Lappeenranta-Lahti University of Technology LUT
School of Engineering Science
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Measuring & Analyzing Methods for Clean-In-Place Processes Development and Optimization

Master's Thesis

2021

Examiners: Ph.D. Eero Kiljunen
Prof. Satu-Pia Reinikainen

Supervisor: M.Sc. Stefan Karlsson

ABSTRACT

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Cleaning processes are an important part of the manufacturing process of pharmaceutical products. Efficient and effective cleaning procedures are essential to maintain the safety and efficacy of manufactured medicines. Many manufacturing equipment are cleaned by using the Clean-in-Place method, without disassembling the equipment on site. CIP processes are automated and various measurement and analysis methods make it possible to obtain additional information about them.

This thesis has been done to Orion Corporation. The literature section introduces the CIP cleaning process, and reviews analyzing methods that could potentially be applied to development and optimization of CIP processes in the pharmaceutical industry. Based on comparison of the potential methods from literature review, the most suitable methods, Raman Spectroscopy and Collo Liquid Fingerprint Technology are chosen for further investigation. The chosen two methods are tested in the experimental part by using laboratory samples, process samples, and real-time in-line data from Orion Corporations Tablet department's equipment's CIP process. Finally, the methods are evaluated and compared with each other.

The results show, that both methods have potential to develop the CIP processes by providing new types of information. Collo appears to be more suitable at several areas compared to Raman, which would require more investigation to achieve the full exploitation. Collo is sensitive, and in CIP process, the sensitivity also causes challenges with temperature and air. Minimizing the impact of temperature and air requires further investigation.

TIIVISTELMÄ

Lappeenranta-Lahti University of Technology LUT
School of Engineering Science
Chemical and Process Engineering

Elisa Kankaanpää

Mittaus- ja analyysimenetelmiä Clean-in-Place prosessin kehitykseen ja optimointiin

Diplomityö
2021
80 sivua, 35 kuvaa, 6 taulukkoa

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Pesuprosessit ovat tärkeä osa lääketeollisuuden tuotteiden valmistusprosessia. Tehokkaiden ja toimivien pesuprosessien avulla varmistetaan, että valmistuslaitteet tulevat puhtaiksi edellisen tuotteen jäljiltä sekä se, että tuotanto on turvallista ja sujuvaa. Monet valmistuslaitteet puhdistetaan Clean-in-Place menetelmällä, paikan päällä purkamatta laitetta. CIP prosessit ovat automatisoituja, ja erilaisten mittaus- ja analyysimenetelmien avulla on mahdollista saada niistä lisätietoa.

Tämä diplomityö on tehty Orion Oyj:lle. Kirjallisuusosassa esitellään yleisesti CIP-pesuprosessit, sekä käydään läpi menetelmiä, jotka mahdollisesti voisivat soveltua lääketeollisuuden CIP-pesuprosesseihin. Kirjallisuusosassa tehdyn vertailun avulla soveltuvimmaksi valittujen mittausmenetelmien, Raman spektroskopian ja Collo Liquid Fingerprint teknologian soveltuvuutta lääketeollisuuden CIP-pesuprosessiin testataan kokeellisessa osuudessa laboratoriossa valmistettujen näytteiden, prosessinäytteiden sekä reaaliaikaisen in-line prosessidatan avulla Orion Oyj:n tablettituotannon valmustuslaitteen CIP-pesuprosessiin. Lopuksi menetelmiä arvioidaan ja vertaillaan keskenään.

Tulokset osoittavat, että molemmat menetelmät ovat potentiaalisia CIP-pesuprosessien kehitykseen antamalla uudenlaista tietoa pesusta, ja osoittamalla että laite puhdistuu niin kuin kuuluu. Collo osoittautuu kuitenkin useammassa kohdassa lupaavammaksi kuin Raman, jonka potentiaalin täydellinen hyödyntäminen vaatisi jatkoselvitystä. Collon potentiaalin täydellisessä hyödyntämisessä on kuitenkin myös haasteita, kuten lämpötilan ja ilmakuplien vaikutukset dataan.

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Espoo, 23th of June 2021
Elisa Kankaanpää

LIST OF SYMBOLS AND ABBREVIATIONS

API	Active pharmaceutical ingredients
CIP	Clean in Place
CIV	Collo Ion Viscosity
COP	Clean out of Place
Collo	Collo Liquid Fingerprint Technology
CP	Collo Permittivity
GMP	Good Manufacturing Practice
EMF	Electro Magnetic field
EMA	European Medicines Agency
ISFET	Ion Sensitive Field Effect Transistor
MIR	Mid-infrared
NIR	Near infrared
NMR	Nuclear magnetic resonance
NSAID	Non-steroidal anti-inflammatory drug
NaOH	Sodium Hydroxide
PLS	Partial least squares
PW	Purified water
Raman	Raman Spectroscopy
RF	Radio frequency
SRV	Symmetric Resonator Viscometer
TOC	Total Organic Carbon
UV-Vis	Ultraviolet-Visual

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1. INTRODUCTION

This master's thesis work has been done to Orion Corporation. Orion Corporation is a Finnish pharmaceutical company that develops, manufactures and markets human and veterinary medicines, as well as the active ingredients of medicines. Orion Corporation is a company founded in 1917, whose mission is to build well-being. Orion Corporation's products are currently marketed in more than 100 countries, the main market area is Finland. Orion Corporation uses several hundreds of different raw materials and excipients to manufacture medicines, as the portfolio of Orion Corporation consist of almost 400 products. The portfolio includes, for example, the tablets, creams, gels, solutions, injections, and inhalators. One of the focus areas of Orion's strategy is quality and safety. In the manufacture of medicines, cleaning processes play an important role in ensuring the high quality of products. With safe and efficient medicines, patients get help to treat their diseases. (Orion, 2021)

In the pharmaceutical industry, efficient and effective cleaning procedures are essential to maintain the safety and efficacy of manufactured medicines. Cleaning process of equipment is part of Good Manufacturing Practice (GMP) for pharmaceutical manufacturing. In particular, the cleaning of equipment that has been in contact with the product is critical step, as any impurities or product residues will contaminate the next batch to be treated. Maintaining a good level of cleanliness minimizes the risk of impurities and microbes entering the product and prevents the risk of cross-contamination between products. (Khater, M., 2015)

The cleaning methods in pharmaceutical industry can be divided into Clean in Place (CIP) and Clean out of Place (COP). CIP process mean cleaning the equipment on site, without disassembly. (Khater, M., 2015) Although CIP processes are automated and validated, it is possible that after some cleaning processes, contaminants will remain on the surface of device, necessitating a repeat of the CIP process. One of the biggest challenges in the cleaning processes is the variability of the physical and chemical properties with a wide range of raw materials from one extreme to other. The purpose of this thesis is to find one or several measuring methods that could provide more detailed information of the changes in the CIP process containing various products and raw materials, so that the process can be developed and optimized to be more effective in the future.

The aim of the thesis

The aim of this work is to examine measuring and analyzing methods that could be utilized in development and optimization of Clean in Place processes. With a suitable method, it is possible to monitor the progress of the CIP process and find out whether changes in product residues, detergents and water concentrations can be seen from CIP process rinses.
The main research question of the thesis is:

- What kind of measuring and analyzing methods can be used to improve the understanding of the CIP process in pharmaceutical industry?

The aim of literature review is to create an overall picture of CIP processes and what kind of measuring and analyzing methods are used for Clean in Place processes in various industries. In addition, potential methods which appears to be potential for this need, but which are not currently used in CIP processes, are searched. Ideally, the measuring or analyzing method could provide better understanding of the process and of its various cleaning steps and their durations. By this, the process then could be developed to increased productivity and competitiveness. The efficiency and water consumption of the process can also be affected by the information obtained from the appropriate analytical method. Efficient CIP process can affect not only water but also other resources such as energy consumption and the amount of used chemicals. By comparing the cost, sensitivity, potential for CIP cleaning, usefulness, and other issues relevant to the CIP process of the analytical methods identified in the literature review, a matrix can be created to detect the most potential methods for this particular application.

The appropriate method may provide more detailed information on certain cleaning steps, such as rinsing only, or all cleaning steps, depending on the method of analysis and its location. Measurements of the cleaning water in the process can provide information about the changes in the cleaning phase and thus their exact duration. The results obtained from the measurements can potentially optimize durations of the rinsing steps so that time is not wasted. The measurement methods are chosen for experimental part based on theoretical evaluation (matrix). Raman spectroscopy and Collo technology are most suitable for the

detecting of the chemicals of the whole process and placing them close to the drain provides accurate information, especially about risings steps and single pass cleaning steps.

The experimental part answers the following questions, which are derived from the main research question:

- Are the selected methods suitable for pharmaceutical CIP process in practice?
- What kind of information selected methods can give about CIP process and how it can be utilized?
- What challenges may rise from the selected methods and how they could be avoided?

In the experimental part, the measurements are made from the samples prepared in the laboratory. The purpose of those samples is to describe the effluent from the prerinse of the tablet department's product equipment CIP process. The aim of the samples is to evaluate the suitability of the selected methods for CIP application. The experimental part is also executed both by taking samples from the process and with the data provided by an in-line sensor to be attached to the pipeline and analyzing the results. The selected measuring methods are Raman spectroscopy and Collo Liquid Fingerprint analyzer. Either of the selected technologies has not been reportedly used in CIP processes monitoring and optimization in the literature.

2. CLEANING IN PHARMACEUTICAL INDUSTRY

In the pharmaceutical industry, cleaning and its validation is very important. The object of Cleaning Validation is to ensure that the production facilities are consistently clean from the remainders of the previous product. It ensures, that cleaning process removes all active pharmaceutical ingredients residues, excipients, the chemicals used in cleaning process and microbial attributes. For the quality of next product to be manufactured, cleaning the equipment to predetermined levels, is essential. (Raj, 2014)

Several regulatory requirements of laws, guidelines, inspectors, and authorities have been defined for Cleaning Validation in pharmaceutical industry. Good Manufacturing Practice (GMP) refers to activities based on good manufacturing practices for medicines and pharmaceutical ingredients, ensuring that the product meet certain quality requirements. In Europe, European Medicines Agency (EMA) oversees the pharmaceutical industry. EMA impacts on EU GMP guidelines with a variety of requirements for cleaning validation. In Finland, Finnish Medicines Agency, Fimea supervises and develops the pharmaceutical industry. Finnish Medicines Act also stipulate that industrial pharmaceutical production may only be carried out with Fimea's permission. (Fimea, 2021) Medicines Act also requires compliance with GMP. Cleaning process equipment is part of GMP for pharmaceutical manufacturing. (Khater, M., 2015)

One of the most important factors to ensure the hygiene in production facilities is the removal of contaminants from all surfaces. Contaminants consists of microbes, active pharmaceutical ingredients and excipient residues, airborne materials such as impurities and particulate matter, lubricants and ancillary materials, cleaning agents and decomposition residues. (Raj, 2014) Cross-contamination means contamination of a starting material intermediate or final product with another starting material or product. As ensuring patient safety is important in pharmaceutical industry, the avoidance of cross-contamination is also extremely important. EU GMP Guidelines chapters 3 and 5 are focused to the avoidance of cross-contamination and those instructions are followed in the manufacture of medicines. (ECA Academy, 2015)

After cleaning and drying the process equipment, a visual inspection must be performed in accordance with legislation and supplementary instructions to ensure that the process equipment is clean after the cleaning. Visual inspection and quantitation are also part of GMP Guidance, and it is not performed until the entire cleaning cycle has been completed and the equipment is dry. The equipment is visually clean when no residue can be detected on its surface. Visual inspection inspects all surfaces of the equipment that have been in direct or indirect contact with the product. Once the visual inspection has been completed and no residues are found on the product contact surfaces, the next production batch can be started. (El Azab, 2020)

3. CLEANING PROCEDURES

In pharmaceutical industry, the types of cleaning processes can be divided into automated cleaning and manual cleaning. Manual cleaning means various wipes and brushes. Automated cleaning can be divided into Clean in Place (CIP) and Clean out of Place (COP). In COP processes, equipment is disassembled and transported to a separate washing cabinet for cleaning. CIP processes take place on site without disassembling the device. In the pharmaceutical industry, every step of the work must be documented so that each batch of medicine can be tracked back to the point of departure. Also, for cleaning, many cleaning details, such as wipes and brushes used for manual cleanings must be documented. In this work, from different types of cleaning processes the focus is on CIP processes.

The residues and contaminants in CIP cleaning system can be attached to equipment surface and pipes by a combination of different physical effects. Three of those affects are: van der Waal's forces, electrostatic effects and mechanical adhesion and they can be termed as soil adhesion. In CIP process, soil adhesion must be subdued by providing four basic parameters' forces counteracting the adhesion. The drug residues attached to surfaces by physical effects are shown in Figure 1. (Seiberling, 2008)

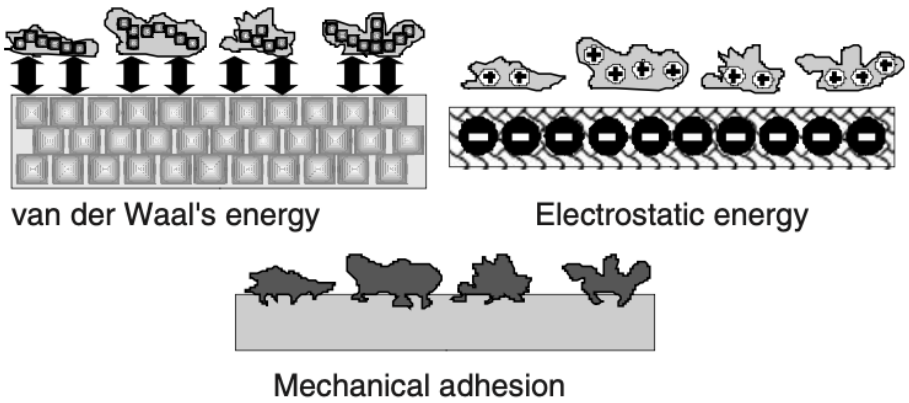

Figure 1 Physical effects to dirt to attached to surfaces (Seiberling, 2008)

In all purification processes such as CIP process, the anti-sticking forces can be divided into four basic parameters which are the cleaning temperature, cleaning time, cleaning kinetics and the chemical activity of the used cleaner. All of those basic parameters are dependent on

each other's and they need to be controlled carefully for successful cleaning. (Seiberling, 2008)

3.1 Clean in Place processes

Clean in Place reflects to complex technology that includes a CIP-compatible process equipment, spray devices, feed and return pipes, vessels for chemicals, CIP skid with chemical feed equipment, and a control system that runs the CIP skid and delivers solution in the correct series at specified flow rate at given composition, pressure and temperature. CIP is designed to automate the essential cleaning processes and avoid disassembling the device into parts, which is time-consuming. CIP processes are used in many different industries, such as food, dairy and beverage processing industries, but in 2018 the largest share of the clean in place market was in pharmaceutical industry. Clean in Place is an efficient way of cleaning in pharmaceutical industry, with strict regulations to ensure product safety and high quality. (Research and Markets, 2019) The three key objects for effective CIP process are to maximize safety by avoiding cross contamination, minimize the cleaning time for save the money by maximizing time for production and optimize thermal efficiency. (Pharma Manufacturing 2007) Figure 2 shows the simplified flow diagram of typical CIP system (Shnayder & Khanina, 2005).

