Automatic buffer preparation system for downstream processes

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Summary

As the market for biopharmaceuticals grows, the demand for continuous downstream processing (DSP) increases. This thesis tackled one of the key factors in successfully creating a continuous chromatography process: the ability to have an automated supply of complex buffer solutions. It aimed to develop and evaluate a Buffer Preparation Unit using an ÄKTA Pure machine, as well as the development of a custom Graphical User Interface to increase the usability of the system. This is done through the combination of the physical configuration of the hardware and the digital implementation of it in Orbit, a custom Python-based software architecture.

The hardware was physically configured to draw from 12 distinct stock solution to mix 10 specific buffers used in the DSP. To bypass the limitations of the standard control software, operations were executed via an OPC UA network using Orbit. Furthermore, the GUI was developed to centralize control of the system, allowing operators to execute pre-defined recipes, automatically calculate the volumetric ratios for manual buffer blends, monitor real time Quality Assurance data, and perform some basic maintenance on the system.

System evaluation identified two main challenges in the system. Firstly, the narrow inner diameters of the multi-valve configuration introduced cavitation during the mixing phase of the buffer preparation, capping the flowrates during this phase to 18-25 ml/min, depending on the position of the buffer flask. While this does not pose a big problem in the stand-alone system, it introduces limitations to up-scaling and integration with the rest of the DSP. Secondly, the internal pH sensor of the system was found to have a static error and relatively large deviations in its readings, making it unreliable which necessitates the use of an external sensor for measuring pH during quality control.

The thesis concludes that the developed BPU shows promise in being used in a real-life setting, despite its physical limitations. With the developed GUI combined with the BPU's ability to automatically prepare buffers on demand, the system has potential to reduce the need mor manual buffer preparation and with further work, it may be integrated into the rest of the DSP, generating a truly automatic buffer supply for the process.

Populärvetenskaplig sammanfattning

I takt med att allt fler användningsområden upptäcks ökar efterfrågan på biologiska läkemedel i form av till exempel enzymer, antikroppar och virus. I många fall har biologiska läkemedel redan blivit standard i behandlingen av sjukdomar som diabetes, HIV/AIDS, samt vissa neurologiska sjukdomar. Dessa läkemedel kännetecknas av att de produceras av celler som har blivit genetiskt modifierade för att producera en specifik farmaceutisk molekyl. Från cellerna kan sedan läkemedlets verksamma komponenter utvinnas och renas upp.

Trots den ökade efterfrågan av biologiska läkemedel är fortfarande framställningsprocessen både dyr och ineffektiv. Utöver stora kostnader associerade med forskning och utveckling av själva läkemedlet, ligger en stor del av produktionskostnaderna, i vissa fall majoriteten av kostnaderna, i uppreningssteget. Det är det steg av processen där den verksamma komponenten ska separeras från allt annat en cell innehåller. Beroende på produkt finns det en uppsjö av tillgängliga metoder för att separera ut och rena upp det biologiska läkemedlet. Ett exempel är jonbyteskromatografi, en process där ämnen separeras baserat på skillnader i laddning, vilket är den centrala separationsprocessen i denna rapport.

I jonbyteskromatografi blandas alla ämnen ut i en saltlösning (mobil fas) som sedan förs genom en så kallad kolonn vilket är en typ av rör packad med ett fast ämne med en laddad yta (stationär fas). Ämnen i den mobila fasen fäster då till ytan i kolonnen till följd av skillnad i laddning mellan komponenterna i den mobila fasen och ytan i den stationära fasen. Därefter sköljs kolonnen med saltlösningar med olika koncentrationer och sammansättningar, så kallade buffertar, för att gradvis få ämnena att släppa från den stationära fasen. Beroende på ämnenas laddning kommer de släppa olika lätt från den stationära fasen, vilket gör det möjligt att separera ut den önskvärda komponenten.

Likt många andra processer inom industrin är det önskvärt att ta fram en kontinuerlig process för jonbyteskromatografi, eftersom detta möjliggör en strid ström av det renade biologiska läkemedlet och minskar dödtiden associerad med att återställa kolonnen efter en separation. För att möjliggöra en sådan kontinuerlig kromatografiprocess krävs även en konstant tillgång till de buffertar som används i separationen, vilket även är denna uppsats fokuspunkt.

Uppsatsen undersöker möjligheten att konfigurera en maskin för att automatiskt blanda upp buffertar genom att blanda ihop olika stamlösningar, högkoncentrerade lösningar av de olika komponenterna som uppgör bufferten, till en färdig buffert. Kvaliteten på de blandade buffertarna, samt de fysiska begränsningarna hos maskinen undersöktes. För att underlätta beställningen av buffertar och underhållet av maskinen har även ett användargränssnitt tagits fram. I detta gränssnitt finns även möjligheten att beställa buffertar baserat på önskad koncentration i slutprodukten.

Resultatet visar att maskinen går att använda för att blanda buffertar av önskad kvalitet, men att det finns övre begränsningar för flödeshastigheten under vissa delar av uppblandningsprocessen, samt att den interna pH-mätaren maskinen har ett statiskt fel i sitt utfall och är inkonsekvent när den jämförts mot en extern pH-mätare. Vidare utveckling krävs för att fullt kunna integrera systemet med resten av kromatografiprocessen och möjliggöra en automatisk påfyllnad av buffertar utan handpåläggning av operatören.

Acknowledgments

I would like to begin by thanking my supervisor Niklas Anderson for being so kind and generous with his time during this project. I would also like to thank the PhD-students, Maja Sondell and Julius Klemens Lorek their kindness and all the help in the lab. Thank you to Bernt Nilsson for the encouragement during this project and all the amazing courses you have taught during my time at LTH. Finally, I would like to thank my family, my partner, her family who has become friends, and all the friends who have become like family for all the support during my years in Lund.

Abbreviations

Abbreviation	Full Name
DSP	Down-Stream Process
BPU	Buffer Preparation Unit
BMS	Buffer Management System
CAC	Continuous Annular Chromatography
FIFO	First-in-First-out
GUI	Graphical User Interface
IC	In-Line Conditioning
IEX	Ion exchange
ILD	In-Line Dilution
HPLC	High-Performance Liquid Chromatography
OPC	Open Platforms Communications
OPC UA	Open Platforms Communications Unified Architecture
REST API	Representational State Transfer Application Programming Interface
QA	Quality Assurance

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1 Introduction

Biopharmaceuticals have revolutionized the pharmaceutical market since their introduction in 1982 as they offer both higher precision and targeting as well as fewer side effects than conventional small-molecule drugs [1]. They include recombinant therapeutic proteins, monoclonal antibodies and vaccines, and are produced in biotechnical processes using methods in molecular biology. The biopharmaceutical market is ever expanding, with an increase in global sales from US$188 billion in 2017 to US$343 billion in 2021, corresponding to an 16% increase per year on average [2, 3], and as of 2023 biopharmaceuticals comprised 27% global pharmaceutical market, with an annual increase of 8%, twice the growth rate of conventional drugs [4].

The production of biopharmaceuticals is generally divided into two steps, upstream processing which aims to produce as high a yield as possible of the desired bioproduct through e.g. fermentation in a bioreactor, and downstream processing (DSP) which aims to isolate the desired bioproduct from the rest of the product stream to achieve a high purity [5]. As DSP accounts for up to 80% of the total production costs of biopharmaceuticals, improving these processes is of particular interest. Recent advances in converting the DSP from batchwise to fully continuous has shown promise in increasing productivity and product quality in combination with decreasing the capital investment cost, facility size and buffer consumption, leading to lowered production costs and environmental impact [6, 7, 8].

The focus of this thesis is on the development of an automatic buffer preparation unit (BPU) for liquid ion exchange (IEX) chromatography in a continuous DSP for biopharmaceuticals.

1.1 Liquid Chromatography

Liquid chromatography is a method of separation which is based on the interactions between the components of a mixture in a liquid phase (mobile phase) and the stationary phase which generally consists of a packed column [9]. Figure 1.1 illustrates the process flow of liquid chromatography and the interactions between components at different magnifications.

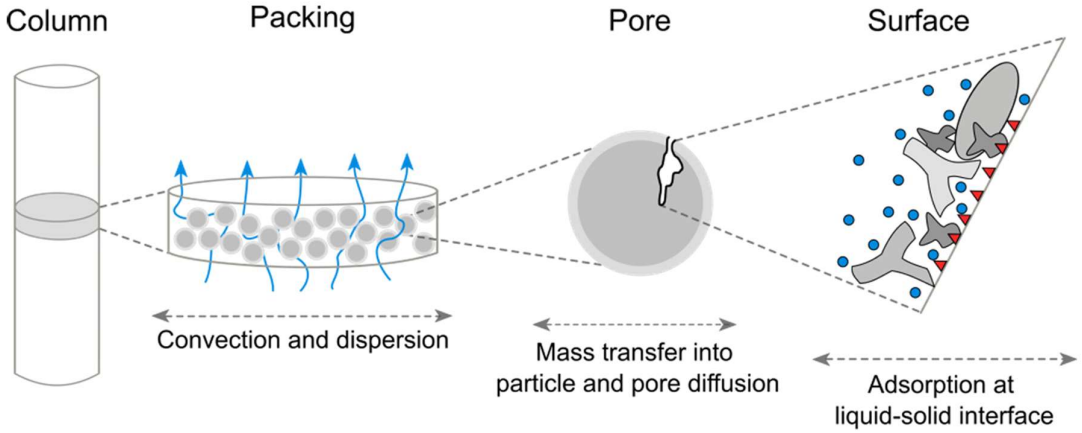

Figure 1.1. Illustration of the interactions of components at different magnifications and the process flow in a chromatography column. Adapted from [7].

Liquid IEX chromatography is a special form of adsorption chromatography, where the separation is based on reversible electrostatic interactions between charged molecules in the mobile phase and the oppositely charged stationary phase [9]. The column is first loaded with the sample, wherein solutes are retained in the solid phase. Subsequently, the column is washed using a weak buffer to ensure that only the solutes relevant to the separation remain retained in the solid phase [10], [11]. The product is then separated from the stationary phase through elution, where a buffer, typically a salt solution, is fed to the chromatography column in a linear gradient. This decreases the product's affinity to the stationary phase and allows it to exit the column. The final step of the process is the equilibration and regeneration step, where strong and weak buffer are in turn passed through the column to ensure it returns to its initial state [7].

1.2 Continuous Chromatography

In the production of biopharmaceuticals, continuous operation refers to the possibility to connect several unit operations in series, to ensure the continuous production and processing of therapeutic molecules over an extended period without any isolated steps occurring in between the unit operations [12], [13].

