anklin, Wolfgang Drahm, and Alfred Rieder, "Coriolis mass flowmeters: Overview of the current state of the art and latest research," *Flow Measurement and Instrumentation*, vol. 17, no. 6, pp. 317–323, 2006. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0955598606000379

[2] N. Bilaniuk and G. S. K. Wong, "Speed of sound in pure water as a function of temperature," *The Journal of the Acoustical Society of America*, vol. 93, no. 3, pp. 1609–1612, 1993.

[3] C. R. Thomas and D. Geer, "Effects of shear on proteins in solution," *Biotechnology Letters*, vol. 33, no. 3, pp. 443–456, 2011.

[4] L. Shuhua and G. Gaizhi, "The application of improved hsv color space model in image processing," in *2010 2nd International Conference on Future Computer and Communication*. IEEE, 2010.

[5] Tianyun Su, Wen Wang, Zhihan Lv, Wei Wu, and Xinfang Li, "Rapid delaunay triangulation for randomly distributed point cloud data using adaptive hilbert curve," *Computers & Graphics*, vol. 54, pp. 65–74, 2016. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0097849315001223