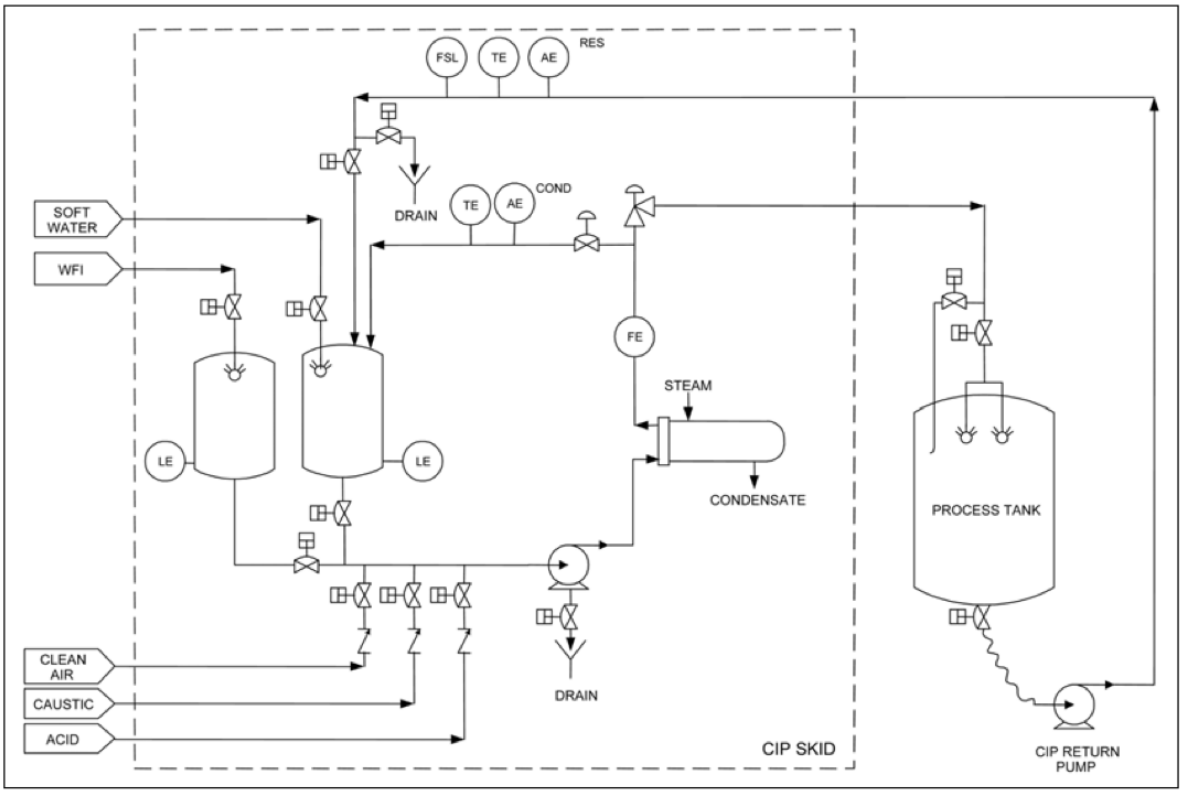

Figure 2 Simplified CIP process flow diagram (Shnayder & Khanina, 2005)

In Figure 2, the CIP skid has two tanks, one for clean water and the other for the preparation and recirculation of cleaning solutions. There are several types of CIP skids and there can be for example several tanks needed to prepared cleaning solutions, so that the same CIP skid can be used for several process equipment at the same time. (Seiberling, 2008) CIP Process looks simply, but in practice it often has the same number of cleaning routes as the equipment has different types of objects to be cleaned. The cleaning routes are repeated in the programmed order and are selected to send wash or rinse solution by means of valve manifolds. (Setpoint Industrial, 2020)

Some instrumentations are commonly used to control CIP process and verify performance. Common measurements include temperature, pressure, conductivity, and flow in pipelines. The temperature is regulated, monitored, and controlled in many specific ways. The temperature can be monitored with a temperature sensor, for example from the CIP supply. Control valves are commonly used to heat and control the temperature of CIP solution. Some CIP processes also use heat exchangers for cooling. The conductivity monitoring systems in CIP

processes are normally used to ensure that appropriate concentration of detergents used is added and does not directly control the dosage of detergents and in final rinse check. Flow-meter and flow control valves are used in CIP process to ensure control of mechanical action. (Seiberling, 2008)

In many CIP processes, the current shortcoming is the lacking in the real-time process monitoring of the changes occurring in the process. Non-optimal control of the parameters of cleaning process and the durations of steps in CIP leads to reduced efficiency and excessive consumption of resources. (Simeone et al., 2018)

Typical steps of CIP process

Typical steps of CIP process in pharmaceutical industry are presented in Figure 3.

Figure 3 Cleaning steps of CIP process (Modified: Seiberling D., 2002; CSIdesigns, 2021)

The purpose of the first step, prerinse, is to remove as much organic fat, carbohydrate, proteinaceous soil, or other solid residue products as possible with warm water. The wastewater

of the prewash rinse is the dirtiest water in the washing process, and therefore the washing water is removed from the process without circulation. (Bremer et al., 2009) The composition of the prewashes varies, in some applications the prewash is done only with tap water, others already use detergents and enhancers in the prerinse. (Seiberling D., 2008)

In every cleaning step, utilized type of water quality (purified or tap water) depends on the type of product to be cleaned. If the product is in category of sterile products, the water used is either PW or even higher quality water. If the product is non-sterile, only the final rinses quality is purified water and water quality in other steps are tap water. (EMA, 2020)

The step 2 of the CIP process is the most important step, alkaline wash. The purpose of this step is to remove protein and grain residues from the surfaces. The temperature of alkaline washing solution is over 70 °C. The duration of the washing phase can vary from ten minutes up to an hour. Alkaline solution wash is done as circular wash, in which the same washing solution circulates in the pipelines for the entire duration of this phase. After alkaline it is the turn of the step 3, post wash rinse where the detergent residues left in the process are removed. Post wash rinse is typically done with tap water and it takes 10 to 15 mins. (Seiberling D., 2008)

The step 4, acid washing, destroys inorganic microbes in pipes and removes mineral and oxide deposits from water and product on the equipment inner surfaces. Acidic wash also helps to neutralize the residues of alkaline. After acid wash, there are also rinse with tap water, step 5, intended to remove acid detergent residues. (Seiberling D., 2008)

The final rinse in step 6 is with high quality, reserve osmosis water. Reserve osmosis removes contaminants from unfiltered water when pressure forces it through a semipermeable membrane. The purpose of rinsing with purified water is to ensure that the equipment is clean after tap water rinse. (Seiberling D., 2002)

3.1.1 The effect of four basics in CIP

Time, action, chemistry, and temperature are the four basics and the relationship between them is the key to a successful cleaning study. Figure 4 shows the right relationship in the cleaning circle.

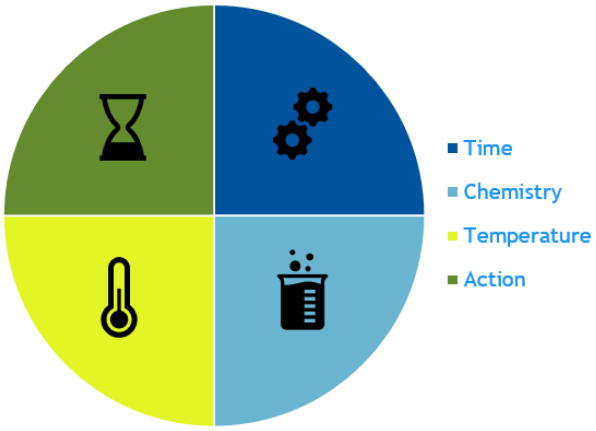

Figure 4 Cleaning circle (Modified: Seiberling, 2008)

The significance of the duration of the washing steps in the CIP process sound simple, but one very important time variable for efficient cleaning is contact time, in which the cleaning agent is exposed to the soil. If the drug residues are not exposed long enough in cleaning cycle it can be more difficult to remove the dirt and soil. The effect of time is seen in both the detergent-containing steps and the sensing steps. It is important to find suitable time relationship between for example alkali wash and initial rinse. The same time ratio can be applied to subsequent wash if it is known that the prerinse has been effective. Excessive washing can cause problems if the detergent has already reached its' carrying capacity, and therefore the remaining dirt can mix and re-settle in the piping or inside the equipment. (Weiner, 2020)

Action is also one of the four basic parameters and refers to the energy needed to properly clean the surface. In the CIP processes, action refers to the flow through the pipelines, and it is highly important, because the speed and the type of flow affects directly to the efficiency of cleaning. Flow also brings the cleaning agents into contact with the dirt on the surfaces.

In some recommendations, the flow rate in CIP process pipelines should be at least four times the product flow. (Stier, 2020)

Chemistry refers to the importance of the cleaning agents selected for the process; therefore, the agents have been selected to be best suited for the cleaning of a particular process and its soil. A wide variety of cleaning agents are available in the industry, and therefore suitable detergents with optimal concentrations are important in the CIP process. The choice of detergents should take account not only the product to be cleaned and the surface, but also the hardness of the water used for washing. (Stier, 2020)

Temperature is the last of four basic parameters in CIP process. The optimal temperature of the rinsing waters, in both acid and base washes, has a large effect on the efficiency of the whole process. The optimum temperature of pre-wash rinse is below 40 degrees, as above that proteins and starches undergo chemical changes. After alkaline wash, the required rinse water volume is reduced by using heated rinse water compared to rinse water in lower temperatures. This is due to heated rinse water's ability to remove detergent residues more efficiently. The lowest temperature water in CIP steps is often used in prerinse. Acidic rinse can be used effectively at lower temperature than base. (Seiberling, 2008)

3.1.2 Products to be cleaned and detergents in CIP process

In pharmaceutical industry, the chemicals used in manufacturing of medicines contains certain drug or a mixture of several drugs. However, all medicines contain three fundamental parts: Active pharmaceutical ingredients (API), excipients and non-pharmaceutical raw materials. (Abrantes et al., 2016) Also, several different chemicals and detergent agents are used in cleaning processes (Khater, M., 2015). APIs, excipients, and other non-pharmaceutical raw materials are washable materials in cleaning processes.

Active Pharmaceutical Ingredients and Excipients

API is the biologically active part of medicine. They usually constitute about 30 % of the drug composition, but the constitute varies according to the composition of the product. API molecules have many chemical functional groups, because they are usually combinations of

several factors that goes beyond the intended therapeutic effect. APIs are created either by isolation from pharmaceutical raw material or by chemical synthesis. One commonly known API is ibuprofen which is propionic acid derivate and non-steroidal anti-inflammatory drug (NSAID). In solid medicine, the form of API is the most thermodynamically stabile crystalline. (International Union of Crystallography, 2014)

Excipients are safe and "inactive" ingredients, that make up the bulk of any drug composition. Pharmaceutical excipients have many tasks. They are used to control the release of API, as carriers of API, to mask the taste of medicine, as a solubilizer or emulsifiers, to ensure accuracy, and in precision and mixing of homogenous ingredients. Common excipient as filler agent and as diluent is lactose. Common excipients used as sweeteners are for example sorbitol, aspartame, and saccharin. (Alvi, 2014) Excipients usually constitute about 50 % of the drug composition. (PCC Group, 2017)

The remaining of the pharmaceutical composition are pharmaceutical preparations and formulations that define the dosage form of the product. There are several forms depending on the safe route of administration. These include for examples, tablets, capsules, creams, gels, inhalations, injections, pastes, and aerosols. (Pharmapproach, 2020)

Detergents in CIP Processes

Because a wide variety of chemical compounds are used in medicines, effective detergents are also needed to remove them from surfaces of production equipment. Important detergents in CIP cycles are caustic soda, acid, and various enhancers. Commonly used caustic soda is sodium hydroxide (NaOH) with concentration range of 1-4% and pH 13. NaOH as a strong alkali is used to remove protein-based biopharmaceutical drugs because of their efficacy in peptidizing the bonds that hold proteins together. Although NaOH is effective in cleaning, its use also involves challenges. The concentration of NaOH must be suitable for the soil to be removing, both too high and too low will not give the desired washing result. Another base used in CIP caustic wash is potassium hydroxide, which is a more expensive option than sodium hydroxide but, in some cases, essential. Potassium hydroxide can be used in situations when the products contain strong alkaline. In addition, an enhancer can be used in

the CIP process to reduce the surface tension of the alkaline detergent and thus improve its dirt removal and transport ability. In acidified wash, commonly used acid is nitric acid with concentration of 0,5%. Also, mixture of phosphoric and nitric acids is applied. (Seiberling, 2008)

3.1.3 Challenges in CIP systems

A failed CIP process is the sum of many factors. In the event of a failed wash, there are still run-off marks from tap water or other residues on the inner surfaces of the equipment or piping. In pharmaceutical industry, residues must not remain and in the worst case, it could contaminate the entire next batch. The main challenges in CIP cleaning are chemicals. Some detergents can cause foaming in the process due to the use of the wrong type of detergent or too low temperature. Also, the reaction of the detergent to some excipients can cause foaming. Excessive foaming can affect to CIP process so that the pumps in the process cannot operate normally. (Chematic, 2019)

The same equipment can be used to produce very different types of products, so the scale of the products to be cleaned in CIP process is also very wide. Currently, there is no detergent which surfactants would foster the removal of all CIP cleanable soils and APIs. (Chematic, 2019)

Since the speed and type of flow are factors affecting cleaning efficiency, too low flows can cause poor cleaning results. Even though there are flow and temperature meters in the process, they are not everywhere, and there may be areas where temperature or flow are not measured. If the sensor does not work as assumed, the incorrect cleaning method may result as impurity in the surface of the equipment causing contamination.

3.2 Environmental impacts

Even though using the alkaline and acidic chemicals in CIP process is necessary, usage produces also large amounts of very high and low pH wastewater (Nishijima et al., 2014). The CIP process can be long as it could take 5 hours to complete the process, and it consumes a lot of water, detergent, and energy during that time (Fan et al., 2017). In Orion Corporation,

during one CIP cleaning recipe used for fluid bed granulator, the total water flow through the process is several cubic meters. Prerinse, which consumes couple of thousand liter of water, during which API residues are removed, is collected in its entirety, and dealt with separately.

Measurement of the analytical method can also affect other challenges than just optimizing CIP process. For example, by analyzing the waste stream of the process, it can be seen when the rinse wash has progressed so far that the pharmaceutical ingredients are no longer released from the device and piping. This information can be used to ensure that no pharmaceutical substances end up in the environment with the effluent. The release of APIs into the environment has adverse effects, for example on aquatic ecosystems. (Strade et al., 2020)

3.3 Economic analysis

The CIP process consumes resources such as energy, water, detergents, and employee time. After the CIP process, if the equipment is not clean enough during the visual inspection, the cleaning must be repeated. Recleaning are directly out of the production time and affect the work of many people, so their economic impact must also be considered. Re-washes affect many resources and stakeholders like a domino effect. The impact on costs in production alone, consist of the resources used for extra washing, lost equipment time, overtime works over the weekend, and the delay caused by extra washing to other stages of the process. In addition, the impact of stakeholders incurs costs in the form of wages, while the extra washing causes extra work for many employees. Re-washing may have affects for example planning of production, deviation handling, storage, and laboratory. In Orion corporation, it has been estimated that the cost of re-washing can be around 2000€.