A key element in achieving a continuous process for biopharmaceutical production is to move away from batch wise DSP through continuous chromatography [13]. There is only one method for truly continuous chromatography in the form of continuous annular chromatography (CAC) [14]. This method includes a circular column which slowly rotates as feed is continuously added. Despite it being the only continuous method, it is not as commonly implemented as its pseudo-continuous counterparts which utilizes multiple columns or flow-through operations and offer increased productivity and product quality in combination with higher flexibility, at the cost of increased complexity [7], [13].

1.3 Buffer Management

The buffers used in chromatography are produced by mixing different salts with high quality water to achieve the right composition, pH, and conductivity for their intended use [15]. These properties must be measured and kept within a narrow interval to achieve a successful separation. This can be a resource demanding task, with respect to both raw materials and energy demand [8], as well as labor [7]. Hence, Isaksson [7] highlights the importance of a well-functioning buffer management system (BMS) and points out its role as a crucial part of a successful DSP of biopharmaceuticals, especially in up-scaling continuous processes to meet the demands of the growing market. Automatic buffer preparation and delivery systems serve to increase the robustness of the process, eliminate bottlenecks associated with manual buffer preparation and lower the need for manual supervision of the buffers, freeing up time for the operators to manage other tasks. Furthermore, Isaksson points out that a BMS could serve to reduce the facility footprint and reduce the consumption of high-quality water and chemicals, allowing for a more sustainable process with lower costs of operations.

At the department of process- and life science engineering at Lund University, a research team led by Nilsson B. and Andersson N. has developed a lab-scale integrated DSP for biopharmaceutical purification [7], [11], [16]. The process consists of and ÄKTA pcc 75 chromatography unit, an ÄKTA Pure 25 for polishing, an ÄKTA Pure 150 for sample collection and an Agilent HPLC system for sample analysis [7]. For this process, Isaksson [17] developed a BPU using an ÄKTA Explorer machine, to enable an automatic and steady supply of buffers.

1.4 The Orbit Controller

A custom software for controlling the lab scale DSP called Orbit has been developed in house at the department of Process- and Life science Engineering at Lund University [18]. Its primary goal is to overcome the inherent limitations of the ÄKTA Unicorn software which is the traditional way of controlling the ÄKTA Chromatography units. As Unicorn is designed to only run a single column in an open loop setup, it lacks the required versatility to be able to control a continuous DSP. Orbit is implemented in Python and uses Open Platform Communications (OPC), Open Platform Communications Unified Architecture (OPC UA) or Representational State Transfer Application Programming Interface (REST API) to connect to the Unicorn server. It is based on the existing methods in Unicorn and is used to send instructions to Unicorn which in turn controls the system, allowing for more advanced process control, process automation and data acquisition [7], [11].

Orbit uses a modular and hierarchical control structure, designed to break down complex bioprocesses in sequential and parallel operations [18]. The basic structure is based on *Processes*, consisting of *Phases*, which in turn consist of a series of *Instructions* and *Events*. A *Process* is created by listing which *Phases* it consists of in the order they are to be executed. In turn, the *Phase* can be divided into a pre-phase and a main phase. In the pre-phase, the initial *Instructions* for the *Phase*, such as valve positions and initial flow rates are defined. This is to ensure that the main phase is started at the correct time. In the main phase the different instructions of the phase are carried out in order, and the phase is ended once the instructions are carried out and the time of the phase is reached. To allow for more advanced control of the system, *Events* may also be added to a *Phase*. *Events* are defined as methods in the *Process* class and are called cyclically during the duration of the *Phase*. They can be triggered by either a time signal or a signal from a sensor. *Events* may be used to e.g. control the level in a tank through a PID controller. Orbit also uses *Loops, Events* which, along with sampling, are run parallel to the *Phases*, as they allow for execution of code independently of the active *Phase*. *Loops* can be used for e.g. safety monitoring or continuous control of the system or to iteratively print process information. Figure 1.2 displays the architecture of an Orbit *Process*.

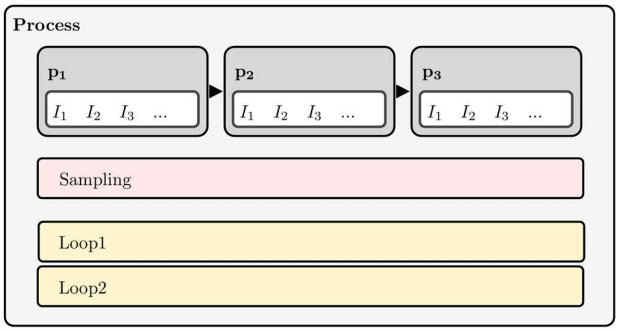

Figure 1.2. Illustration of the Process architecture. A process consists of a sequence of phases (p_1, p_2, p_3) which in turn consist of a series of instructions (I_1, I_2, I_3). Sampling and Loops are run in parallel with the phases. Adapted from [18].

1.4.1 Digital Twin

A digital twin is a virtual, computerized representation of a physical system. [19]. They are used to simulate the physical system digitally and enable for real time synchronization of sensor data, e.g. UV-signals or pH-values, as well as advanced process control. In orbit, the physical units of the lab scale DSP, such as valves, pumps, sensors or tubes, are represented as

objects in the code, allowing for a detailed digital representation of the physical setup [17], [20]. Each object has assigned attributes and methods, which correspond to their physical counterparts, such as maximum and available volume for the *Flask* objects [17]. Using the digital twin present in Orbit, it is also possible to monitor the physical system through information about valve positions and flow rates and through that information calculate states which are not measured, such as the real time available volume in a buffer flask.

1.4.2 Buffer Management System

Using the digital twin of the DSP present in Orbit, Isaksson et.al. [17] produced a Buffer Management System (BMS) to enable automatic monitoring, preparation, delivery and quality control of the buffers used in the process using an ÄKTA Explorer machine as the buffer preparation unit (BPU).

The BMS has three main components: *Client, Engine* and *Runner* [17]. The *Clients* consist of buffer-consuming chromatography units. They use the digital twin to monitor the volumes of their buffer flasks in real time and are tasked with sending an order to the *Engine* once the volume of a buffer has passed a critical threshold level. In turn, the *Engine* acts as a central administrator, receiving orders from the connected *Clients* and placing them in a first-in-first-out (FIFO) queue. Once the *Runner*, the part which handles the physical execution of the orders using the BPU, is free for a new order, it receives a command from the *Engine* to prepare and deliver the requested buffer to the correct *Client*. Once delivery is done, the *Engine* sends a signal to the *Client* to update its volume status in the digital twin. The BMS also has a manual mode, allowing an operator to place specific buffer refill orders for the *Engine,* using one of the pre-defined recipes for buffers present in Orbit. Figure 1.3 shows a graphical representation of the BMS, illustrating the flow of information as well as the hardware used.

In the 2023 paper by Isaksson et.al. [17], the performance of the BMS during a 10-day period was tested. During the test a total of 55 orders were placed, resulting in a total volume of 38 l, by the two clients. It was found that the average preparation and delivery time for one liter of buffer was 76.1 min, which corresponds to a daily buffer preparation capacity of 18.9 l, where as a BPU with higher possible flow rates, such as the ÄKTA Pure, potentially could prepare up to 150 ml buffer/min, corresponding to a daily capacity of 216 l [17], [21]. During the test run, orders were placed manually on day 1 and 5 to fill all the client buffer flasks, resulting in a maximum total queue length of 8 orders, and a total wait time of almost 7 h, where 365 min was the queue time and 55 min was the preparation and delivery time [17]. During the test run, no buffer was completely depleted.

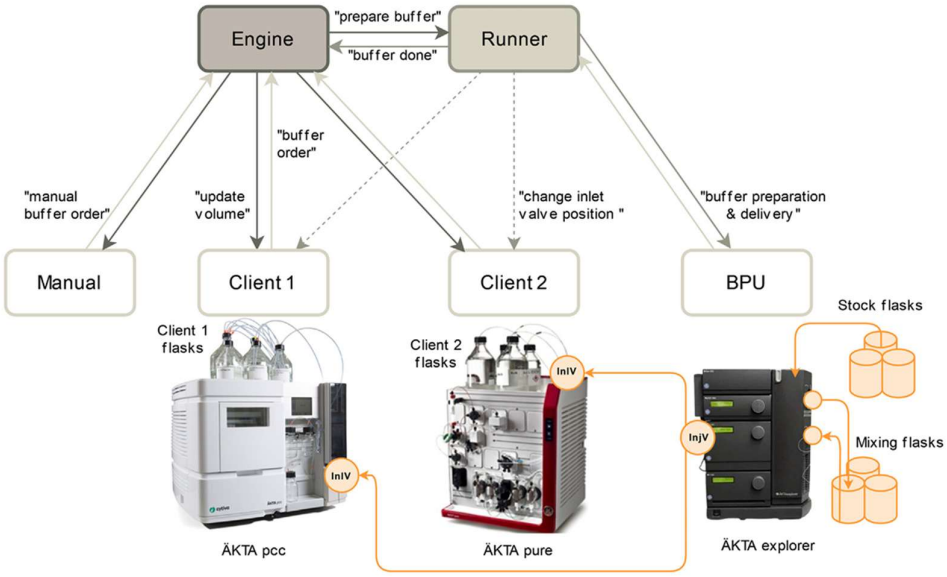

Figure 1.3. The physical representation of the BMS with two Clients connected, showing the flow of information between the Clients, Engine and Runner, as well as the hardware used. Adapted from [17].

Even if the BMS operating on an ÄKTA Explorer did not experience the issue of buffer depletion, the ÄKTA Explorer machine is old hardware, no longer sold by Cytiva [22]. This may potentially pose future problems associated with increased downtime, increased costs of system maintenance, system integration and skill gaps [23], [24].

There is also still room for improvement of the system to decrease the risk of a client not receiving an order in time. In developing a new BPU which allows for higher flow rates, such as the ÄKTA Pure, the time for both preparation and delivery could be reduced. This would result in shorter queue times and in turn reduce the risk of total buffer depletion.

Furthermore, the system could be further upgraded by improving the user experience for the operator by adding a Graphical User Interface (GUI) for control of the BPU. This would allow for a more intuitive manual order placing.

1.5 Aims and Outline

The aim of this thesis is to develop and evaluate a BPU on an ÄKTA Pure 150. This by a combination of physical configuration of the ÄKTA Pure machine, creating the necessary flow paths needed for buffer preparation and the digital implementation of the system in Orbit, creating a digital twin of the new system. The system will be evaluated to identify hardware limitations in both maximum possible flow rates and sensor accuracy. Additionally, a GUI will be developed for the system. The GUI will aim to increase the usability of the BPU and allow for real time control of the system.

The thesis will detail the materials, hardware modifications, and software development methodologies used to construct the BPU and the GUI in Chapter 2. Chapter 3 presents the evaluation of the system, including flow path limitations, software performance, sensor calibration and quality of the prepared buffers. Chapter 4 outlines the proposed future work, and Chapter 5 summarizes the conclusions of the study.

2 Methods

The development and implementation of the BPU on the ÄKTA Pure consisted of three main parts. Firstly, the system configuration, calibration and evaluation of the ÄKTA Pure to make it able to perform the buffer preparation and identify potential system bottlenecks. Secondly, to build a digital twin of the new BPU in orbit and to implement and modify the existing system for automation and control to make it compatible with the ÄKTA Pure. Thirdly, to create a GUI for increased usability and a more intuitive control of the system for the operators.

2.1 System configuration

An ÄKTA Pure 150 was used as the BPU. It was modified by installing additional valves and modifying the standard flow path of the system. Figure 2.1 displays an overview of the BPU. The buffer stock flasks were connected to the inlet valves of pump A and B. Water was connected to the inlet valve of pump A (A1) for dosing water to the buffers, and to a versatile valve for use in the system wash. Using the column valve and the loop valve, two tubes were connected to each buffer flask. One used as an inlet to the flask and one as an outlet. The injection valve was used to switch between using the loop valve and the column valve, depending on which position the buffer being prepared had.

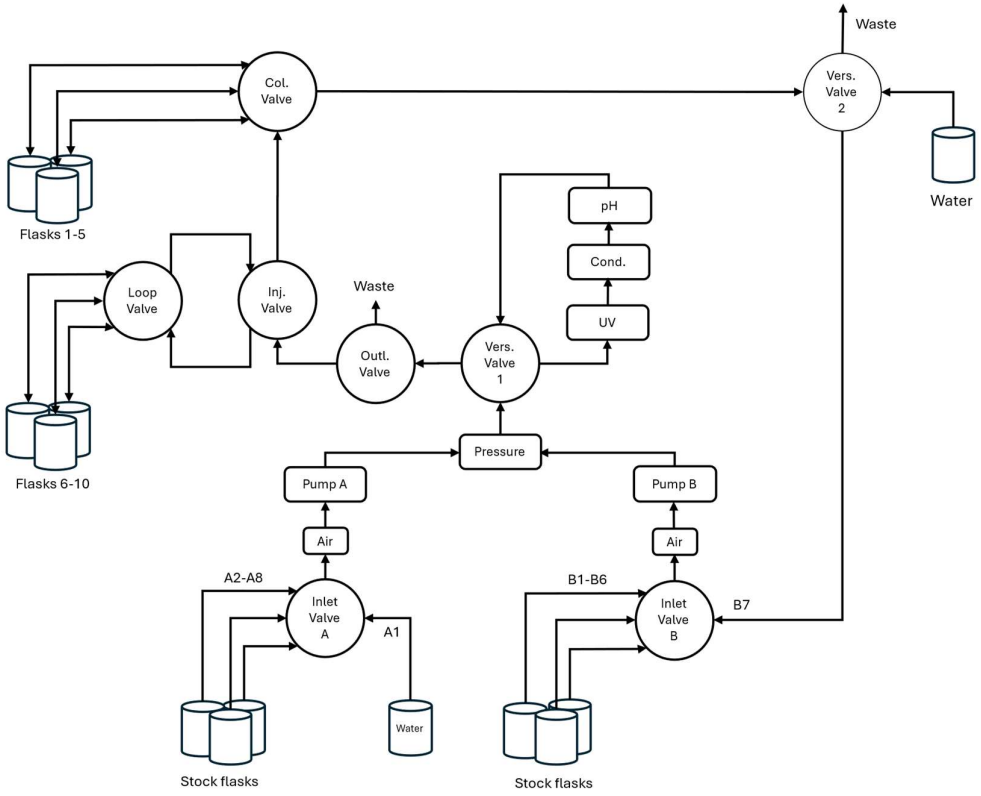

Figure 2.1: An overview of the BPU, showcasing the detailed layout of the system. Vers. Valve are the versatile valves, Outl. Valve is an outlet valve, Inj. Valve is the injection valve and Col. Valve is the column valve. UV, Cond. And pH are the UV, conductivity and pH-sensors.

2.2 Buffer Preparation

The buffers were prepared using In-Line Conditioning (IC), in which the system utilizes highly concentrated, single-component stock solutions that are dosed and then mixed with water [15]. Unlike In-Line Dilution (ILD) methods, which rely on multicomponent buffer concentrates whose maximum concentrations are strictly limited by the common ion effect, IC isolates the components. This allows each individual stock solution to be prepared at much higher concentration factors, limited only by the absolute molar solubility of that specific chemical in water. Typically, the stocks are concentrated to around 10 times the concentration of the final buffer.

There is, however, an upper limit to these stock concentrations dictated by the hardware. When developing a lab-scale BPU, similar to the one being developed in this work, Isaksson et al. [17] found that too high stock solution concentrations require very small volumetric doses to reach the target buffer formulation. This, in turn, negatively impacts the achievable dosing accuracy of the system's pumps. To ensure reliable and precise buffer preparation, they established a volumetric threshold, choosing stock concentrations that ensured the required dosing volume never dropped below 1 mL when preparing a 200 mL batch of finished buffer. This limitation in the stock solution concentrations was also used in this case.

The required volumes were calculated using pre-defined recipes and the known concentrations of the stock solutions. All recipes used in the system were provided by the PhD-students in the research group and were based on what they needed for their research. A total of 10 buffers, using water and 12 different stock solutions, were used in the system and are displayed in Table 2.1. The desired concentration, pH and conductivity of the buffer solutions were also provided. As the buffer pH is affected by the temperature of the solution, all flasks used in the buffer preparation, as well as the finished buffers, were stored in room temperature [15].

Buffers 1-5 as well as 10 had to be adjusted for pH using HCl. This was done by preparing 200 ml of each buffer, controlling the pH and then slowly adjusting the pH to the desired value and then calculating the required concentration or HCl. The pH adjustment was especially important in buffers 4 and 5, as the pH must be roughly 9 when the $MgCl_2$ stock is added to prevent precipitation of the salt. Furthermore, the concentrations of the phosphates in buffer 6, as well as the concentration of HAc in buffer 7 had to be changed slightly to achieve the correct pH value of the buffers. In adjusting the HAc concentrations of the buffer, they were prepared without any HAc, and then HAc was added slowly until the right pH was reached.

To prevent volumetric overshooting during preparation, a flow reduction *loop* was implemented in Orbit. During the dosing of stocks and water, this loop automatically reduced the flow rate to 10 ml/min when 10 ml remained and further reduced it to 2 ml/min when 2 ml remained.

Table 2.1. List of all the buffers and solutions prepared by the BPU. Specified with target concentrations of all components in mM and the target pH.

Buffer no.	Buffer/ solution	Buffer composition	Target pH
1	Capture Buffer A	25 mM Tris 100mM NaCl	7.8
2	Capture Wash Buffer	25mM Tris 1000 mM NaCl	7.8
3	Capture Elution Buffer	100 mM Glycine 100 mM NaCl	< 3
4	Polishing Buffer A	20 mM BTP 2 mM $MgCl_2$	9.0
5	Elution Buffer B	20 mM BTP 250 mM NaAc 2 mM $MgCl_2$	9.0
6	Equilibration Wash 1 (Prism A)	16.2 mM Na_2HPO_4 3.8 mM NaH_2PO_4 150 mM NaCl	7.4
7	Wash 2 (Prism A)	47.4 mM NaAc 2.6 mM HAc	6.0
8	Capture Elution and Regeneration	2.0 mM NaAc 48 mM HAc	3.5
9	CIP (Prism A)	500 mM NaOH	-
10	Agua Recovery Buffer	10 mM Glycine	2.5

The buffer preparation process followed the general scheme shown in Figure 2.2. First each stock was dosed based on the calculated volume from the recipes using the flow path shown in Figure B1 in Appendix B. Then, water was added to the flask to fill the remaining volume (Figure B2). After the dosing of stock solutions and water the buffers were mixed in the flask using a magnetic stirrer and recycled through the system using pump B, passing the sensor pack. Once the buffers had been properly mixed the system was washed through using water to cleanse it from any remaining buffer before a new order could be placed. The flow paths for recycling and system washing are shown in Figure B3 and B4 respectively.

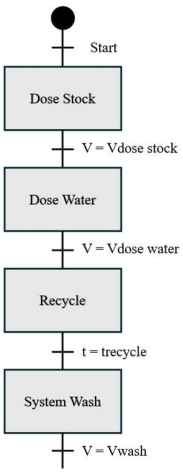

Figure 2.2. General scheme of the buffer preparation process. First Stocks are dosed until the volume of each stock has been reached respectively, then water is added to fill the remaining volume in the flask. After addition of stocks and water, the liquid is recycled for a predetermined period of time before the system is flushed with a set volume of water.

2.3 System calibration

To ensure reliable results from the sensors, as well as correct flow rates generated by the pumps, the system was calibrated. Calibration was performed on the pH-sensor and the pumps, and the internal system volume was calculated.

2.3.1 Calibration of the pH-sensor

Calibration of the pH sensor was done in accordance with the user manual for the ÄKTA Pure system [25]. Standard pH buffers with the pH of 7 and 4 were used. The internal pH sensor was compared to an external reference sensor.

2.3.2 Calibration of the pumps

To be able to ensure that the calculated volumes of buffers were accurately dosed, the pumps had to be calibrated. This was done using a correction factor which was calculated according to Equation 1.

$$Correction\ factor = \frac{Theoretical\ water\ weight}{Actual\ water\ weight} \quad (1)$$

Using one pump at the time, 650 ml of water for was transferred using a flowrate of 100 mL/min. This was performed three times for pump A and B respectively and the mean value of the actual weight of delivered water was calculated for each pump. The density of the water was calculated based on the temperature of the water. The scale used had an accuracy down to 0.1 g and a measurement error of ± 0.1 g.

2.3.3 Internal system volume

To ensure that the correct volume of buffers was added, the internal volume of the system was calculated. This was done using the inner diameter and length of certain tubes as well as the internal volumes of the valves and sensors in the relevant flow path. Upon preparing the buff-

ers, this volume is a part of the recirculation loop and must thus be considered when calculating the volume of stock solution required to achieve the proper buffer concentration.

2.4 Buffer quality control

The quality of the mixed buffers mixed by the BPU was controlled and compared to buffers mixed by the PhD students. The buffers were controlled based on pH and conductivity using both the inline sensor pack and external sensor equipment. For each buffer, 200 ml were prepared three times, and the average pH and conductivity was noted along with their relative standard deviation.

2.5 Graphical User Interface

To increase the usability of the BPU and provide easier real time control, a GUI was developed using the PyQT5 framework. In developing the GUI, googles' AI, Gemini, was used as an aid to generate segments of code based on prompts describing the desired features of the GUI.

The software architecture utilized multithreading, where the main thread was dedicated to user interactions and logging, while a QThread background worker was used to handle the continuous hardware communication via the OPC UA protocol in Orbit. A state management system was implemented using JSON-files to be able to track the real time volumes in the physical flasks. Furthermore, a hardware teardown logic was implemented to allow the operator to safely abort operations mid run, stopping all pumps and releasing all network sockets without corrupting the Unicorn server connection.