4. POTENTIAL MEASURING METHODS FOR CIP PROCESS

Potential measuring methods for measuring the waste stream of CIP washes can be divided into in-line types that can obtain data over longer period and to those that can analyze samples taken from the field. Any in-line sensors, that must withstand CIP, must be resistant to harsh conditions, high acid, and base concentrations. Not all sensitive analytical sensors are made for harsh conditions. (Edwards et al., 2015) A potential, suitable method of analysis should be able to show several things about different steps of CIP process such as physical and chemical changes. Ideally, the potential method is also able to indicate the moment in each cleaning step in the CIP process when the change stabilizes. For example, in the case of prerinse, potential method could show the moment when the change is stabilized for the level of the detergent solution. However, this moment is challenging to see directly, if the cleaning steps include several routes that are traversed one at a time. An appropriate method could potentially achieve the following benefits: Reduced water usage, sanitizer consumption, wastewater cost, cycle times, operating costs and increase in available process time documented sanitizer concentration rapid return investment.

4.1 Potential measuring methods for real-time measurements

Most analyzing and measuring methods are laboratory-based, expensive to perform and require sample transport to laboratory. In terms of long-term monitoring, it is time consuming to transport samples to the laboratory for examination and wait for their results. (Li et al., 2014) Therefore, different types of measurement methods have been developed, where the measures could be installed in-line, at-line and on-line. In in-line measurements, the sample is measured directly from the process. At-line measurements means that the sample is removed from the process stream but still analyzed in its immediate vicinity. On-line measurement means a measurement in which the sample is diverted out of the process stream but can still be returned to the process.

4.1.1 Conductivity measurement

In studies, the most frequently emerged analytical method for CIP monitoring is conductivity measurement. Since the detergents used in CIP have higher conductivity than the rinsing water, conductivity measurement is a logical choice. Conductivity is not specific measure, as it measures the concentration of ions in solution, but cannot make difference in between initial electrolyte and ion. In CIP Process, conductivity is most commonly used in-line to monitor completeness of rinses and sometimes also the concentration of sodium hydroxide. (Emerson, 2010)

There are two types of conductivity meters utilized in CIP processes. One is a contacting sensor and the other is inductive sensor. Most of the contacting conductivity sensors consist of two electrodes, but sensors with four electrodes exists. Four electrodes sensors are much more efficient than two-electrode sensors, and the working principle is different. In the two electrodes conductivity analyzer, the measurement is based on the current by applying an alternating voltage to electrodes. Ions are made to move by an electric fiend creating current. Resistance of the solution is calculated by using Ohm's law. (Emerson, 2010) According to Ohm's law, the current flowing through a resistive circuit is thus greater the higher the voltage between the ends of the conductor is– the voltage is directly proportional to the magnitude of current. In a four-electrode sensor, the voltage drop caused by electrolyte resistance is measured between inner electrodes as the analyzer injects current between the outer electrodes. Inductive conductivity sensor is also called toroidal sensor. Working principle of toroidal sensor is the following: using an alternating voltage causes an ionic current in the solution around the sensor, and the voltage causes the ionic current to flow in a ratio proportional to the conductivity of liquid. The use of an inductive sensor is limited to samples with a conductivity greater than 15 μS/cm. (Emerson, 2010) Table I shows the different conductivity methods and ranges of them.

Table I Ranges of different conductivity sensors (Emerson, 2010)

Conductivity sensors	Range, µS/cm
Two-electrodes	0.01 to 50,000
Four-electrodes	1 to 1,4000,000
Toroidal sensor	> 15

Conductivity measurement is an inexpensive alternative to the method of analysis and can provide information from even quite sensitive changes in solution concentration and is able to detect water quality well. However, by measuring the conductivity from the CIP process, it is not possible to distinguish and further define whether it is a solid, API, excipient, or a detergent.

4.1.2 Total Organic Carbon measurement

Total Organic Carbon (TOC) is used in CIP process to validate, that the water of the final rinse is on the required purity-level. It is fast and simple measuring method for detecting low levels of organic compounds. There is several in-line type of TOC analyzers, but all of them oxidize organic carbon and measures CO2. Different techniques and detection methods can have large difference in accuracy. Several TOC sensors on the market that are suitable for CIP processes, use Mid-infrared (MIR) technology, which takes several minutes to obtain a reliable TOC result. The faster technology in used for this application by implementing MIR is based on External Cavity Quantum Cascade Laser. Quantum Cascade Lasers is a semi-conductor laser, meaning it can produce five parallel beams. It is based on transitions between lanes in a heterogeneous structure. (Siegmann-Hegerfeld et al., 2013) In addition to TOC measurement based on MIR technology, TOC can be based on membrane conductometer technology. The principle of membrane conductometer technology is, that the membrane forms a protective membrane-like barrier to interfering ions which allows a more accurate CO2 analysis and therefore more accurate TOC reading. (Sievers, 2021)

Like conductivity measurement, TOC is a non-specific analyzing method, so while it can detect every compound in the CIP process that contains carbon such as APIs, excipients, and detergents, it cannot determine specific compounds. TOC reports the sum of all organic carbon and is therefore very suitable for assessing water quality. TOC meter that can follow the process in-line, in real time, is more expensive than the conductivity meter. (Neumeyer, 2020)

In-line measurement method, that combines Total Organic Carbon & conductivity verification analyzers, can be used to the detect if the process vessel is clean. The strength is that most organic and inorganic substances in the CIP process can be detected by two efficient analytical methods. Conductivity detects inorganic substances and TOC detects organic compounds that cannot be detected by conductivity. (Hach, 2016)

4.1.3 Turbidity sensors

Turbidity sensors are commonly used in dairy industry CIP processes in prerinsing, to confirm that the piping system has been flushed clean. There are several working principles for turbidity sensor. Most of them are based on near infrared (NIR) absorption. The transition in turbidity sensor used in CIP processes are from 730 to 970 nm. (Li et al., 2014) Turbidity is simple and basic indicator for measuring water quality. Any samples that pass through a particular light source will affect the turbidity of the sample. In the measurement of the water quality in the final rinse of the CIP process, turbidity meter is not as efficient as for example TOC, as the process contain only high purity water where the particle size and the amount of particles are small. (Hach, 2021)

Studies have presented consistency measurement as well optical backscatter in which the light source is modulated with a low square waves, only on a frequency of tens Hz. Low-frequency source in turbidity sensor might be more efficient detection method because it mitigates the effect of ambient light and external disturbances that may interfere with the NIR based turbidity sensors. (Kirkey et al., 2018)

4.1.4 pH sensors

From all quality measurements in processes more than 30 % are pH measurements. One potential measuring method for pH measuring is Ion Sensitive Field Effect Transistor (ISFET). ISFET measures the concentration of ion solution from the liquid. The ion concentration is changed to pH. It offers an alternative to places such as CIP process where it is not possible to use conventional pH analyzer with glass probe. ISFET sensor is commonly used to improve the stability of the CIP process. The working principle of ISFET sensor is based on the change of normal field effect transistor. (Elprocus)

Some pH, conductivity, turbidity, and TOC sensors measure also dissolved oxygen. For measuring the pressure of oxygen that is dissolved in the sample, there can be used dissolved oxygen sensors. In CIP processes, these dissolved oxygen sensors could be used in a final rinse, such as a TOC sensor, to detect water purity. There are two types of dissolved oxygen sensors, optical and electrical and both are used for controlling wastewater treatment. (Mershon, 2016)

4.1.5 Near-infrared spectroscopy

Infrared (IR) spectroscopy is based on absorption of electromagnetic radiation at certain wavelength. When the infrared radiation is absorbed into the bonds of molecule, they begin to vibrate. IR can be divided based on the wavelengths at which the electromagnetic radiation absorbs. For liquid samples, more suitable infrared spectroscopy is NIR instead of middle infrared (MIR). MIR has stronger light absorption of water than NIR, so NIR is more suitable infrared spectroscopy technology for liquid samples. That is why NIR has been used in CIP measurements. NIR covers the transition from 780 to 2526 nm. (Li et al., 2014) NIR absorption-based photometer can be used in CIP processes to detect the precise process interface. Typically, this kind of analyzer is installed at the returning point of CIP. NIR technology can detect API, detergents and excipients. (Sarraguca & Lopes, 2009)

4.1.6 Viscosity and density sensors

With viscosity, huge amount of process fluid can be obtained in molecular level. In-line vibrating resonators are on the market for monitoring and optimizing CIP processes and detecting cleaning end points in dairy industry. (Rheonics, 2021) These sensors can determine the density and viscosity of a liquid. The cleaning process is monitored by monitoring the viscosity and density of the detergents. By following the change, a certain cleaning step can be seen, when the change in viscosity no longer occurs. Many vibration-based sensors on the market use lateral vibration. Some of the vibrating resonator sensors on the market use vibrates in torsion, which is more stable and more isolated alternative to lateral vibration. Torsional resonators, which are particularly sensitive to the viscosity of liquids, are shaped of cylindrical and vibrate parallel to their own surfaces. The working principle of the in-line sensor is, that one end of the torsional resonator is immersed to the liquid to be measured and Symmetric Resonator Viscometer (SRV) measures the density and viscosity of process liquid. (Rheonics, 2015)

In industry, there are several different technologies for density and concentration measurement. One potential method for fluid density measurement is the hydrostatic pressure differential. The working principle of hydrostatic pressure differential density sensor is, that the hydrostatic pressure is applied on a capacitive density transmitter. (Smar, 2021)

4.1.7 Refractometer

In-line refractometers are for measuring the optical index. They are used to measure the concentration of the solution, but the density can also be calculated. Refractometers are commonly used in food & beverage industries' CIP processes for product identification in liquids (Flexim, 2009). Refractometry is based on the fact, that as the density of substance increases, its refractive index rises in the same proportion. The working principle behind one refractometer on the market is critical angle measurement. The main components of the refractometer are light source that sends rays in different angles, prism which receives rays of light and process interface and image detector. Figure 5 shows the working principle behind one refractometer on market. (Vaisala, 2020)

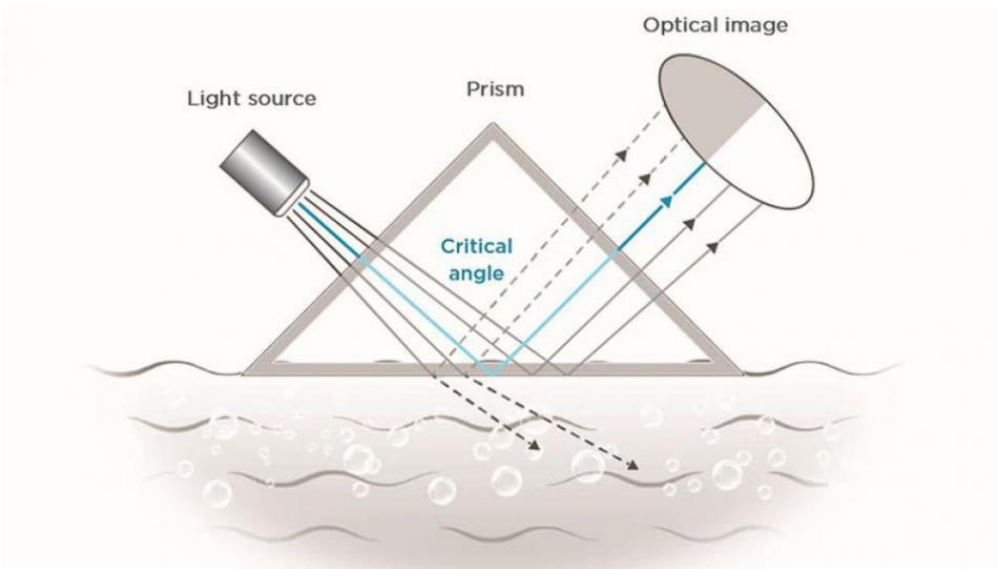

Figure 5 The principle of critical angle measurement refractometer (Vaisala, 2020)

Even if refractometer is commonly used in other industries, it might not be suitable method for CIP measuring in pharmaceutical industry, because the liquid should be optically homogenous and preferable isotropic. (Vaisala, 2020) In CIP processes, there might be some API and excipient residues, that might be in solid phase.

4.1.8 Ultraviolet-Visual spectroscopy

There are Ultraviolet-Visual spectrometers on the market that can measurement in-line measurements and where reliable matrix detectors record the produced spectrum in seconds. UV-Vis measurements are based on the Lambert Beer law. According to Lambert Beer law, the absorption of electromagnetic radiation depends exponentially on the amount of absorbent. In some studies, UV-Vis measurements have been able to determine the dynamics of CIP cleaning. The onset of the alkaline wash after prerinsing in a CIP process is rapid and even purification temperature could have been lower. Challenge of the UV-Viv monitoring is that the technology is not suitable for entire CIP process, because in acidic wash there are typically minerals, which are not absorbed by UV light. (Berg et al., 2017) UV-Vis technology has been able to detect API in the cleaning process art low concentrations in some studies. (Spoerk et al., 2020)

UV-Vis technology is also utilized in an optical multi-angle led spectrometer method on the market, where in addition to UV-Vis the third pulsed light led is NIR. This non-contact, in-line multipoint spectral measurement method can be used to measure turbidity, color variability, temperature, UV for non-visible pollution and detection of contaminants. Its advantage is that the LEDs can measure the optical density at up to 6 different wavelengths, from 365 to 860nm. In the CIP process, this LED technology can be utilized throughout the process for example accurately identify the duration of each phase. (Indatech, 2020)

4.1.9 Low-field NMR spectroscopy

One potential measuring method for detection and identification of cleaning agents in the CIP process, such as sodium hydroxide and nitric acid, is low frequency nuclear magnetic resonance (NMR). Low-field NMR technology can also give a better understanding of the deposits formed on the inner surfaces of the device. In low-field NMR the charged atomic nuclei of hydrogen 1 and carbon 13 isotopes begin to precede at their characteristic Larmor frequencies. Larmor precision means the rotation of the axis of rotation about an axis parallel to external magnetic field. The technology is based on the relaxation behavior of active nuclei in a magnetic field and it is non-invasive. The frequency of low-field NMR is 20-100 MHz (Fysun et al., 2019) There are no studies on the suitability of NMR Spectroscopy for the whole CIP process, but there are studies showing the suitability for detecting APIs in addition to detergents. In some process's NMR has been used to detect organic compounds in wastewater.

4.1.10 Collo Liquid Fingerprint Technology

Collo Liquid fingerprint inline measurement could be suitable for analyzing and optimizing the CIP process in real time. In its technology, Collo utilizes a radio frequency (RF) resonator that emits a narrowband Electro Magnetic field (EMF) into the liquid. The generated EMF short-range field is directional, and the frequencies used in it are as harmless as the signals used in mobile phones. The measurements are based on an electromagnetic field that vibrates the molecules and the particles of the liquid. Collo also measures the temperature. The liquid fingerprint is measured by Collo Permittivity (CP) and Collo Ion Viscosity (CIV). CP is used

to detect physical changes such as changes in phase and particle size. CIV is more sensitive for chemical changes such as chemical concentrations and impurities. (Collo Whitepaper, 2020) The EMF passing through the liquid stream is presented in Figure 6.