Finally, an automated Quality Assurance (QA) data parser, a script that converts the raw sensor data into a machine-readable format, was added. An offset was introduced to the sensor data to account for hardware drift. The average values of the measured buffer properties were calculated and presented after each run in the GUI.

3 Results & Discussion

Applying the methods outlined in section 2 above, a BPU was developed on an ÄKTA Pure machine, and a GUI was developed to increase the user experience. Here, the results from the implementation of the physical system, the development of the digital twin and the development of the GUI will be presented and discussed.

3.1 System configuration

The system was successfully configured and was able to perform the buffer preparation as expected. There was, however, one key bottleneck discovered in the system which might be a problem if the BPU is connected to a client system and integrated with the BMS in the future. Upon exploring suitable flowrates for the system, it was discovered that cavitation occurred during the recycling phase if the flowrate was too high. To counteract this, flow path upstream of the pump was configured to be as short as possible with a minimal number of valves and as high a diameter as possible for the tubes, without compromising the functionality of the system. This enabled a flowrate up to 25 ml/min for buffer flasks 1-5 (connected to the column valve). The limit for flasks 6-10 was on the other hand lower as they were connected to the loop valve, which added both more valves and tubing to the flow path, increasing the pressure drop and lowering the maximum possible flow rate to 18 ml/min. To compensate for the lower allowable flow rate for flasks 6-10, the duration of the recycling phase was increased to allow for complete mixing of all the components.

While a theoretical hydrodynamic analysis could potentially provide insight into the exact pressure drops leading to the observed cavitation, standard steady-state balances such as Bernoulli's equation, which assumes steady state operation [26], were deemed inadequate for this specific system. The ÄKTA Pure utilizes dual-piston pumps [25], which generate a highly dynamic flow. Consequently, the instantaneous peak flow rates, and their corresponding pressure drops, are significantly higher than the set average flow rate, invalidating steady-state assumptions. Furthermore, the exact internal geometries of the valves are unknown, and the flexible nature of the tubing results in energy dissipation through movement during pump pulses, making calculations of minor pressure losses highly approximate at best. Instead, the empirical cavitation thresholds of 25 ml/min and 18 ml/min serve as practical design constraints in for the BPU.

This is not a major problem in the context of the BPU, as the buffers were able to mix properly during the recycling phase. It does, however, pose a problem if the same flow path upstream of the pump were to be used to deliver buffer to a client, as the upper limit of the flow rate leads to long delivery times. To fix this with the current setup, tubing with larger diameters would need to be installed between the column valve and the injection valve, as well as between the injection valve and the loop valve. This would, however, probably only increase the maximum flow rate slightly. Another option is to add a third tube to each buffer flask and use an additional pump for stock delivery. This would on the other hand require the addition of an additional water inlet for purging the tubes downstream of the buffer inlet valve.

3.2 Stock solution concentrations

In evaluating a reasonable concentration of the components in the stock solutions, two aspects were considered. Firstly, the rule of thumb mentioned above, that the stock solutions should

have around 10 times the concentration of the component in the final buffer. Secondly, the concentration which corresponds to 1 ml of stock solution being required to mix 200 ml of the buffer was calculated, this due to limitations in the physical pumps. Initially, the pump started at 100 ml/min for a fraction of a second before flow reduction *loop* lowered the flow to 2 ml/min when sending an order for 1 ml. To further safeguard from potential overshooting a further level of safety was added to the system. If the calculated required stock solution volume was 10 ml or lower, the initial flowrate was set to 10 ml/min and if it was 2 ml or lower, it was set to 2 ml/min, lowering the risk of overshooting due to an initial pulse at a high flowrate for low volume orders. In testing the systems, it was found that both pumps could accurately deliver exactly 1 ml of water over a series of runs. Based on these two considerations, each stock concentration was evaluated, and the chosen concentrations are displayed in Table 3.1.

Table 3.1. The range of target concentration in the final buffer for each component, the maximum concentration each component may have in the stock solution to meet the requirement that no more than 1 ml should be added to a 200 ml order of buffer, the chosen stock solution concentration and the minimum order volume for each stock solution when a order of 200 ml buffer is made.

Component	**Target range [mM]**	**Maximum concentration [mM]**	**Chosen concentration [mM]**	**Minimum order volume (200 ml order) [ml]**
Tris	25	5243	1000	5.24
NaCl	100-1000	20 972	4000	5.24
BTP	20	4194	200	21.0
$MgCl_2$	2	419	400	1.05
HCl	4-7.44	839	500	1.7
NaAc – Low	2	419	400	1.05
NaAc – High	47.4 - 250	9941	2000	4.97
Na_2HPO_4	16.2	3398	500	6.80
NaH_2PO_4	3.8	797	500	1.59
HAc	2.6-48	545	400	1.36
Glycine	10	2097	200	21.0
NaOH	500	104862	5000	20.1

The amounts of salt or highly concentrated stock solution added to each stock solution flask were calculated using the molecular weight of the salt or the concentration of the solution. Calculated amounts of each component are presented in Table A1 in Appendix A.

3.3 System calibration

The system was calibrated according to the methods described above. Both the pumps and the pH sensor could be calibrated.

3.3.1 pH-sensor calibration

The pH-sensor was successfully calibrated. According to the manual, the calibrated electrode slope should be at least 80% and the asymmetry potential at pH 7 should be within the interval ± 60 [25]. After calibration, the calibrated electrode slope was 100.884% and the asymmetry potential at pH 7 was -52.205 mV.

There was, however, a clear difference between the internal pH sensor and an external one in the lab, both in consistency and pH reading. Both sensors were calibrated before the runs were made and should therefore display the same values. Since the external pH sensor is used frequently in the lab and stored properly in a storage buffer, it was deemed the more accurate reading.

Once the difference between the pH-sensors were noticed, the measured pH for both the internal and the external pH sensors were noted for each run during the process of preparing buffers and fine tuning the recipes. Over a series of 30 runs, it was found that the external pH sensor on average gave a higher reading of the pH of 0.178 with a standard deviation of 0.067. The difference between the internal and external pH sensors ranged from pH 0.08 to pH 0.3. This means that the internal sensor both had a notable stationary error and a substantial variability in its readings, making it unfit for buffer quality evaluation.

The inconsistency of the internal pH sensor might stem from the fact that liquid is constantly flowing past the sensor, becoming increasingly more well mixed during the recycle phase when the measurements are taken, making the sensor unable to converge properly during the time span of the phase. However, it was found that the problem persisted even when the duration of the recycle phase was increased, and that the pH sensor had reached a steady reading during the original recycling phase duration, ruling out this conclusion. Another possible explanation is that the internal pH sensor was not properly stored in between runs. After a finished run the system is flushed with deionized water, making the pH electrode sit in contact with the water in between runs. This leads to decreasing salt concentrations in the pH electrode membrane due to osmosis, causing the drift in the pH signal [25]. To counteract this, the pH sensor should be stored in a storage solution containing a 1:1 mixture of standard pH 4 buffer and 1 M potassium nitrate solution. That solution was however considered too cumbersome in relation to using the external pH sensor for quality control, and hence, the internal pH reading is to be seen as an approximate reading of the buffer pH rather than the pH of the final product.

3.3.2 Pump calibration

Table 3.2 displays the result of the calibration runs for the pumps. The average weight delivered was 649.7 g, corresponding to a volume of 651.3 ml for pump A, and 650.1 g, corresponding to a volume of 651.7 ml for pump B.

Table 3.2. Pump calibration runs with pump A and B. An order of 650 ml was placed for each pump, and the delivered weight was noted. Using the delivered weight, the volume was calculated using the density of the water. During the run, the temperature of the water was 23 ^{o}C, which corresponds to a density of 0.99762 g/ml [27].

Run	Delivered weight, Pump A [g]	Calculated volume, pump A [ml]	Delivered weight, Pump B [g]	Calculated volume, pump B [ml]
1	649.8	651.4	650.2	651.8
2	649.7	651.2	650.1	651.7
3	649.7	651.2	650.1	651.7
Average	649.7	651.3	650.1	651.7

Using the average delivered volumes for each pump, the correction factor was calculated to 0.9980 and 0.9974 for pump A and B respectively. Table 3.3 shows the delivered weights and volumes after the correction factor was implemented.

Table 3.3. Validation run of the pumps with the correction factor implemented.

Run	Delivered weight, Pump A [g]	Calculated volume, pump A [ml]	Delivered weight, Pump B [g]	Calculated volume, pump B [ml]
1	648.4	649.9	648.4	649.9
2	648.6	650.1	648.4	649.9
3	648.5	650.0	648.4	649.9
Average	648.5	650.1	648.4	649.9

Given the density of the water, 648.45g of water corresponds to the ordered 650 ml. This would correspond to a reading of either 648.4 or 648.5 on the scales, but as it also had a measurement error of ± 0.1g, a delivered volume of 650 ml could render a reading of 648.3 to 648.6 g. Hence, all the measured weights lie within an acceptable margin for the accuracy of water delivery.

3.3.3 Internal system volume

The total volume of all the tubes, valves and sensors in the relevant flow paths for each buffer flask is displayed in Table 3.4 below.

Table 3.4. Internal system volume for all buffer flask positions.

Buffer no.	Internal system volume [ml]
1	13.55
2	13.55
3	13.55
4	13.55
5	13.55
6	13.79
7	14.79
8	15.80
9	15.80
10	15.80

The reason for the difference in the system volume is twofold. Firstly, buffers 6-10 were connected to the loop valve for delivery and recycle, which added 0.24 ml of system volume due to the internal volume of the loop valve and the tubes connecting the injection valve and the loop valve. Secondly, the difference in length of the inlet and outlet tubes to the buffer flasks, which had to be longer for flasks 7-10 as they were placed further from the BPU in the physical setup.

3.4 pH adjustment of buffers

Table 3.5 displays the volumes of HCl required to adjust the pH of buffers 1-5 and 10, as well as the corresponding concentrations of HCl in the final buffers.

The required volume of HAc to achieve the correct pH in buffer 7 was 0.89 ml for 200 ml buffer, which corresponds to a buffer concentration of 1.77 mM HAc. This lowered HAc concentration in buffer 7 lead to only 0.95 ml of the stock solution being required to mix 200 ml of the buffer, hence putting it below the design specification mentioned above.

Table 3.5. Added volume and corresponding concentration of HCl to achieve the correct pH in buffers 1-5 and 10

Buffer no.	Buffer name	Volume 0.5 M HCl added [ml]	Concentration of HCl in buffer [mM]
1	Capture Buffer A	7.15	16.2
2	Capture Wash Buffer	7.6	17.2
3	Capture Elution Buffer	15.2	33.3
4	Polishing Buffer A	3.9	9
5	Elution Buffer B	4.75	10.9
10	Agua Recovery Buffer	4.466	10.1

When testing the correct concentration of the phosphates in buffer 6, multiple runs were made with different concentrations of Na_2HPO_4 and NaH_2PO_4. The total concentration of phosphates was kept constant at 20 mM. The buffers were prepared using the BPU and the pH was tested using the external pH sensor. Results are displayed in Table 3.6.

Table 3.6. Resulting pH from different concentrations of the phosphates in buffer 6. The pH reading from the external pH sensor is displayed.

Concentration of Na_2HPO_4 [mM]	Concentration of NaH_2PO_4 [mM]	pH
17	3	7.27
17.2	2.8	7.34
17.3	2.7	7.35
17.4	2.6	7.44
17.35	2.65	7.42

The achieved concentration relationship of the phosphates in buffer 6 does not correspond to the theoretical value. According to the Henderson–Hasselbalch equation, the pH of an acid-base pair is:

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right) \quad (2)$$

For the $H_2PO_4^- - HPO_4^{2-}$ pair, pK_a is 7.21 at 25 ^{O}C [28]. This implies that the theoretical concentration quotient should be 1.55, corresponding to 12.2 mM of Na_2HPO_4 and 7.8 mM of

NaH_2PO_4. This most likely indicates an improper mixing of stock solutions, and that their true concentration differs from what is used for the basis of calculation. It has no direct impact on the system as is, but it does mean that the recipes might have to be recalibrated once the stocks run out and new stocks are mixed.

The corrected concentrations were added to the buffer recipes in orbit to ensure that the BPU prepares buffers with the desired properties. The final recipes for all the buffers are presented in Table A2 in Appendix A.

3.5 Quality control of prepared buffers

The resulting pH and conductivity for all buffers prepared by the BPU using the corrected recipes are displayed in Table 3.7.

Table 3.7. Target and resulting Conductivity in mS/cm and pH for all buffers prepared by the BPU using the corrected recipes.

Buffer no.	Buffer/ solution	Target	Result
1	Capture Buffer A	Cond: 12.22 pH: 7.8	Cond: 12.30 ± 0.65% pH: 7.81 ± 0.10%
2	Capture Wash Buffer	Cond: 96.99 pH: 7.8	Cond: 87.82 ± 0.22% pH: 7.81 ± 0.16%
3	Capture Elution Buffer	Cond: 14.7 pH: < 3	Cond: 14.53 ± 2.0% pH: 2.79 ± 1.4%
4	Polishing Buffer A	Cond: 0.7 pH: 9	Cond: 1.28 ± 3.01% pH: 8.98 ± 0.45%
5	Elution Buffer B	Cond: 16.7 pH: 9	Cond: 17.45 ± 0.26% pH: 9.05 ± 0.24%
6	Equilibration Wash 1 (Prism A)	Cond: 17.5 pH: 7.4	Cond: 18.06 ± 0.090% pH: 7.38 ± 0.13%
7	Wash 2 (Prism A)	Cond: 3.22 pH: 6.0	Cond: 3.83 ± 0.37% pH: 6.07 ± 0.64%
8	Capture Elution and Regeneration	Cond: 0.322 pH: 3.5	Cond: 0.40 ± 5.1% pH: 3.53 ± 0.71%
9	CIP (Prism A)	Cond: - pH: -	Cond: - pH: -
10	Agua Recovery Buffer	Cond: 2.4 pH: 2.5	Cond: 2.32 ± 3.4% pH: 2.52 ± 0.75%

Most buffers were able to be prepared with consistent quality, with a pH value close to the target value. The conductivities of the prepared buffer did on the other hand not match the target value with the same accuracy, but after discussion with the PhD-students it was concluded that the exact value of the conductivity is not as important as the exact value of the pH, and that the main goal regarding conductivity is to keep it in the right range. The only buffers which exhibited a relative standard deviation higher than 1% in pH or conductivity over the three test runs were buffers 3, 4, 8 and 10.

The larger relative standard deviation for the conductivity of buffers 4, 8 and 10 may simply be an effect of the low absolute value of the conductivity of these buffers, as the absolute standard deviation for the buffers were 0.039, 0.021 and 0.078 for buffer 4, 8 and 10 respectively, which are all in line with the general trend of the absolute value of the standard deviation for conductivity of the buffers when excluding Buffer 2, which had a much larger target conductivity and Buffer 3.

It was found that the Capture Elution Buffer (Buffer 3) quickly displayed some flocculation as well as some deposits on the bottom of the buffer flask after mixing. The same phenomenon was observed in the Agua Recovery buffer (Buffer 10), but at a lower extent, as well as in the Glycine stock flask, indicating that glycine is not suitable to be stored at room temperature for longer periods of time. It would probably be better to either store the Glycine stock in a refrigerator to extend its lifetime and connect it to the BPU when needed, to mix small volumes of glycine stock on demand, or to mix the stock without the addition of glycine using the BPU and then add glycine manually.

The degradation of Glycine may also be an explanation to the comparably large deviations in the results of the quality control on buffer 3, as degradation of Glycine probably affects the buffering effect of Glycine itself which consequently likely affects the conductivity of the solution. This also implies that the corrections made to the recipes may not be applicable when using a freshly prepared and correctly stored glycine stock solution.

The same phenomenon was, however, not observed in the results for Buffer 10, which also contained glycine but was more consistent in conductivity and pH during the three runs. A probable explanation is that the concentration of Glycine in Buffer 10 was much lower than in Buffer 3, 10 mM and 100mM respectively, making the effect of the degradation of the Glycine stock impact the quality of the buffer less.

Despite less than 1 ml of HAc being added to Buffer 7 when preparing the 200 ml used for the quality control of the buffers, it was consistent in both conductivity and pH, indicating that the system was able to accurately deliver small volumes of stock solution during the preparation. It would probably still be good to decrease the concentration of the HAc stock solution for future iterations of the system to lower the risk of compromising the quality of the buffer when mixing low volumes.

3.6 Graphical User Interface

To increase the usability of the BPU and allow for more intuitive control of the system, a GUI was developed and implemented. The interface was designed to facilitate the interaction between the operator and the underlying Orbit and OPC UA architecture, overcoming the inherent limitations of the standard Unicorn Software.

3.6.1 Operational modes and manual buffer preparation

The GUI was divided into an Order Configuration segment and a Live Process monitoring segment (Figure 3.1). Within the configuration segment, the system enables the execution of pre-defined recipes (Table A2), as well as a Manual Mode for dynamic buffer preparation.

In the Standard Recipe mode, the operator can choose from the existing pre-defined recipes using the drop-down menu and select the final volume of the prepared buffer. There is also an option to choose the system mode. If the process is run in test mode, a simulation of the buffer preparation is run using the digital twin in Orbit. If it is run in real mode, the system will connect to the ÄKTA Pure machine through OPC UA and perform the buffer preparation using the BPU.

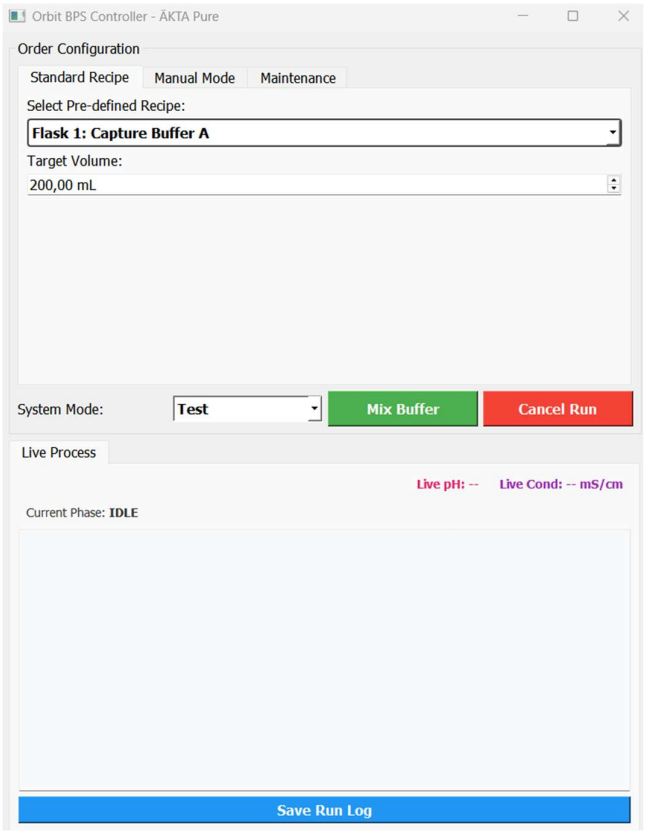

Figure 3.1. The starting window when opening the GUI. At the top there is an Order Configuration segment, wherein the user can select between using the standard recipes, preparing buffers using manual mode, or performing maintenance on the system. In the active Standard Recipe tab, there is a drop-down menu to select one of the pre-defined recipes and a bar in which the target volume of the buffer can specified. In the middle there is a drop-down to choose the system mode, a button to start the process and a button to cancel an ongoing run. At the bottom the live process segment is displayed, where information about the active phase, the live pH and conductivity is shown. The live process segment prints out process information during an active run and has a button that may be used to save the run log.

In the Manual mode, the operator specifies a target flask, selects the desired components and inputs the target concentrations of the components in mM (Figure 3.2). The backend logic then automatically calculates the required volumes of each stock solution to reach the desired concentration.

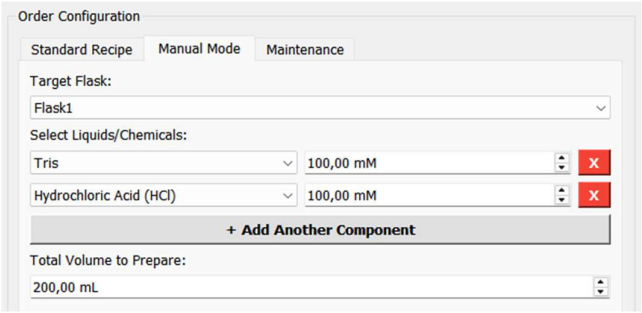

Figure 3.2. Contents of the Manual Mode tab in the Order Configuration. First a target flask is chosen, then the desired components of the buffer are chosen. Components can be added using the "+Add Another Component"-button and removed using the red buttons to the right. The desired total volume of the finished buffer is then added to the bottom.

A maintenance tab was also added to the Order Configuration segment to allow the operator to perform system washes, analysis of a selected buffer using the inline sensors, and to purge the tubes connected to the buffer flask (Figure 3.3). Flow paths for all operations are shown in Figure B4, B5 and B6 respectively.