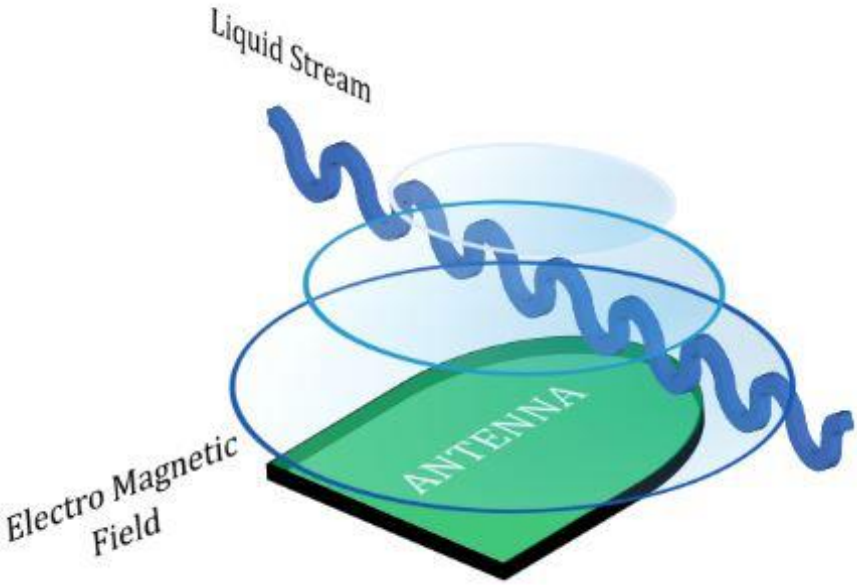

Figure 6 Collo technology Electromagnetic field vibrates molecules of the liquid stream (Collo Whitepaper, 2020)

From the EMF, Collo technology measures the relative permittivity of the dielectric constant. There is two parts of dielectric constant, the real and the imaginary part. The difference between these two is that the real is strongly interlinked with physical properties of substance and the imaginary is strongly interlinked with the dissolved chemical. Sensitivity of the Collo sensor is typically from 0,1 PPM to 1 PPM. Collo technology can detect liquids, solids and chemical impurities. (Collo Whitepaper, 2020) In other industries, Collo technology is used for example real-time water quality controlling, ensuring, and monitoring. (Collo, 2020)

4.1.11 Raman spectroscopy

In addition to infrared spectroscopies, the commonly used vibrational spectroscopy method is Raman. The difference between two of those vibrational method is that Raman measures scatter of electromagnetic radiation instead of adsorption. In pharmaceutical industry,

Raman spectroscopy is used for identification. One important phenomenon in Raman spectroscopy is Rayleigh scattering. In Rayleigh scattering, the particle size is thought to be smaller than the wavelength of the radiation. Raman scattering, in turn, is based on changes in the photons of light energy. When the chemical bonds in a molecule meet the photons of light, the energy of the photon's changes. The photon receives or releases energy in scattering depending on whether the molecule is excited by radiation or returns to its ground state. Figure 7 shows the scheme of Raman and Rayleigh scattering processes. (Fernandes, 2016)

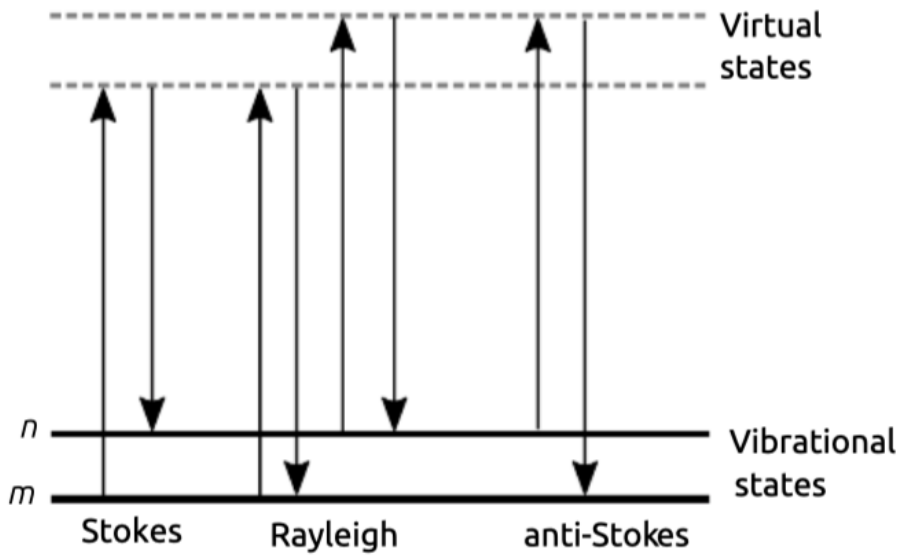

Figure 7 Scheme of Raman and Rayleigh scattering (Fernandes, 2016)

Figure 7 shows the energy changes of different photons situations. Photons whose energy rises from the ground to state *m* are Stokes's scattering, while photons whose energy level falls to the ground state *m* from the higher vibrational state *n* are anti-Stokes scattering. (Fernandes, 2016)

APIs as a cleanable product in CIP process contain conjugated or aromatic domains with strong Raman scattering properties. Since the Raman spectroscopy has very poor scattering property of water, it makes Raman more superior vibrational spectroscopy technology than NIR for monitoring API formulation liquid samples such as wastewater from CIP process. (Alula et al., 2018) The challenge of Raman spectrometry is fluorescence, which interferes with Raman when it is strong. Fluorescence reflects the phenomenon in which molecules of

matter absorb a photon and after a short time, emit a lower energy photon with longer wavelength. However, the substances in the washing process contain relatively few fluorescent compounds, so Raman spectroscopy could be suitable for analyzing and optimizing the CIP process by taking samples.

In addition to the laboratory, there are in-line Raman spectrometers available, that monitor the process in real time, which can be utilized for example to monitor the quality of process water. (Marqmetrix, 2020) In-line Raman could be suitable for analyzing and optimizing the CIP process in real time.

Machine learning is now part of the optimization of many processes and can also be utilized for CIP optimization. It can also be utilized in combined with any of the above methods. Combined with ultrasonic measurement, machine learning has yielded effective results and has been able to predict fouling up to 99% accuracy. (Escrig Escrig et al., 2020)

4.2 Comparison of different methods

Table II shows the applicability of different methods to the CIP process in the pharmaceutical industry. Green indicates the high potential of that method in that region, yellow the weaker potential and red the weakest.

Table II Matrix of analyzing methods potential for CIP process

	Cost	Sensitivity	Potential in CIP	In-line measuring	Commonly used	Usefullness	Detects solids	Detects API's	Detects detergents + excipients	Detects water quality
Conductivity										
TOC										
Turbidity										
pH sensor										
Refractometer										
Density, Viscosity										
NIR										
UV-Vis										
Low-field NMR										
Raman										
Collo										

The Table II can be viewed by comparing different analytical methods and it shows, that Collo, Raman and NIR are the most potential analyzing methods. Lower price analysis methods include conductivity and pH-sensor. More expensive analysis methods are Turbidity, refractometer, density, viscosity and Collo sensors, that could cost tens of thousands of euros. The most expensive methods are NIR, Low-field NMR and Raman spectroscopy where prices for the analyzers for the process can reach very high. Sensitivity in the matrix refers to how small variations can be detected by the analytical method. pH variations may be small, but they are still very rough variations compared to other matrix analysis methods. The best sensitives are obtained with NIR, Raman, low-field NMR and Collo. The potential in CIP process in the matrix describes how well that method of analysis is suitable for the CIP process and how well can it provide more information about the process. The most potential methods for CIP process are NIR, Raman and Collo analyzer. The least versatile information is provided by the pH sensor and refractometer. In-line versions that allow to follow process in real time can be found on the market from every other technology, expect from low field NMR, which has online versions on the market instead of inline versions.

Although various vibrational spectroscopy methods have taken up space in the field of research in recent times, more traditional methods for CIP process monitoring such as conductivity, TOC, turbidity, and pH are still much more commonly used. Usefulness describes the extent to which information can be obtained for the entire CIP process, or whether a particular method of analysis provides information only for a particular cleaning step. For example, in the term of whole CIP process, a lot more information is obtained with a Collo technology than with pH sensor. Since the aim of the work is to detect changes, it is important that the chosen analytical methods also detect solids in the process, APIs, excipients, detergents, as well as the purity of water. The changes can best be detected by using NIR, Raman and Collo.

Based on the matrix and the properties of different technologies, NIR, Raman and Collo could be best suited for real time monitoring of the CIP process. These methods would provide a lot of new useful information about the process. NIR and Raman have been used in many similar applications, such as process water quality controlling. In this work, Raman spectroscopy was chosen from these two, because it has been used more often for the analysis

of liquids and possibility for a suitable laboratory-scale Raman spectroscope was available. In addition, Collo was chosen for experimental parts measurement as it is new and exciting technology.

EXPERIMENTAL PART

Based on the introduction the aim of the experimental part is to find out:

- Are the selected methods suitable for pharmaceutical CIP process in practice?
- What kind of information selected methods can give about CIP process and how it can be utilized?
- What challenges may rise from the selected methods and how they could be avoided?

Based on the literature, the chosen methods, Raman spectroscopy and Collo technology, were used to detect changes in the CIP process. In pharmaceutical industry, concentrations of product residues in cleaning processes can be very small, and therefore, the sensitivity of the methods was also investigated. In experimental part, in addition to the selected methods, pH and conductivity measurements were used to tell the basics about the samples taken from the process. The obtained results are analyzed and finally in the end of the chapter, the selected methods are compared. The Figure 8 shows the contents of the experimental part.

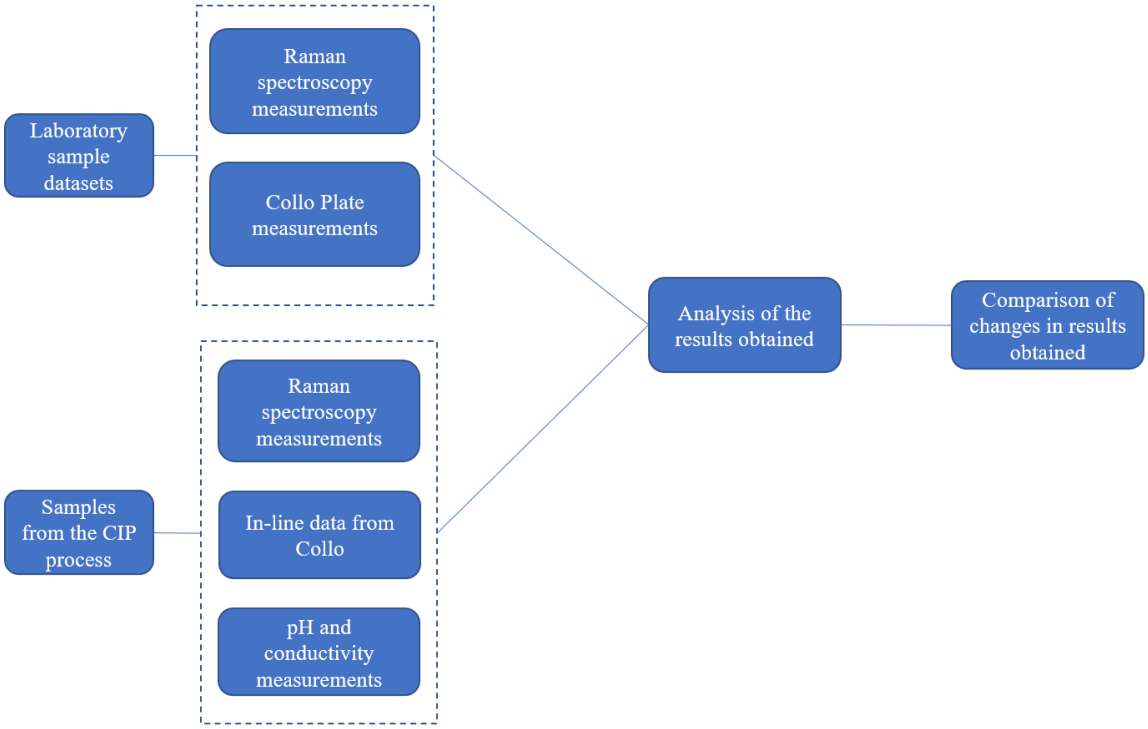

Figure 8 Contents of the experimental part

In this work different analysis methods are used to recognize changes in the CIP-process. The results are expected to be able to present a moment in the rinsing phase where no further change occurs, and the rinsing step has stabilized. If no stabilization occurs, it can be said that the washing steps is too short in duration. The challenge in this are the cleaning routes, where water and detergent solutions flow at certain flowrate to certain routes, making direct stabilization very challenging to see. The suitability of different methods for CIP process is also measured by its ability to detect the product residues, excipients and chemicals used in the process, and the difference between purified and tap water.

5. MATERIALS AND METHODS

The experimental part is performed both by taking samples and analyzing the data obtained from them and with the data provided by an in-line sensor to be attached to the pipeline. The selected measuring methods were Raman spectroscopy and Collo Liquid Fingerprint technology. Raman spectroscopy is performed with Kaiser Raman Rxn 2 Hybrid with wavelength 785 nm and IO-12 probe. Raman measurements are proceeded in laboratory and Collo as an in-line measurement from CIP process. Neither of the selected technologies has been reportedly used in CIP processes in the literature. Regarding Raman spectroscopy, its suitability for monitoring and optimizing CIP processes is studied particularly, as there is no evidence of its use in this type of study. Collo is a new method and has not been used in this type of applications before. Both analyzes are performed for CIP processes in tablet department's product equipment at Orion Corporation's Turku Plant.

5.1 Raman spectroscopy measurement

Raman measurements were performed on two different types of samples. The first datasets were prepared in the laboratory at different concentrations to simulate the prerinsing step of the CIP Process. Other Raman spectroscopic analyzes were performed on samples taken from the CIP process.

5.1.1 Preparation of samples

Simulated cleaning solutions were prepared for the three selected products in laboratory. Two samples were made for each of the three. The first samples included API and excipients based on the composition of the product. The second samples included only API, however containing the same proportion of API as the first ones. The purpose of the samples was to find out how well the Raman spectrometer can detect the spectrum at different concentrations. The solution was prepared to correspond to the prerinsing step of tablet department's product equipment CIP cleaning according to the washing recipe. Solutions for 0,5, 1, 2 and 4 mg/ml were prepared for each product and for the API solution alone. Altogether there were 4 samples at different concentrations, Table III lists datasets.

Table III Dataset's name, API and excipients of the simulated cleaning solutions

Dataset	API	Excipients	0,5 % API mg/ml	1 % API mg/ml	2 % API mg/ml	4 % API mg/ml
1	A	Yes	0,375	0,734	1,493	2,985
2	A	No	0,375	0,734	1,493	2,985
3	B	Yes	0,109	0,218	0,437	0,873
4	B	No	0,109	0,218	0,437	0,873
5	C	Yes	0,357	0,714	1,429	2,857
6	C	No	0,357	0,714	1,429	2,857

5.1.2 Sampling from the process

Samples were taken from the process using the sampling valve shown in Figure 11. A specific time point for taking the samples had been calculated in advance, as samples were desired form a particular CIP process cleaning route. Samples were taken from the cleaning processes of tablet department's product equipment from two different products using different cleaning recipes. In the cleaning process following the first recipe, the active

ingredient of the product to be washed was API B. In the second cleaning process using the second recipe, the active ingredient of the product to be washed was API C. The first cleaning recipe had several recirculation steps, so samples were obtained from only two rinses. In the cleaning process with second recipe, samples were obtained from four different cleaning steps (prerinse, rinse after alkaline wash, rinse after acid wash and final rinse). All samples were taken from the same cleaning route, the first being taken immediately at the beginning of the route and the second at the end of the route. Table IV shows the lists of samples from the cleaning process.