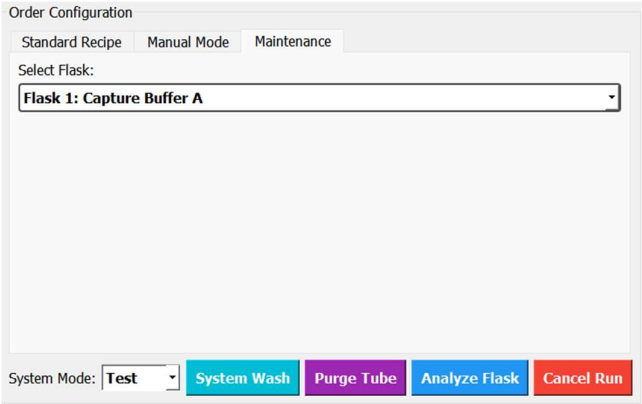

Figure 3.3. Contents of the Maintenance tab. At the top the target flask may be chosen. At the bottom, the desired system mode can be chosen, and buttons for system wash, purge tube, analyze flask, and cancel run are displayed.

During an analysis cycle, the selected buffer is passed through the sensor package and exits through waste port of the outlet valve, consequently wasting 20 ml of the buffer (flow path in Figure B5). The reason for this is that the system is filled with water after a completed run, which would otherwise be added to the buffer flask, diluting it and compromising the properties of the entire solution. The cycle is finished with a system wash phase to prepare the system for preparation of a new buffer.

3.6.2 Real time monitoring and quality assurance

By utilizing a background QThread to handle OPC UA communication, the main interface provides a continuous live feed of the active phase and real time sensor readings which are printed in the Live Process tab.

As noted in section 3.3.1, the internal pH sensor displayed a systematic error when compared to an external reference sensor. To counteract this, the average static offset of 0.178 was implemented within the GUI data parser. The interface intercepts the raw pH data from Orbit and applies the calibration offset before displaying the value to the operator. Hence, the pH value in the GUI differs from the one displayed in Unicorn.

At the end of a buffer preparation or analysis cycle, the GUI calculates a rolling average of the pH and conductivity based on the data during the last 5 seconds of the recycle phase and displays it as a quality assurance message at the bottom of the Live Process segment (Figure 3.4)

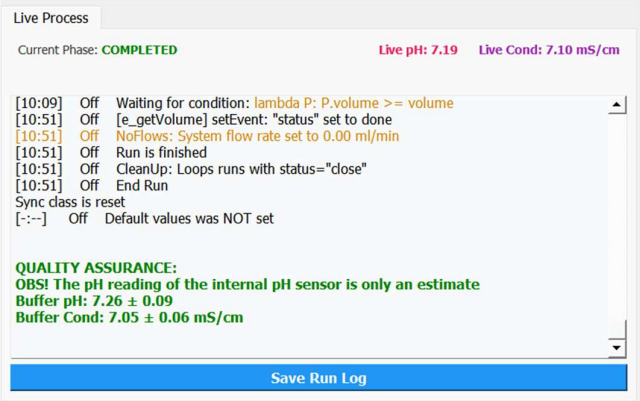

Figure 3.4. Displays the print of the end of a run made in test mode. The current phase, live pH and live conductivity are displayed at the top. Process information, as well as quality assurance information is printed. At the bottom the button for saving the Run Log is displayed.

To more accurately estimate the uncertainty of the system, the standard deviation of the process variations of the pH reading was calculated and combined with the precision error of the pH calibration presented in section 3.3.1, using the root sum square method:

$$\sqrt{\sigma_{process}^2 + \sigma_{calibration}^2} \tag{3}$$

3.6.3 System Safety

To allow for safe operation of the system, an emergency stop function was integrated into the interface. If a run needs to be aborted, a "Cancel Run" command can be initiated, which sends a safe-stop signal to Orbit. This halts all active phases, stops the pumps, and releases the OPC UA network sockets, thereby preventing server corruption and allowing the system to be safely reset.

4 Further Work

This project has successfully demonstrated that the configuration and operation of an automatic BPU using an ÄKTA Pure is possible. There are, however, several key steps remaining to integrate the BPU with fully autonomous DSP network which were deemed outside the scope of this work. Further work should focus on system integration with the BMS, hardware optimization, comparative benchmarking as well as further development of the system software and GUI.

4.1 Integration with the Buffer Management System

The primary objective for future development of this system should be to integrate it with the existing BMS architecture to allow for truly continuous operation, as the current system only works as a standalone BPU which can only handle manual orders. In connecting the physical system to the clients in the DSP and integrating the digital twin of the system within the BMS, the BPU can act as the *Runner*, automatically receiving buffer preparation orders from the *Engine* once the client buffer flasks has passed their critical level and deliver the prepared buffer to the client. Integration with the BMS would also pose for possibilities to further develop the GUI, integrating it with the BMS to display and alter the order queue.

4.2 System benchmarking and performance evaluation

Once the new BPU has been integrated with the rest of the DSP and the BMS, it can be benchmarked against the previous ÄKTA Explorer BPU to evaluate the real-world system capacity. As mentioned in section 1.4.2, the ÄKTA Explorer BPU had an average preparation and delivery time of 76.1 min/l, corresponding to a daily capacity of 18.9 l. While it is mentioned that this system theoretically has a maximum capacity of 216 l/day, testing of the integrated system must be performed to assess the actual capacity of the new BPU and evaluate the actual reduction in queue times and preparation duration. This evaluation will also be vital in investigating the scalability of the continuous lab scale DSP setup.

4.3 Flow path and hardware optimization

To fully utilize the higher possible flow rates in the ÄKTA Pure system, the hardware bottlenecks, identified and described in Section 3.1, must be assessed. As noted, there is an upper limit to the possible flowrates in the recycle phase flow path (Figure B3), as the system exhibits cavitation for flow rates higher than 25 ml/min for flasks 1-5 and 18 ml/min for flasks 6-10. Future work should therefore explore alterative system configurations to find a work around. By installing tubing with larger inner diameters between the column, injection and loop valves to ensure higher allowable flow rates and a more efficient buffer delivery to the client or introducing a third tube to each buffer flask paired with an additional dedicated delivery pump could bypass these restrictions entirely and significantly reduce buffer delivery times to the client systems, but this would have to alter the delivery protocol developed for the previous BMS.

4.4 Automated Sensor Maintenance

The systematic drift and inconsistency of the internal pH sensor, likely caused by prolonged exposure to deionized water between runs, remains a challenge. Further iterations of the system could explore an automated storage protocol, where the system is programmed to draw

and hold a dedicated storage solution within the sensor flow path during idle periods. This would prolong the lifespan of the electrode and increase the reliability of the internal pH readings, eliminating the need for quality control using an external pH sensor. If the accuracy of the internal pH sensor would be increased further functionality could also be added to the system, such as continuous logging of the buffer pH and conductivity and a warning system which notifies the operator if the prepared buffer deviates too much from the target pH or conductivity.

4.5 Improved system control

Another area for improvement of the system could be to add more levels of automated system control. This could, for example, be integrated by adding a *loop* in Orbit that monitors the live pH and conductivity readings during the recycle and system wash phases. This would allow the recycle phase to be active until the pH and conductivity reading have stabilized as this indicates that the buffer is completely mixed. This would add an extra level of certainty to the quality of the buffer and possibly allow for shorter recycling phases. Similarly, if the same principle is applied in the system wash phase, allowing it to be active until a stable reading on the conductivity sensor at around 0 is reached, indicating that the internal system tubing is free from any salts. In doing so, the risk of improper flushing of the system or too long system wash phases would be reduced.

5 Conclusions

This project successfully demonstrated the adaptation of an ÄKTA Pure system into a standalone BPU for continuous downstream processing of biopharmaceuticals and by utilizing the Orbit software architecture, the inherent limitations of the Unicorn control software was successfully circumvented. The developed system managed to prepare buffers with properties close to the targets set beforehand and for most buffers, the variations in pH and conductivity was small. To increase the consistency in the quality of the prepared buffers, the glycine stock should be stored properly, and the concentration of the acetic acid stock should be lowered to increase the required stock volume when preparing small volumes of buffer.

Hardware evaluations did, however, reveal that there are limitations to the maximum possible flow rates during the recycle phase of the process, as too high flow rates caused cavitation upstream of the system pump. Furthermore, it was found that the internal pH sensor had a static error and a relatively large standard deviation when compared to a reference sensor, rendering its readings unreliable.

The development of a custom GUI for the BPU improved system usability. The interface was able to both allow the user to order buffers based on pre-defined recipes and to create their own custom recipes in the manual mode. A correction of the pH reading was successfully added to account for the inaccuracy of the internal pH sensor in the automatic quality assessment of the prepared buffers by applying a static software calibration offset. Additionally, the GUI allowed for routine maintenance of the system, reducing the need to manually configure the system in the Unicorn software.

Despite the physical limitations of the system, it shows promise to be used in a real-life setting to automatically prepare buffers based on manual orders, reducing or even eliminating the need for manual buffer preparation in the lab. Furthermore, the system has a potential to be integrated with the existing BMS, allowing for truly automated buffer preparation in a lab scale DSP for biopharmaceuticals.

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Appendix A – Mixing instructions and recipes

Table A1. Mixing instructions for all stock solutions. Including target concentration for stock solution, target volume for stock solution, molecular weight of the components or the concentration of a more concentrated stock solution used for mixing, and the amount of the component needed to mix the stock solution.

Component	Stock concentration [M]	Target volume [l]	Molecular weight/ concentrated stock concentration	Amount
Tris	1	1	121.14 g/mol	121.14 g
NaCl	4	1	58.44 g/mol	233.76 g
BTP	0.2	1	282.34 g/mol	56.468 g
NaAc	0.4	1	136.08 g/mol	54.432 g
NaAc	2	1	136.08 g/mol	272.16 g
Na_2HPO_4	0.5	0.5	177.99 g/mol	44.4975 g
NaH_2PO_4	0.5	0.5	156.01 g/mol	39.0025 g
HAc	0.4	1	60.05 g/mol	24.02 g
NaOH	5	1	40 g/mol	200 g
Glycine	1	1	75.07	75.07 g
HCl	0.5	1	5 M	0.1 l

Table A2. Final recipes of all the buffers and solutions prepared by the BPU. Specified with target concentrations of all components in mM and the target pH.