Table IV List of samples from the tablet department's product equipment CIP process

Sample	API in product to be cleaned	Cleaning step	Sampling time from route	Seconds after route valve opens
1	B	Prerinse	Beginning	35
2	B	Prerinse	End	95
3	B	Postwash-Rinse	Beginning	35
4	B	Postwash-Rinse	End	95
5	C	Prerinse	Beginning round 1	30
6	C	Prerinse	End round 1	90
7	C	Prerinse	Beginning round 2	30
8	C	Prerinse	End round 2	90
9	C	Postwash-Rinse	Beginning round 1	30
10	C	Postwash-Rinse	End round 1	90
11	C	Postwash-Rinse	Beginning round 2	-
12	C	Rinse	Beginning	30
13	C	Rinse	End	90
14	C	PW Rinse	Beginning	30
15	C	PW Rinse	End	90

5.1.3 Kaiser Raman

Laboratory measurements for measuring datasets were made with Raman spectrometer in Fermion Oy's Espoo plant.

Figure 9 presents the Raman spectrometer used in laboratory measurements.

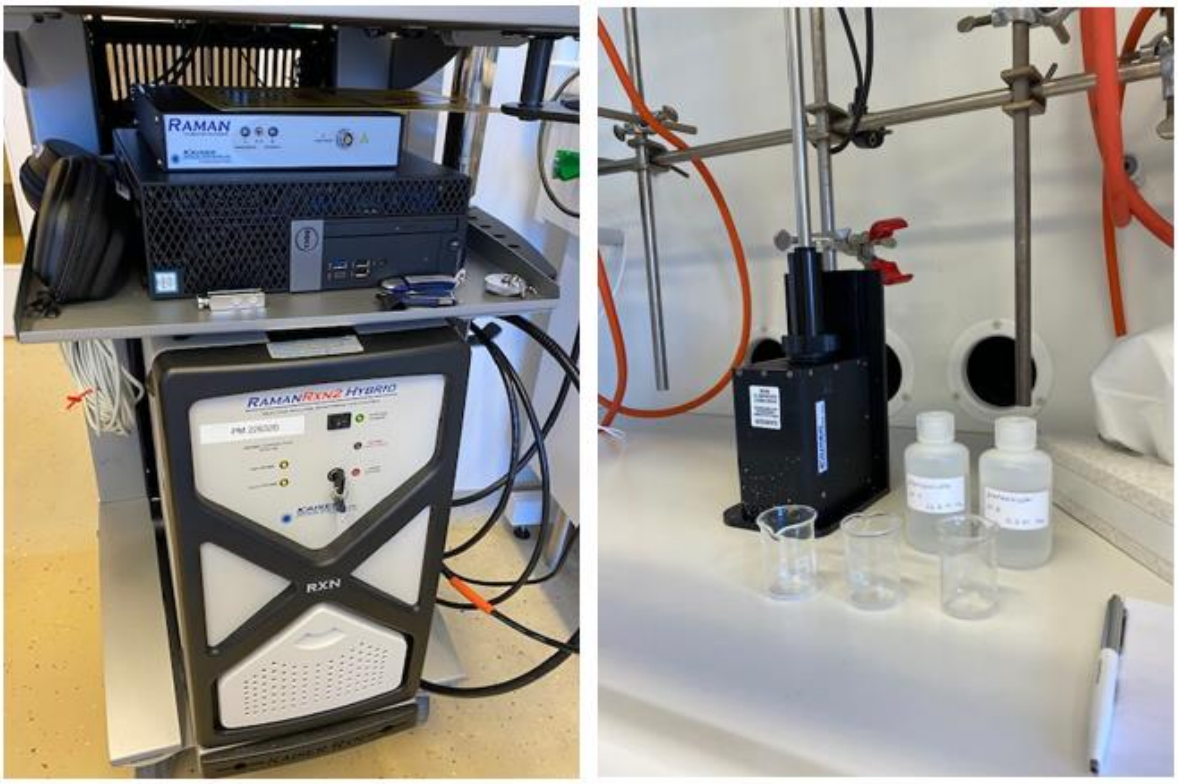

Figure 9 Left picture is Kaiser Raman Rxn 2 Hybrid which is used in measurements. The picture on the right is from the used sample platform to which the sensor comes from the Raman device.

The Raman spectrometer used for the measurements is Kaiser Raman Rxn 2 Hybrid and the used probe was IO-12 probe. The laser used in measurements was Invictus Laser with power of 400 mW and wavelength 785 nm. The measurement parameters were optimized to fit the sample by exposing the sample to a laser for 30 seconds with accumulations 2. Pixel fill aimed at 50 %. The measurements were protected from light on a sample substrate, because the Raman spectra are disturbed by light coming outside.

A small amount of sample was poured into a beaker glass. The beaker glass was placed on a sample tray and the sensor was placed in the center of the sample solution. The sample of the lowest concentration of each dataset always measured first, and the sample of the highest concentration was measured last. The same was repeated for all 24 laboratory samples. For the second sample to be measured, several measurements were taken by turning the beaker glass slightly between measurements. But since the solution was completely homogenous and obtained spectra were similar to each other, it was decided that one measurement was always sufficient for one sample.

Kaiser Raman measurements for process samples were implemented with the same parameters as laboratory samples. One sample, where there was a lot of solid, the exposing time was 10 seconds with 6 accumulations. Process samples were in glass flask, and measurements were performed directly from the flasks. The flask was wrapped in foil for the duration of measurement and three measurements were made on each sample.

5.1.4 PLS model for the data sets

Partial least squares (PLS) models (Li et al., 2001) were performed on the datasets using MATLAB. The PLS regression shows the important changes in response variable, which in that case is concentration. For laboratory samples, there were only four response variables for the datasets, with concentrations 0.5, 1, 2 and 4, which is little for a good PLS model. However, PLS models were made for experiment and learning and therefore the small amount of response variables could be ignored. In PLS models, spectrums are an explanatory variable. All the datasets have been centrally corrected, centered, and scaled in MATLAB before the PLS Regression and the latent variable in all datasets is 1.

5.1.5 Collo Plate In-line analyzer

Collo liquid fingerprint measures were done with Collo Plate analyzer.
Figure 10 shows the Collo plate analyzer and a computer, which transmits real-time measurements data. Analyzer and the computer are connected to each other’s with a 10-meter-long cable.

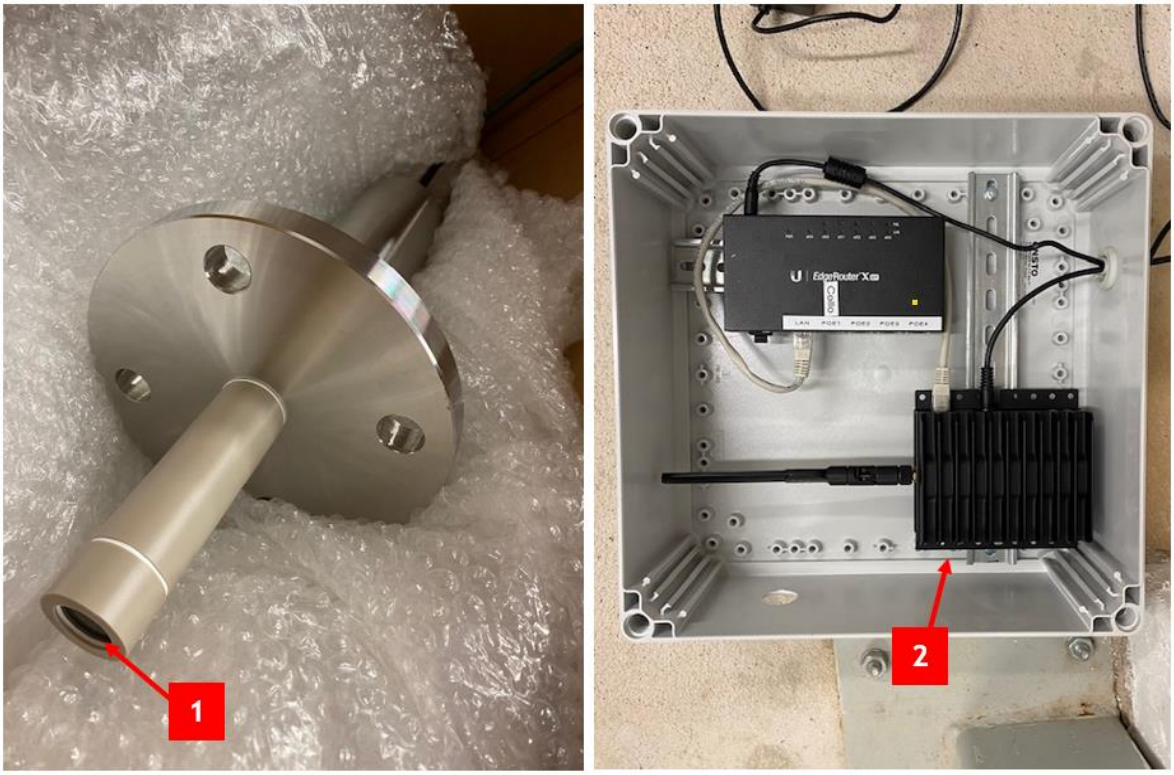

Figure 10 Collo Plate analyzer and computer for transmitting real-time measurement data. The point 1 indicated by the arrow is the sensor that measures the fingerprint of the fluid. The point 2 is the computer which transmits real-time measurement data.

In Figure 10, point 1 indicates the sensor that measures the fingerprint of the fluid. Point 2 is the computer which transmits real-time measurement data. Collo Plate in-line analyzer is installed into the outlet pipe of the tablet department's product equipment CIP process at the Orion Corporation's Turku Plant. The outlet pipe leads to the change valve, from where the wastewater containing API goes to the collection and other wastewater to the drain. The pipe is bent so that the head of the Collo Plate sensor is surrounded by liquid during CIP process. Figure 11 shows the sampling valve for taking samples for Raman measurements and Collo plate sensor in the pipeline.

Figure 11 Sampling valve (1) and Collo Plate analyzer (2) in the pipeline.

The Collo Plate analyzer is active all the time. From the data measured by the sensor, it is possible to see when the pipe is empty and when the cleaning process and its various steps are in progress. Analyzer measures the liquid in every 2 seconds. The computer (shown in Figure 10) transmits real-time measurement data to the cloud, from which it is sought for analysis.

To measure Collo fingerprints of the samples prepared in the laboratory, the Collo Plate Analyzer was removed from the tube and the measurements were made using a grapple and tripod. The samples were poured into a beaker and the Collo Plate sensor was placed from the side so that no air bubbles could be formed to the sensor. For each sample, the sensor was held still for one minute. The beaker and sensor were rinsed with water and dried thoroughly in between the samples.

5.1.6 Methods used for in-line process data

Since the Collo Plate sensor is sensitive, the air bubbles in the process stream can also cause noise to the graphs. For each point in the in-line data, a median has been calculated by applying three points before and three points after to reduce the noise.

In addition, a dashed line can be drawn in the figures of the data from the in-line measurements to illustrate the difference between the obtained measurement data and the calculated level of the cleaning solution (and tap water in step 7) at the same temperature. The level has been calculated using the measured values obtained from Collo for detergent solutions and water by calculating the slope. Slope *x* is calculated with Equation (1).

$$x = \frac{CP_1 - CP_0}{T_1 - T_0} \tag{1}$$

where:

T_1	Upper limit temperature of the reference range
CP_1	CP value at temperature T_1
T_0	Lower limit temperature of the reference range
CP_0	CP value at temperature T_0

The slope *x* can be used to calculate the theoretical level for a given detergent solution CP_i (and tap water) at the temperature measured at each step with Equation (2).

$$CP_i = CP_0 + x * (T_i - T_0) \tag{2}$$

where:

T_i	reference temperature to be considered
CP_i	CP value at temperature T_i

5.2 Conductivity and pH measurements

Conductivity and pH measurements were made on process samples to get more information from the CIP process. pH measures were done with Mettler Toledo SevenExcellence S400 pH-meter. Before the measurements, calibration was made with three buffer solutions in pH 4, 7 and 10.01. The electrode used in measurements was Viscous Pro-ISM. Between the samples, the electrode was rinsed with purified water between samples. Conductivity measures were performed with Mettler Toledo Checkmate conductivity sensor.

6. RESULTS AND DISCUSSION

This chapter discusses the results obtained from Raman spectroscopy, pH, conductivity, and Collo Plate analyzer measurements. The suitability of Raman and Collo for the pharmaceutical CIP process was investigated by measurements. The aim was to find out whether changes in products, detergents and water concentrations can be seen from CIP process rinses. The methods were also challenged even at very low concentrations. The goal of the conductivity and pH measurements was to obtain more information about the cleaning process to support the measurements obtained with Raman and Collo Plate sensor. Results from measurements have been presented in previously mentioned order.

6.1 Results from Raman measurements

This section presents data obtained from Kaiser Raman Rxn 2 Hybrid with IO-12 probe measurements. First are the results from laboratory samples, then the results from the process samples. In all figures based on the data obtained from Raman, the spectrums have peaks of ranges of 378, 418, 578 and 750 cm^{-1}. These peaks are caused by the IO-12 probes high-purity sapphire window (Kaiser optical systems, 2021), and therefore they can be unnoticed.

6.1.1 Laboratory samples Raman spectrums and PLS regressions

Results from Raman measurements are plotted with MATLAB and Excel. For PLS regression plots, PLS model have been created for the datasets. Figure 12 shows the Raman spectrums (orange) and PLS regression (blue) of dataset 1.

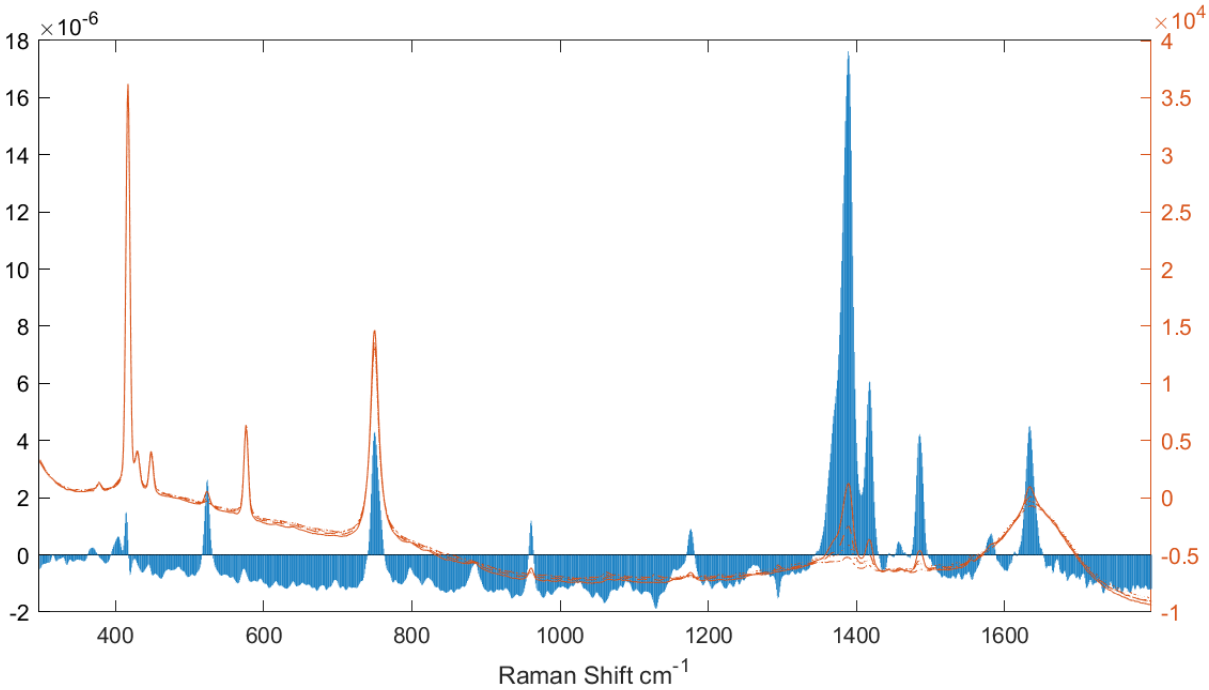

Figure 12 Raman spectrums (orange) and PLS regression (blue) of dataset 1 with API A and excipients.

All the spectra of the samples at different concentrations of the dataset 1 are superimposed in orange the Figure 12. The PLS regression shows that the important changes in concentration variations are at 524, 751, 961, 1389, 1418, 1484 and 1636 cm^{-1}. The orange spectral plots show the clearest changes at different concentrations at the point 1418 cm^{-1} where there is also the largest change according the PLS regression plot. In that point is also the largest peak of product API. The change at 751 cm^{-1} might have gone unnoticed, as one of the background peaks is at the same point. However, the clear change shown by the PLS regression showed that the API concentration of dataset 1 also changes at that point.

Figure 13 shows the Raman spectra and PLS regression for same API but without excipients from the dataset 2.

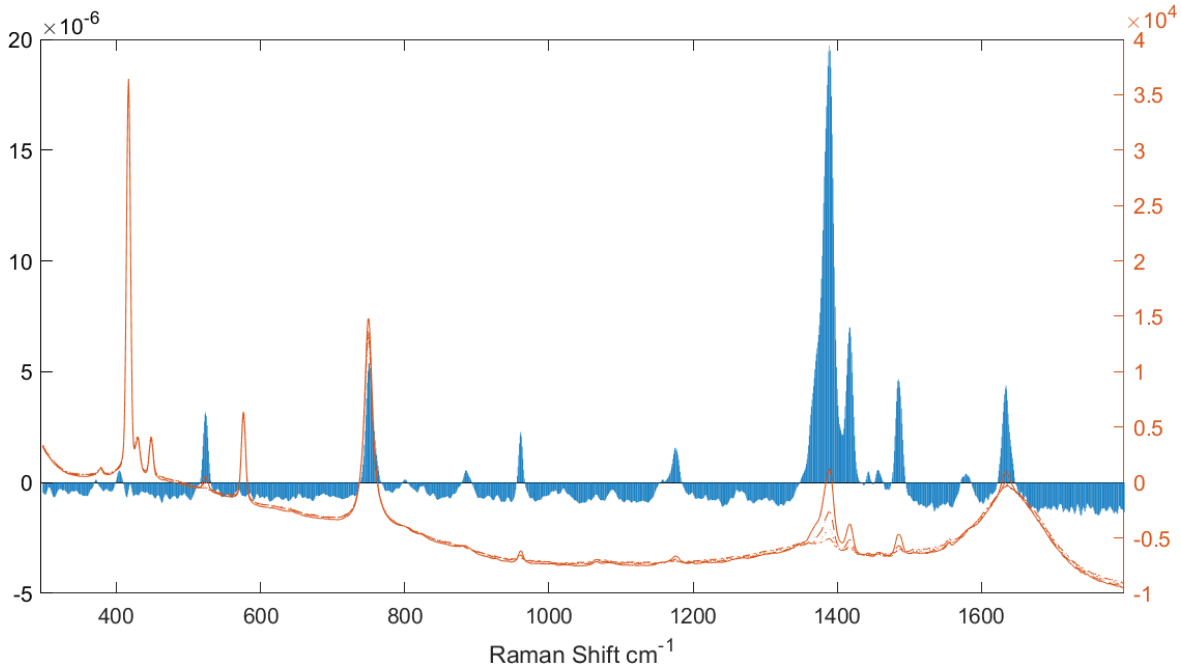

Figure 13 Raman spectrums (orange) and PLS regression (blue) of dataset 2 with API A and without excipients

The spectra and PLS regression of Figure 13 correspond to those of Figure 12, although in dataset 2, there was no excipients. The excipients have good Raman scattering, and the reason why they do not appear in the spectral images is that the device is not tuned to such a large wavelength range. The settings of the device were set to measure only up to certain wavenumber range. There would probably have been differences between datasets 1 and 2 in the wavenumber ranges of 2750-3000 cm-1 as the excipients contained in dataset 1 have peaks in those wavenumbers. The excipient content of this product was also only about 25%, so that the API peaks stand out more clearly.

Figure 14 shows the Raman spectrums and PLS regression of dataset 3.

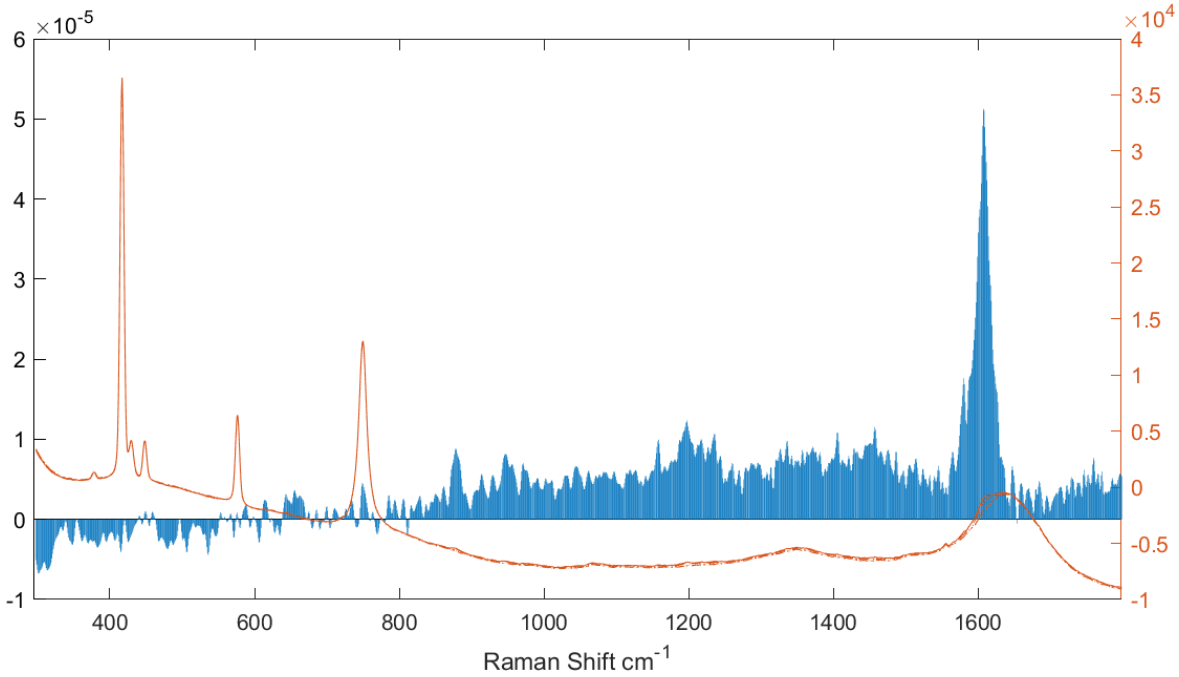

Figure 14 Raman spectrums (orange) and PLS regression (blue) of dataset 3 with API B and excipients

Dataset 3 have significantly lower concentrations and changes in concentrations than other datasets, but PLS regression at least partially distinguishes the API peaks clear. In Figure 14 the only significant peak at which the concentration changes are at wavenumber 1610 cm^{-1}. Rest of the PLS regression peaks under 1 might be the peaks from excipients, because in this product, excipients have a lot of small peaks on area 900-1500 cm^{-1}. Concentrations in this sample were very low, at a minimum of about 10 mg API per 100 ml of liquid. But compared to previous product, this product has much higher excipient content, 78%. In this spectrum, API peaks should appear not only at 1610 cm-1 but also at 1670 cm^{-1}. (Spectrabase, 2021) Possibly at higher concentrations, a clearer peak would have begun to form at wavenumber 1670 cm^{-1}.

Figure 15 shows the Raman spectrums and PLS regression of dataset 4.

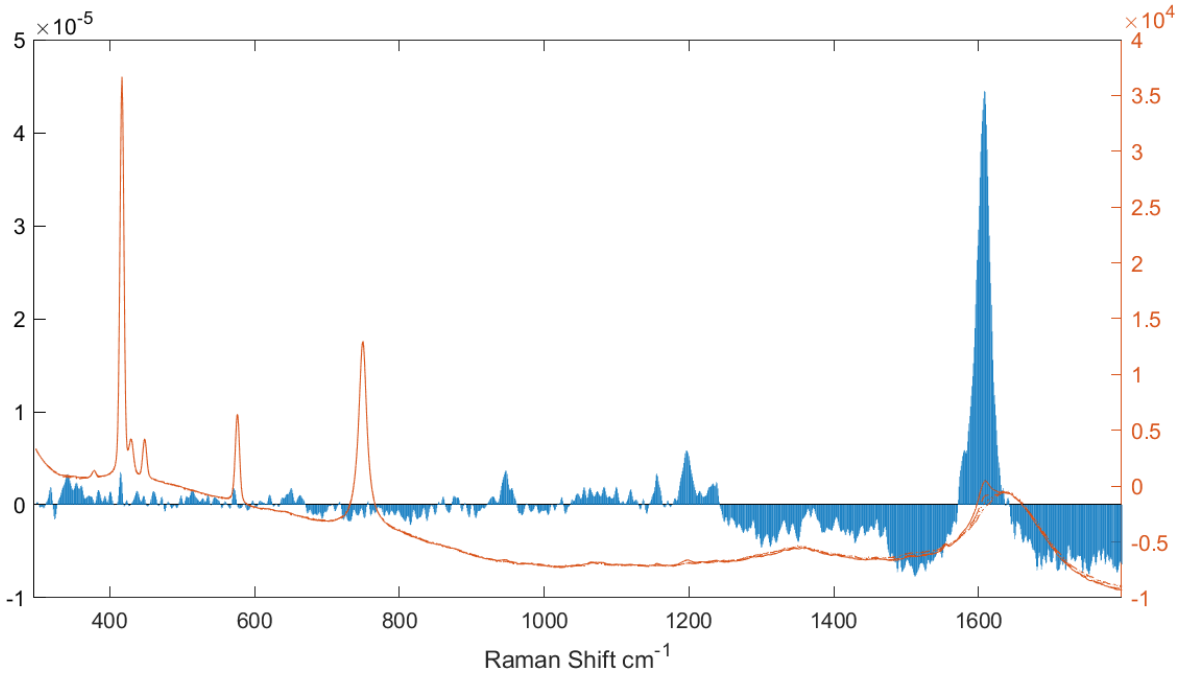

Figure 15 Raman spectrums (orange) and PLS regression (blue) of dataset 4 with API B and without excipients

The spectrums of Figure 15 correspond to those in Figure 14, although in dataset 4, there were no excipients. The lacking in excipients can be seen when comparing PLS regression of both figures. In excipient area 900-1500 cm^{-1} in Figure 15 there are not as much small peaks, as in Figure 14, because excipient peaks are usually shown in that area. Figure 16 and Figure 17 shows the Raman spectrums and PLS regression of dataset 5 & 6.

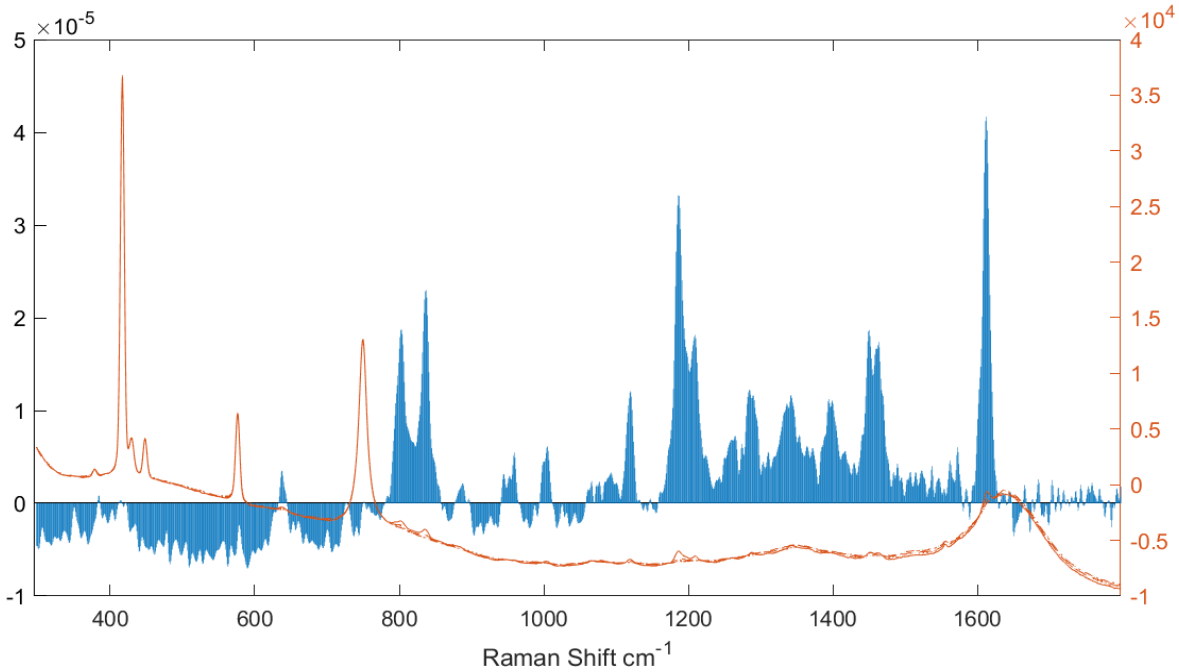

Figure 16 Raman spectrums (orange) and PLS regression (blue) of dataset 5 with API C and excipients

The PLS regression shows that the important changes in concentration variations are at 639, 802, 835, 1186, 1210, 1451 and 1612 cm^{-1}. Those peaks are products API peaks. In this product, small excipient peaks are also at 900-1500 cm^{-1}, although the clearest excipient peaks are at 1220-1500 cm^{-1}.