Buffer no.	Buffer/ solution	Buffer composition	Target pH
1	Capture Buffer A	25 mM Tris 100mM NaCl 16.2 mM HCl	7.8
2	Capture Wash Buffer	25mM Tris 1000 mM NaCl 17.2 mM HCl	7.8
3	Capture Elution Buffer	100 mM Glycine 100 mM NaCl 33.3 mM HCl	< 3
4	Polishing Buffer A	20 mM BTP 8.97 mM HCl 2 mM $MgCl_2$	9.0
5	Elution Buffer B	20 mM BTP 250 mM NaAc 10.88 mM HCl 2 mM $MgCl_2$	9.0
6	Equilibration Wash 1 (Prism A)	17.35 mM Na_2HPO_4 2.65 mM NaH_2PO_4 150 mM NaCl	7.4
7	Wash 2 (Prism A)	47.4 mM NaAc 1.77 mM Hac	6.0
8	Capture Elution and Regeneration	2.0 mM NaAc 48 mM Hac	3.5
9	CIP (Prism A)	500 mM NaOH	-
10	Agua Recovery Buffer	10 mM Glycine 10.1 mM HCl	2.5

Appendix B – Flow paths

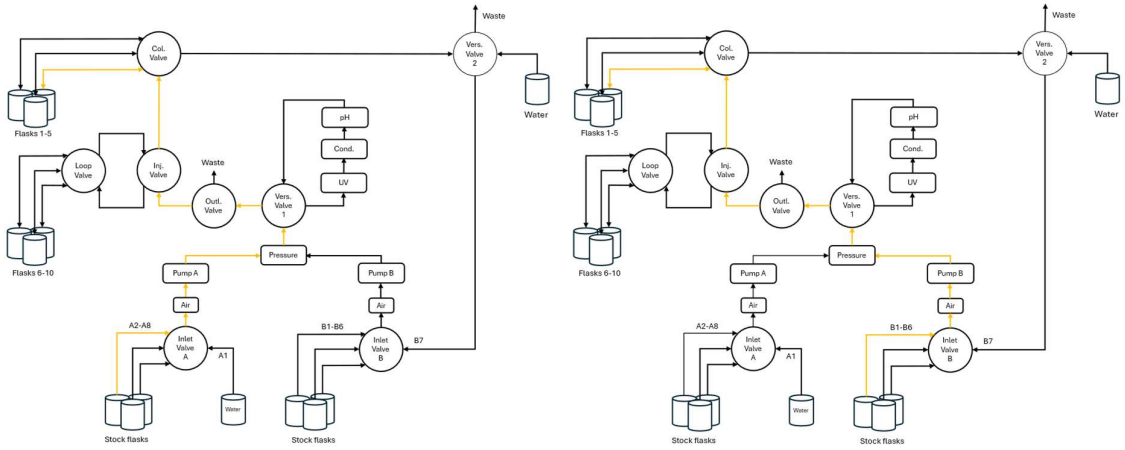

Figure B1. Dosage flow path for stocks using Pump A (left) and Pump B (right) marked in orange. The stock is taken from its flask using the pump, passing through the pump inlet valve, the air sensor, pump, pressure sensor, Vers. Valve 1 and the Outl. Valve. Depending on the buffer flask position, the stock is either passed through the Inj. Valve and added to the buffer flaks using the Col. Valve, or through the Loop Valve.

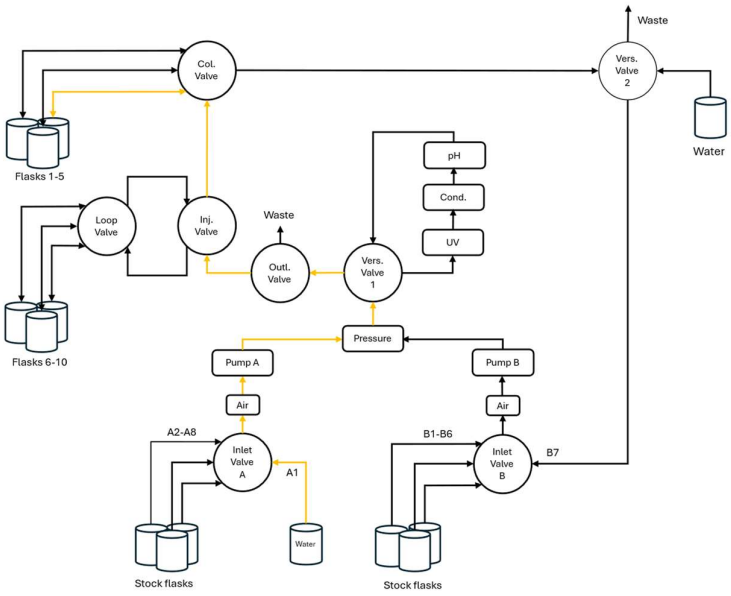

Figure B2. Dosage flow path for water using Pump A marked in orange. Water is taken from its flask using the pump, passing through the pump inlet valve, the air sensor, pump, pressure sensor, Vers. Valve 1 and the Outl. Valve. Depending on the buffer flask position, the water is either passed through the Inj. Valve and added to the buffer flaks using the Col. Valve, or through the Loop Valve.

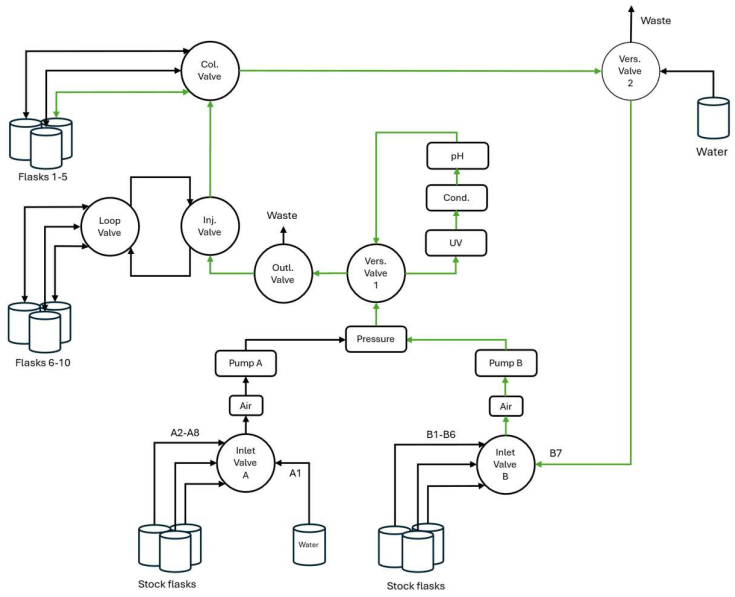

Figure B3. Flow path for recycling of buffer during mixing marked in green. The buffer is pumped from its flask, passing Vers. Valve 2, Inlet Valve B, Pump B, the pressure sensor, Vers. Valve 1, the sensor package, back to Vers. Valve 1 and then Outl.Valve on to Inj. Valve. If the buffer is positioned in flask 6 – 10, it is passed to the loop valve and pumped back into its flask, otherwise it is pumped back into the flask through the Col. Valve.

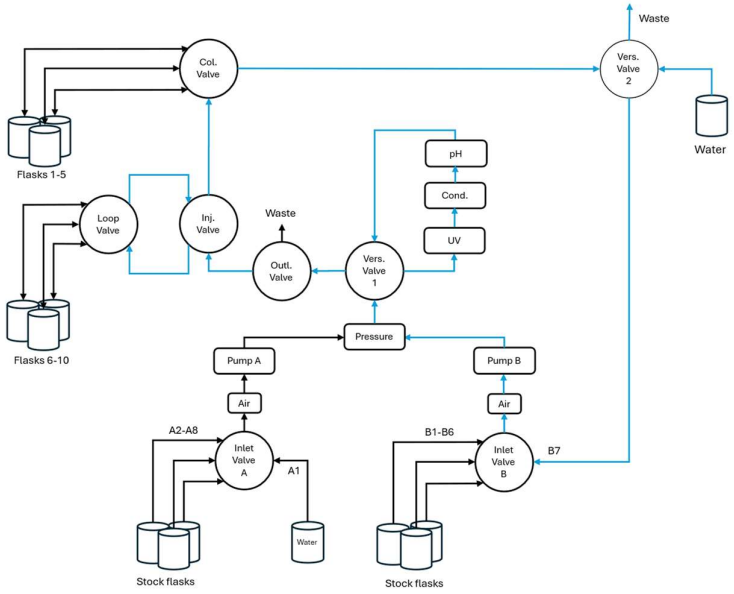

Figure B4. Flow path for system wash marked in blue. Water is taken from a flask connected to Vers. Valve 2 and then pumped through the same flow path as when recycling. The flow exits the system through Vers. Valve 2.

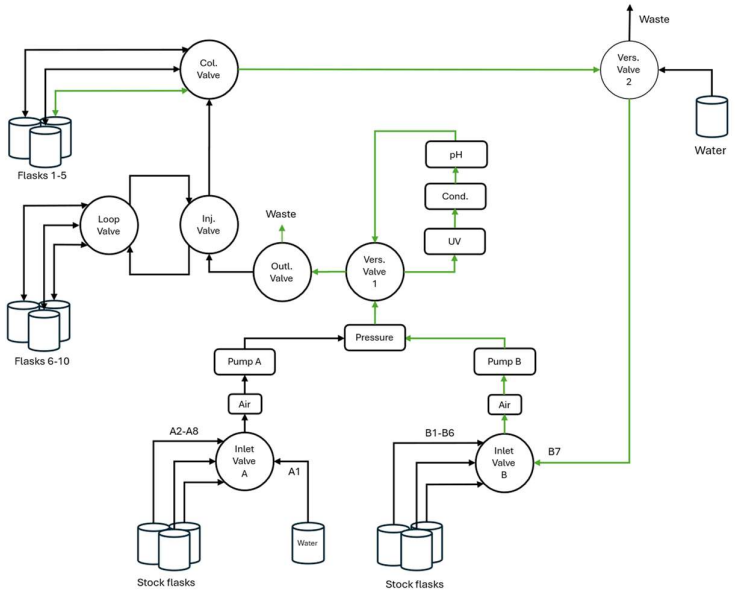

Figure B5. Flow path for buffer analysis using the inline sensor package marked in green. The buffer is pumped from its flask, passing Vers. Valve 2, Inlet Valve B, Pump B, the pressure sensor, Vers. Valve 1, the sensor package, back to Vers. Valve 1 and then Outl.Valve where it exits the system goes to waste. If the buffer is positioned in flask 6 – 10, it is passed to the loop valve and pumped back into its flask, otherwise it is pumped back into the flask through the Col. Valve.

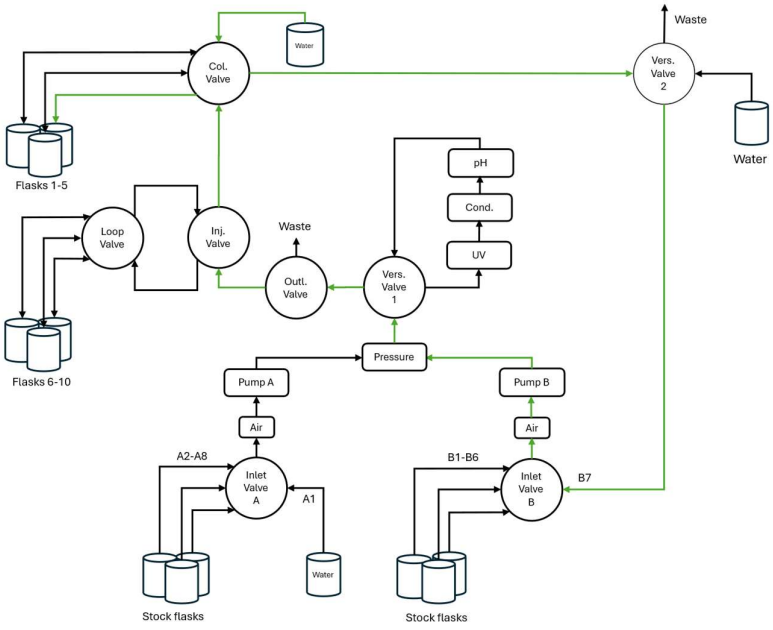

Figure B6. Flow path for purging the tubes of buffer marked in green. The buffer outlet tube is placed in an external water flask. The flow follows the same path as for the recycle phase shown in Figure B3, but skips the sensor packs.

Appendix C – User manual GUI

User manual – Graphical User interface for Buffer Preparation Unit

Axel Sundström

2026-04-13

Table of Contents

1 Introduction

To control the Buffer Preparation Unit (BPU), a graphical user interface (GUI) has been developed to allow the user to control the machine. It offers the ability to mix buffers from a list of predefined recipes, manually mix buffers based on desired concentrations in the final buffer, and to do some maintenance such as washing the system, purging the tubes connected to the buffer flasks, and use the inline sensors for analysis of a prepared buffer.

2 GUI Layout

The GUI is divided into two main segments (Figure 2.1). The top segment (Order Configuration) for controlling the system is divided into three tabs: Standard Recipe, Manual Mode and Maintenance. Here, the specifications for the desired buffer or operation can be selected and run. The bottom segment contains information about the live process. It displays the current phase, the live pH and conductivity during a run, and the process information that is normally printed by Orbit within the terminal.

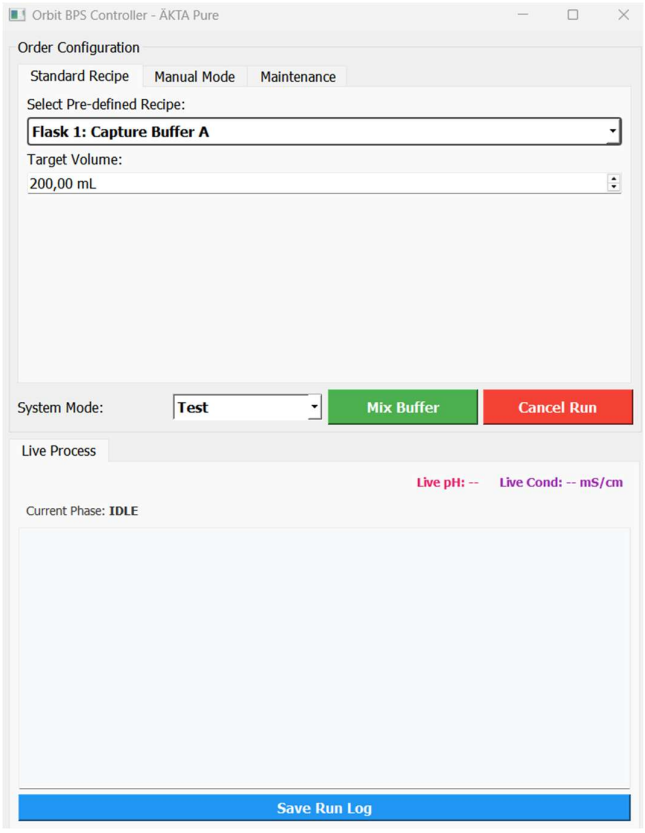

Figure 2.1. The starting window when opening the GUI.

3 Order Configuration

In the Order Configuration segment of the GUI, the settings for the system and the specifications for the prepared buffer are defined.

3.1 System modes

Before any operations are run, an appropriate system mode must be selected from the drop-down menu at the bottom of the Order Configuration segment.

- **Test mode:** Runs a digital simulation of the system. Valves will not shift position, and the pumps remain turned off. The system generates randomized data for pH and conductivity. Useful for testing recipes and verifying software behavior.
- **Real mode:** Connects the system to the ÄKTA Pure hardware via OPC UA and prepares the buffers in the physical flasks.

3.2 Standard Recipe

In the Standard Recipe tab (Figure 3.1), buffers can be prepared based on pre-defined and validated recipes. The tab contains:

- **Select Pre-defined Recipe:** Choose which buffer you want to prepare from the drop-down menu.
- **Target Volume:** Define the volume of buffer you want to prepare. The volume can either be changed by manually entering the desired volume or by using the arrows on the right-hand side of the menu

Figure 3.1. Contents of the Standard recipes tab in the Order Configuration.

3.3 Manual mode

In the Manual Mode tab (Figure 3.2), buffers can be mixed manually by specifying the target flask and the desired concentration of components in the finished buffer. The tab contains:

- **Target flask:** Choose which buffer flask you want to mix the buffer in.

  NOTE! If the target flask has been used to mix another buffer, the inlet and outlet tubes connected to the flask must be purged beforehand (See 3.4 Maintenance)

- **Select Liquids/Chemicals:** Define the desired chemicals in the buffer and their target concentration in mM. More components may be added by pressing the "+ Add Another Component" button. If "Just Water" is selected, concentration may not be defined.

- **Total Volume to Prepare:** Define the volume of buffer you want to prepare. The volume can either be changed by manually entering the desired volume or by using the arrows on the right-hand side of the menu.

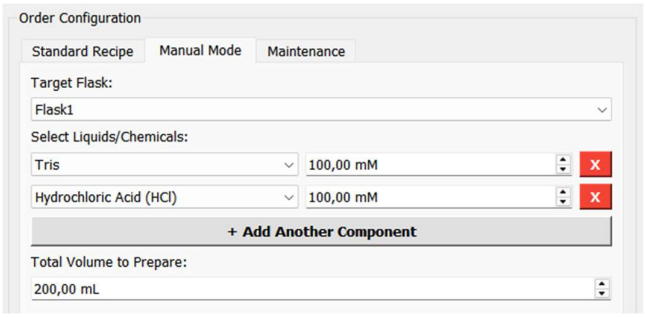

Figure 3.2. Contents of the Manual Mode tab in the Order Configuration. Here two arbitrary chemicals have been chosen, both with a target concentration of 100 mM.

The required volumes of stock solutions will be calculated automatically, and dosed first, after which the remaining volume will be filled with water.

NOTE: It is not possible to mix buffers of a higher concentration than stock solutions. It is recommended to always try a new recipe in test mode before running it in real mode.

3.4 Maintenance

In the Maintenance tab (Figure 3.3) some miscellaneous operations can be found. The tab contains:

- **Select Flask:** Choose which buffer flask to perform the operation on.
- **System Wash:** Purge the system with water to reset it in preparation of buffer mixing. Useful if e.g. a run for needs to be aborted or if the system has been operated manually using Unicorn (Figure B4). For System Wash, the selected flask has no impact.
- **Purge Tube:** Purges the tubes connected to the flask. Before the tubes are purged, the outlet tube in the buffer flask must be placed in an external bottle containing water. Water will be taken from the outlet tube, pass the system and exit through the inlet tube (Figure B6). This is useful if the buffer in a flask has gone bad or between mixes of manual buffers, as the old buffer sits in the flask inlet and outlet tubes.
- **Analyze flask:** Pumps the buffer from the chosen buffer flask through the sensor package and out into waste. This consumes 20 ml of the buffer (Figure B5).

  NOTE: The inline pH sensor is not accurate, and the reading should be considered as an approximation.

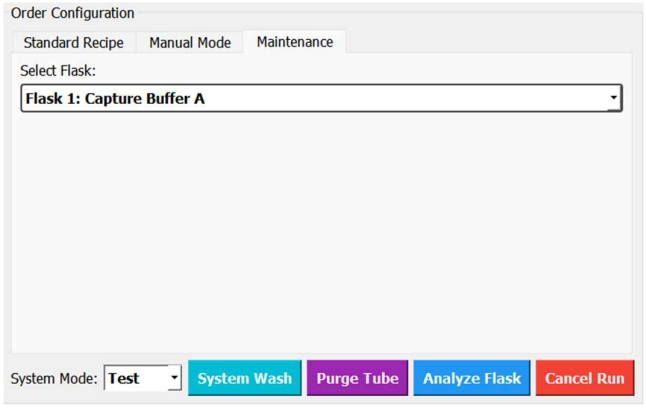

Figure 3.3. Contents of the Maintenance tab in the Order Configuration.

4 Monitoring and Quality Assurance

In the bottom segment of the GUI is the Live Process tab (Figure 4.1). This tab shows information of the current run, including the active phase, the live pH and conductivity readings (in real mode), as well as the information orbit normally prints in the terminal window. At the end of each run, there is a print containing information of the average pH and conductivity during the last 5 seconds of the recycle phase, together with their standard deviation. For pH a static error found during the calibration of the system is added to display a more accurate estimate of the actual buffer pH. This segment also contains a button to save the run log as a HTML file.

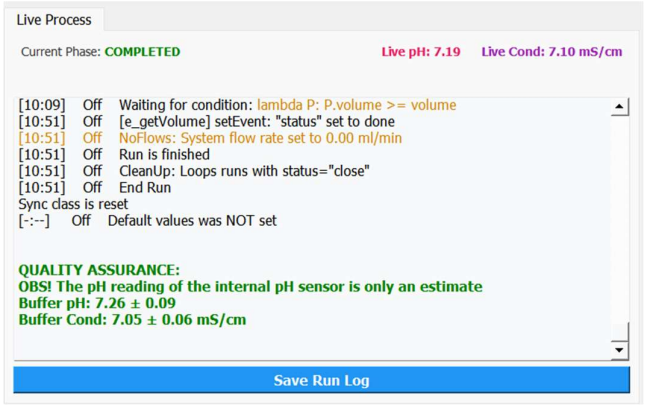

Figure 4.1. Displays the print of the end of a run made in test mode. The current phase, live pH and live conductivity are displayed at the top. Process information, as well as quality assurance information is printed. At the bottom the button for saving the Run Log is displayed.

5 Operating instructions

Below follows step by step instructions for preparing a buffer using the GUI.

1. Select standard recipe or define a recipe in manual mode
2. Select target volume
3. Select system mode (standard setting is test mode)
4. Press the “Mix Buffer” button. The system will start by dosing the stocks, followed by adding water. Then it goes through a recycle phase to ensure that the buffer is properly mixed, before performing a system wash to prepare the system for the preparation of a new buffer.
5. Wait for the run to finish before preparing a new buffer

If for any reason an active run needs to be cancelled, there is a “Cancel Run” button, which instantly stops all pumps and disconnects the GUI from the hardware.