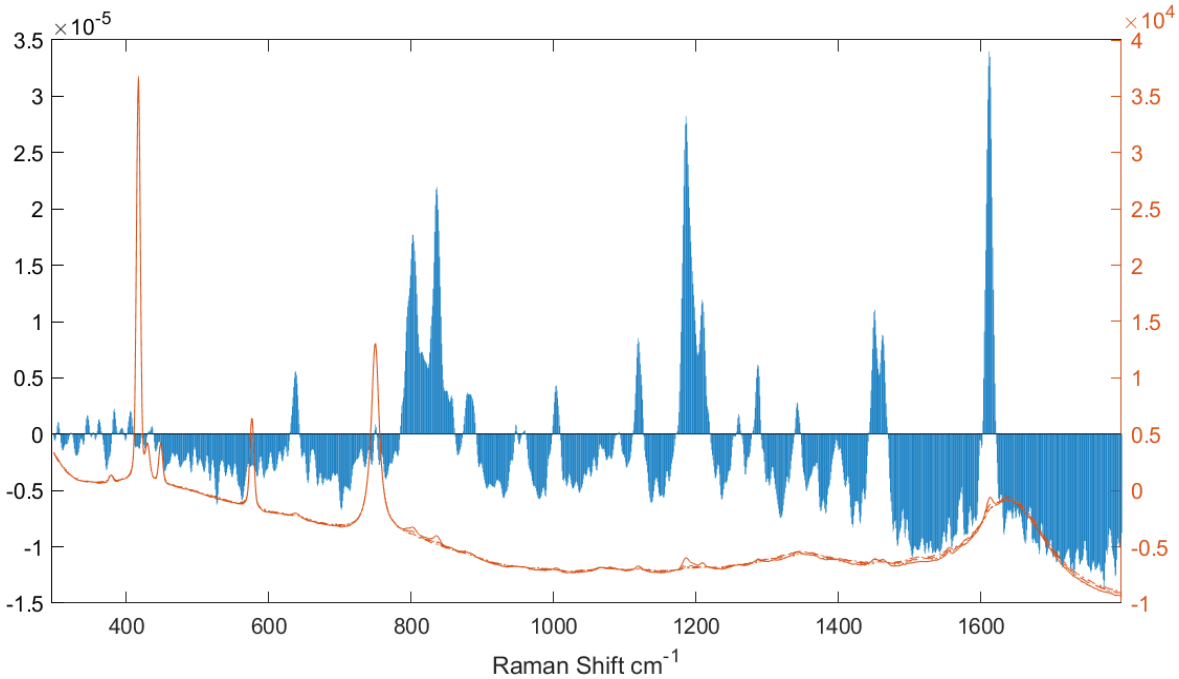

Figure 17 Raman spectrums (orange) and PLS regression (blue) of dataset 6 with API C and without excipients

As noticed earlier, when comparing datasets 3 and 4 in Figure 14 and 15, the same phenomenon can be seen here in comparing datasets 5 and 6 in Figure 16 and Figure 17. The spectrums are similar to each other, but the difference between with and without excipients can be seen in PLS regressions. The clearest difference in excipient peaks in Figure 16 and Figure 17 is at the area of 1220-1500 cm^{-1}.

Comparing all the figures obtained from the spectra, it can be noted, that the detergent peaks cannot be seen. This is because the peaks of the NaOH-based detergent solution should appear in the wavelength range above 3500 cm^{-1}. From all of this, it can be concluded, that from the point of view of the CIP process, changes in the detergent could be monitored by Raman spectroscopy, if the different wavelength range is used. Also, the variations at API concentration can be detected even at a relatively low concentration, so Raman spectroscopy can be used to obtain accurate information about the CIP process prerinse. Although the amount of data was small, the PLS models were good and reliable.

6.1.2 Process samples Raman spectrums

For process Samples 1-15 (Table IV), the Raman measurement were done three times for each sample to get reliable information of the spectrum. The spectrums of the samples are done with Excel from the data obtained from Kaiser Raman. The results are presented according to the order of the cleaning steps in the CIP process. At first, is presented results from samples from cleaning process with first cleaning recipe. After that one can find results from samples from cleaning process with second recipe. The Samples 1-4 are from the CIP process after product containing API B. Figure 18 shows the Raman spectrums of Samples 1 and 2 from prerinse.

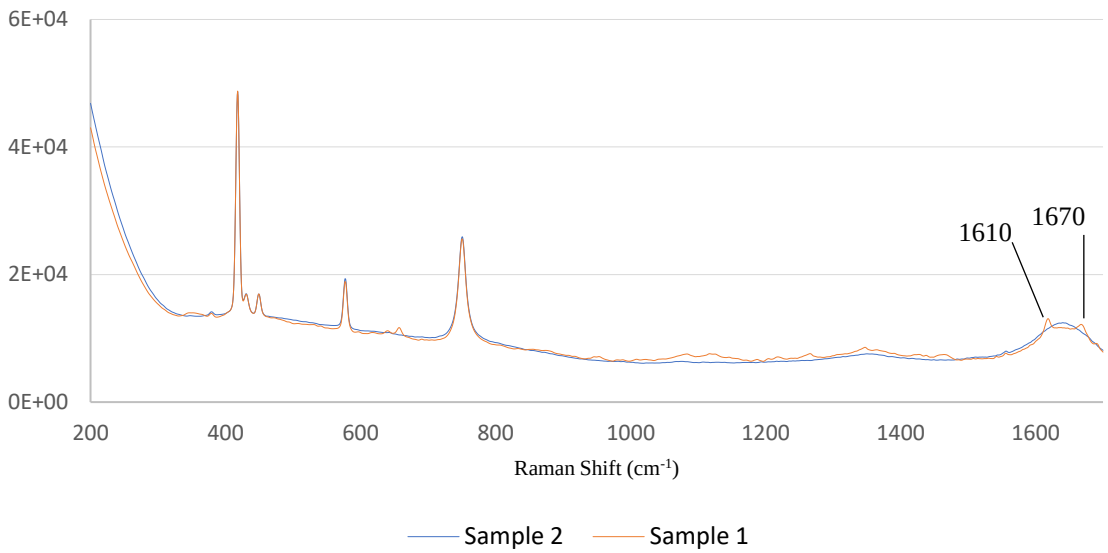

Figure 18 Raman spectrums of Sample 1 with API B from the beginning of prerinse and Sample 2 with API B from the end of prerinse.

Figure 18 shows, that biggest changes in spectra are in the same areas as in Figure 14 (*Raman spectrums and PLS regression of dataset 3 with API B and excipients*) at range of 1610 cm^{-1} and 1670 cm^{-1}. In this product, there are lot of excipients relative to the number of API, which is why the peaks in the range of 1100-1500 cm^{-1} are so clear. In the beginning of the first round of prerinse (Sample 1), most of the product residues are removed. At the end of the prerinsing (Sample 2), it is no longer possible to visually distinguish the corresponding points in spectrum. Figure 18 shows, that the prerinse quickly flushes out the largest product

residues in the CIP process. When Sample 1 was taken, the process sample contained foam. Additional foaming was not observed in the other samples of this CIP process. From this it could be concluded that one of the excipients or API causes foaming when reacting with the detergent.

Spectrums of sample 3, taken from the beginning of rinse after alkaline wash, and Sample 4, from the end of the same rinse, look the similar to the spectrums of sample 2, so they are not shown in the figure. However, the spectrum of Sample 3 is shown together with the spectrum of purified water (PW). Spectrums of Sample 3 and PW are shown in Figure 19.

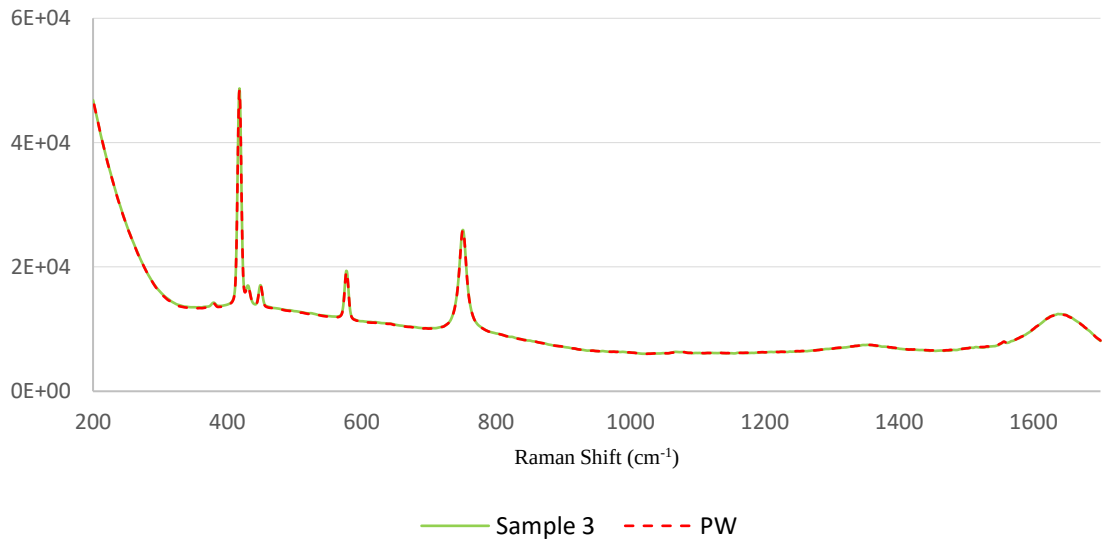

Figure 19 Raman spectrums of PW and Sample 3 from the beginning of the rinse after alkaline wash of CIP process after product with API B.

Figure 19 shows that difference between purified water and tap water rinse after strongly alkaline wash cannot be detected with Raman, at least in this wavelength range. Pure water has most intense feature at 3410 cm^{-1} (StellarNet, 2019) so it was quite expected, that changes in water would not be visible in this area. Aqueous sodium hydroxide, which is the basis of the alkaline detergent also has most intense feature at 3600 cm^{-1} (Stefanski et al., 2018). In CIP process, that means, that only changes in prerinse can be monitored with Raman in this wavelength range.

The following Figure 20 and Figure 21 show the spectral graphs of the samples taken from the CIP process after the production of the product containing API C. In Figure 20 are presented spectrums of the samples from the prerinse. In Sample 5, from the beginning of the first round of prerinse, almost half of the 200 ml glass bottle sample were solid. Consequently, the sample was mixed thoroughly before measurements. Sample 6, at the end of the first round of prerinse, also contained solids, but significantly less than the sample taken at the beginning of the same round. Both Samples 5 and 6 from first round of prerinse foamed when samples were taken. In Samples 7 and 8 from the second round of prerinse there was neither solids nor foam.

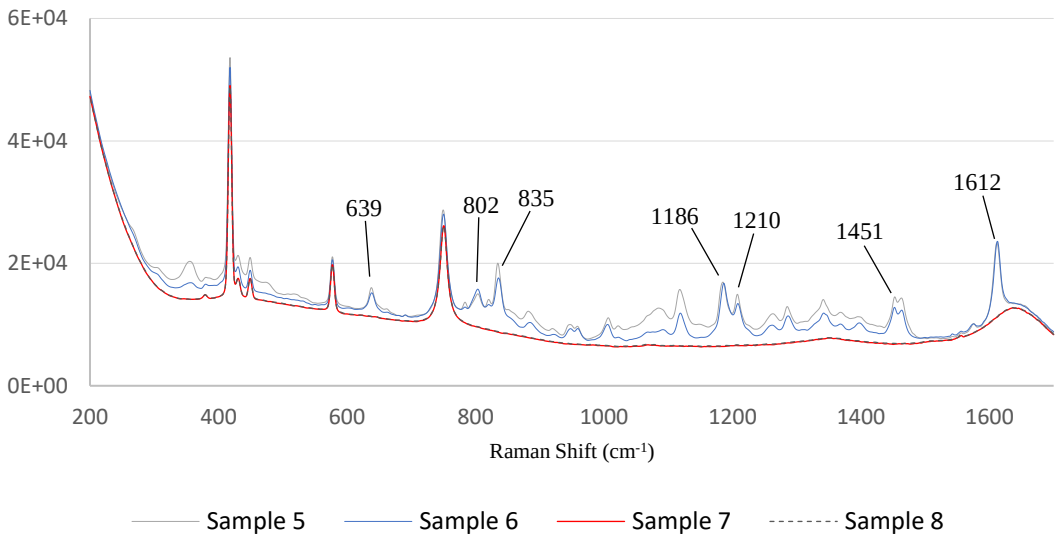

Figure 20 Raman spectrums of prerinse after production of product with API C. Samples 5, 6 are from first round of prerinse (beginning and the end) and Samples 7 and 8 from the second round of prerinse.

Figure 20 shows, that after the first round of prerinse after product containing API C, the spectrums do not have any remarkable peaks, so excipients and API are removed. The marked bumps in Figure 20 are the peaks of API C and the other changes in the spectrums of Samples 5 and 6 are peaks of the excipients. In the beginning of prerinse first round (Sample 5), the concentration is higher than in the end of first round (Sample 6). In the second round of the prerinse, there is no more visually perceptible excipients or API's left and the spectrums of Samples 7 and 8 looks the same than PW spectra in Figure 19.

In Figure 21, is presented spectrums of samples 9, 10 and 11, which are from rinse after alkaline wash, Samples 12 and13 from rinse after acid wash and Samples 14 and 15 from PW rinse. All those samples contained no solids or foam.

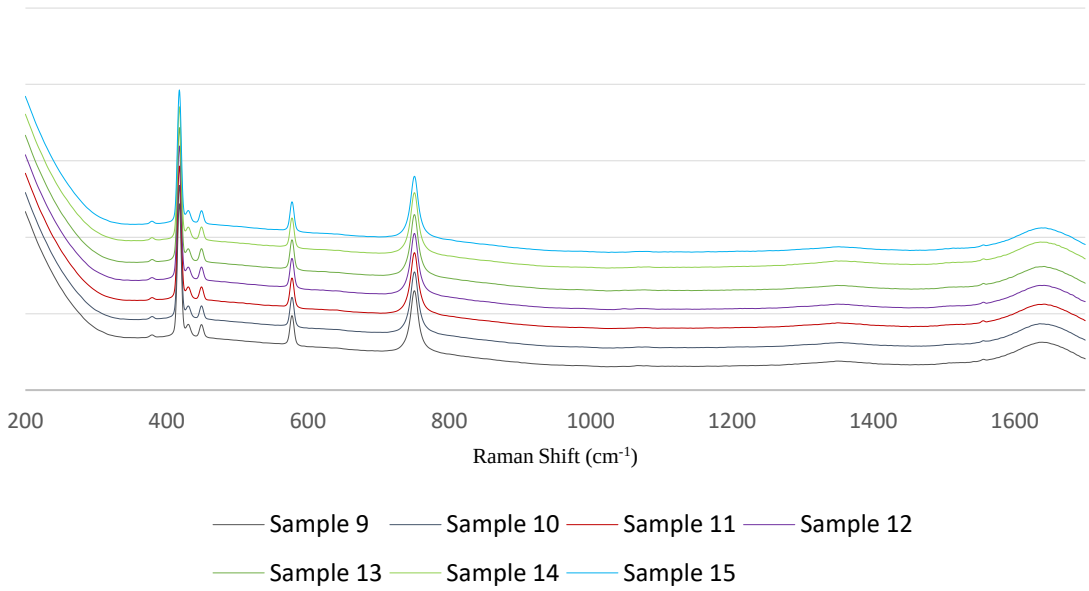

Figure 21 Raman spectrums of Samples 9, 10, 11 from tap water rinse after alkaline wash. Samples 9 and 10 are from first round (beginning and end) and Sample 11 from the beginning of second round. Samples 12 is from beginning of tap rinse after acid wash and sample 13 from the end of it. Samples 14 and 15 are from purified water rinse, beginning and end.

In Figure 21, the spectrums have been moved to different locations so that every spectrum is visible as they were completely on top of each other. Figure 21 shows, that even though the Samples 9,10 and 11 are tap water rinses after the caustic wash and Samples 12 and 13 are the tap water rinse after acidic wash, they all look similar to Samples 14 and 15 which are from rinse with PW. So at least in this wavelength range, samples of alkaline, acid, or water of different purities cannot be distinguished by Raman, so it appears not to be a suitable method to monitor the entire CIP process.

6.2 Process samples pH and conductivity measurements

In addition to the Raman measurement presented in the previous section, conductivity and pH measurements were performed on samples taken from the process. The results from the pH and conductivity measurements are shown in Table V. Table V shows the different acidic, strongly alkaline, mildly alkaline, and neutral measurement results at different colors.

Table V Results from process samples pH and conductivity measurements

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Conduc-tivity µS/cm,	637	666	319	155	886	-	-	-	788	244	174	169	158	30	3,6
pH	7,2	10,2	10,6	8,9	7,1	7,3	12,7	12,7	11,3	10,3	9,3	5,8	7,4	7,3	7,1

It can be seen from Table V that Samples 1, 5 and 6, taken from the first round of the prerinse of the CIP process, have significantly more neutral pH than Samples 2, 7 and 8 taken from the second round of the prerinse.

In Samples 1 and 2, which are taken after product containing API B, the detergent solution consist of water and an enhancer with pH of 12,9. Prerinse detergent solution after product with API C (Samples 5, 6, 7 and 8), consist of water, alkaline detergent with pH 14 and enhancer with pH 12,9. Since the pH of the prerinse after product with ABI B is initially lower than the one, after product with API C, it is clear, that even in the second round of the prerinse Sample 2 pH is not as high as in Samples 7 and 8.

It seems that in the first round of prerinse most of the active pharmaceutical ingredients and excipients, used in the manufacturing of the product, neutralize the pH of detergent solution but are also rapidly eliminated. This same thing can be seen from Figure 20, where according to the Raman spectrum, the second round of prerinsing appears to no longer have product and excipient residues. The conductivity meter of the strongly alkaline process sample could not be measured.

Comparing the pH and conductivity of Samples 3 and 4 with the corresponding rinse step after alkaline washing of the second product (Samples 9, 10 and 11), it can be observed that in Samples 3 and 4 the alkaline detergent has been washed away more efficiently. This can be seen especially when comparing the measurement results from Samples 4 and 11, from the end of the rinses, to tap water conductivity in Turku, which is 140-150 µS/cm (Turun vesihuolto, 2020). Also, pH value in Sample 11 is still higher than in Sample 4. This may be due, for example, to the fact that in the latter wash, an alkaline detergent is already used in the prerinse, while in the prerinse of the first only enchanter is used. For this reason, there are more alkaline detergent residues in samples 9, 10 and 11. The removal of the detergents from the process is also seen in the Samples 12 and 13, rinsing after acid washing, where the conductivity in Sample 13 is quite close the conductivity of water.

Even though the pH is same in Samples 5 and 15, it can be seen from conductivity, that the Sample 15 is purified water and Sample 5 contains a lot of product residues. In all samples, conductivities decrease as assumed, as cleaning progresses. The result of pH and conductivity measures shows that the final rinse of CIP process is effective, and the outgoing water is clean.

6.3 Results from Collo Plate analyzer

Collo Plate analyzer measures the effluent flowing in the process in real time. The results presented in this paragraph have been obtained in two ways. Major of the measurements were done as an in-line measuring, but for the measuring of datasets 5 & 6 prepared in the laboratory (Table V), the sensor was removed from the piping and measurement was done manually.

Collo Plate analyzer measures the fluid every 1-3 seconds. From one CIP process, the amount of collected data is large. In-line data was accumulated in the cloud, creating a new file for each date, If the CIP process continued overnight, the data of the entire wash was retrieved from several files.

6.3.1 Results from laboratory samples

In Figures 22-25 and 27, x-axis shows the Collo Permittivity (CP) and y-axis Collo Ion Viscosity (CIV). The datasets in Figure 22 and Figure 23 have been measured by holding the Collo plate analyzer in sample for one minute. From the data imported from the cloud, the period of one minute corresponding to each measurement moment has been searched for, from which the values have been extracted.

Fingerprints of samples from the datasets 5 and 6, PW, W and air measured by using Collo Plate analyzer, are presented in Figure 22.

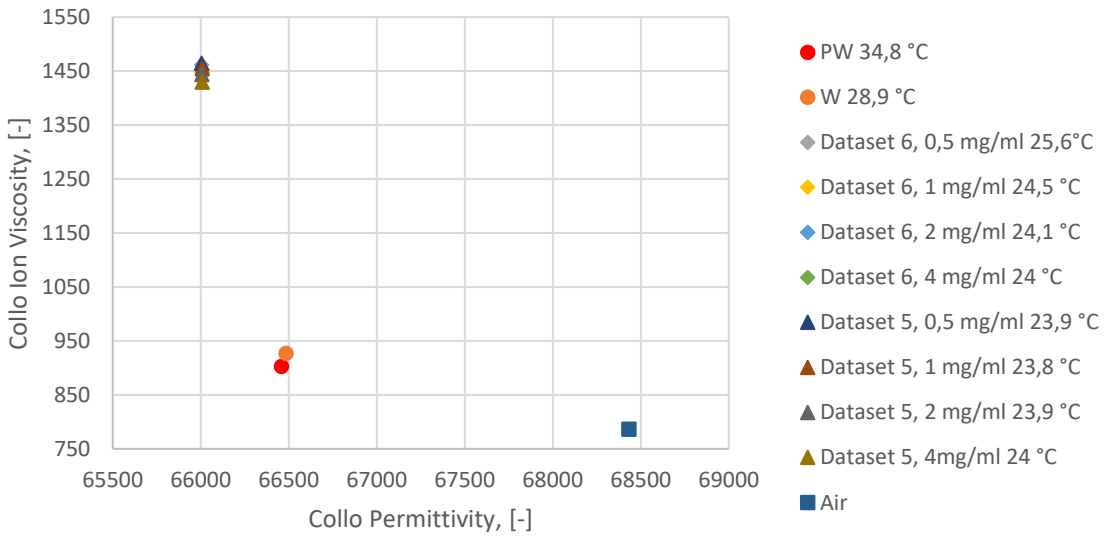

Figure 22 Collo fingerprints of the PW and W, air, and samples from datasets 5 with API C and excipients and 6 with API C without excipients.

In Figure 22, Collo fingerprints of PW and W are shown red and orange dots. Collo fingerprint of air is shown in dark blue square marker, the fingerprint value of air has been measured by ColloidTek Oy. The fingerprints of the measured datasets 5 and 6 are in totally different area compared to water samples. The air fingerprint is at a significantly different area compared to the fingerprints of liquids. Figure 23 and Figure 24 shows the fingerprint of dataset samples and water samples more closely.

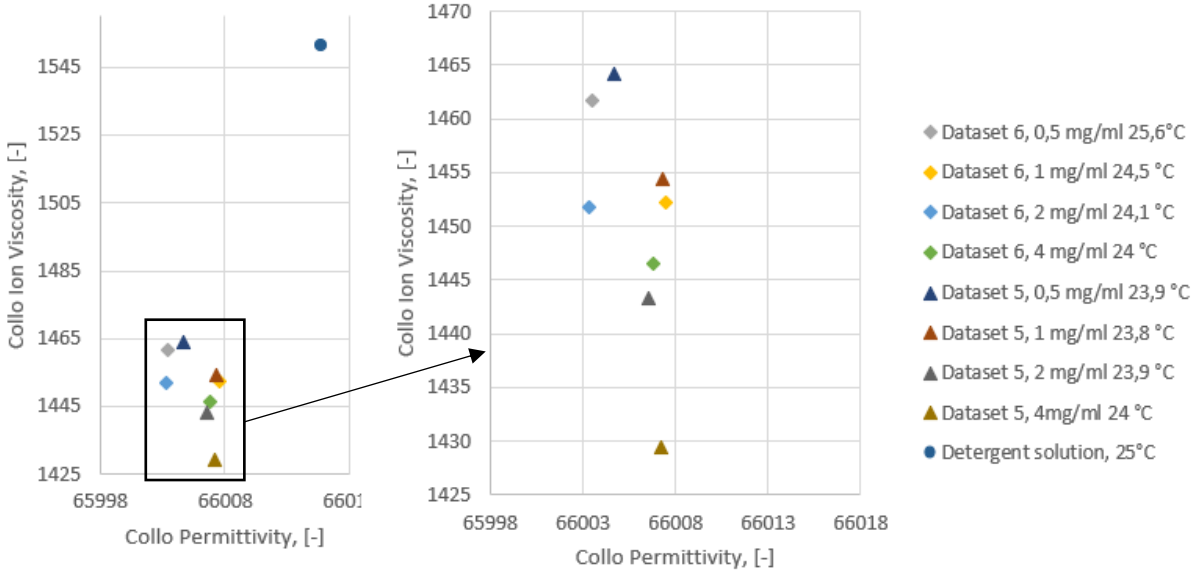

Figure 23 Collo fingerprints of the samples from datasets 5 with API C and excipients and 6 with API C without excipients. Blue dot in figure is the fingerprint of detergent solution of prerinse in 25 degrees, measured by ColloidTek Oy.

In Figure 23, blue dot shape marker is the fingerprint of the detergent solution in prerinsing that has been measured by ColloidTek Oy. It can be seen from the figure, that the lower the concentration in the sample is, the closer it rises in CIV values to the level of the detergent solution. Correspondingly, the higher the concentration in the sample, the farther from the detergent the concentration is. This might be due to the phenomenon observed in the pH measurements conducted earlier, that the product residues neutralize the detergent solution.

Figure 23 also shows that dataset with excipients (Dataset 5) are further from the level of detergent solution than the samples without excipients (Dataset 6). In addition, the dataset with excipients is further from each other, compared to the dataset without excipients. However, overall, the fingerprint area is small between all the samples, although the differences in concentration are quite large. Therefore, it might be challenging to observe the changes in the removal of product residues from the wide in-line data of one initial cleaning step of the CIP process. Figure 24 shows the Collo fingerprints of water in different purity and temperature.

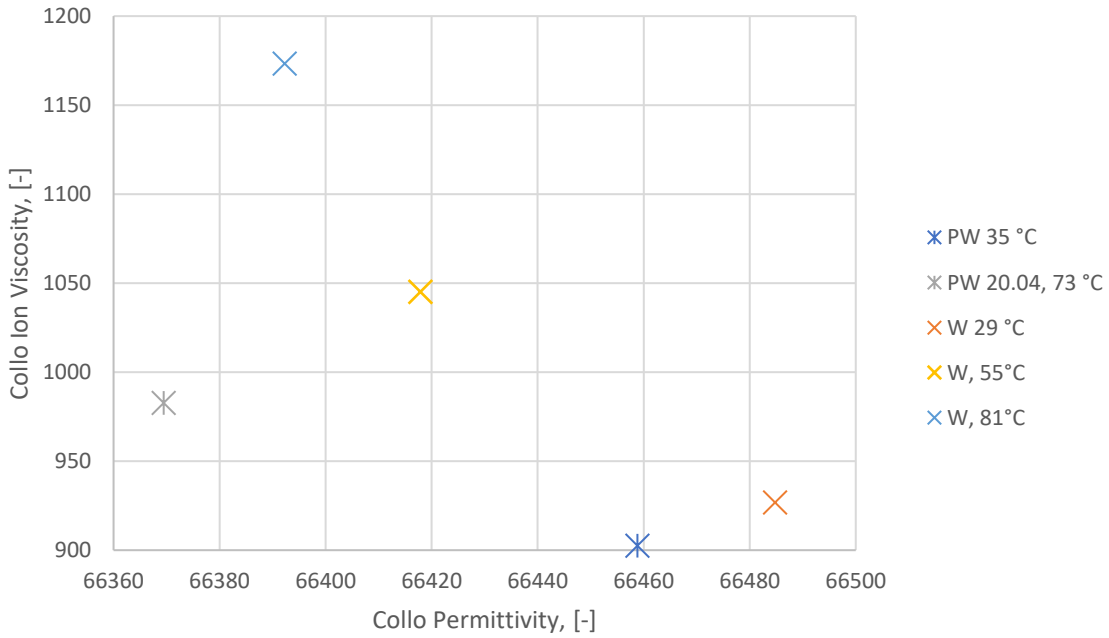

Figure 24 Collo fingerprints of the tap water and purified water in different temperatures.

Figure 24 shows, that Collo Plate analyzer can also detect the difference between water in different purities. The fingerprint values of water in 55 and 81 degrees have been measured by ColloidTek Oy. The fingerprint of tap water at a temperature of 29 degrees is higher in CIV value than the fingerprint of purified water measured at a higher temperature (35°C). The same phenomenon is more intense at higher temperatures when the purified water CIV value stays significantly further away from the high temperature (73°C) compared to CIV value of tap water (81°C). It can also be seen in Figure 24, that as the temperature rises, the CP value decreases.

Figure 24 also shows that the temperature has a large effect on CP and CIV values. When looking at the fingerprints of water at temperatures 29, 55 and 81 in Figure 24, it is observed that when the temperature increases, CP decreases and CIV increases. In term of the CIP process, this means that comparing samples at different temperatures is not reliable without temperature compensation.

6.3.2 Results from process samples' moments from in-line data

The fingerprints for process samples (Table IV) presented in the Figure 25-27 are searched from the in-line data, from the same moment, as when the corresponding samples have been taken from the process. Fingerprints for samples searched from in-line data are shown in Figure 25.

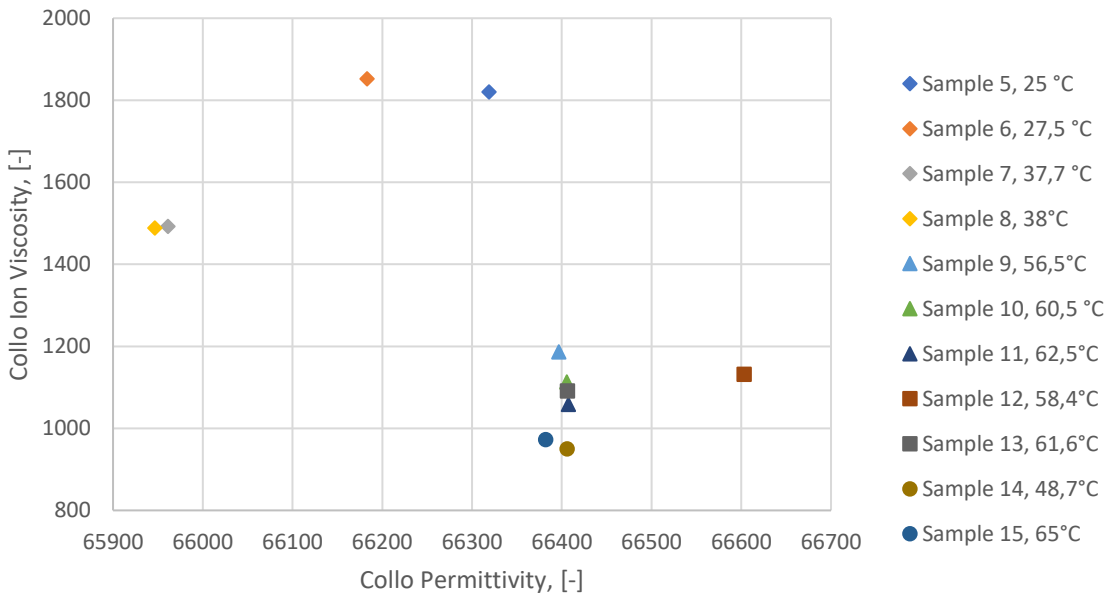

Figure 25 Fingerprints for specific process samples from the CIP process in-line data after production of product with API C.

Figure 25 shows, that fingerprints of rinse steps after alkaline and acidic washes (Samples 9-13). These fingerprints are on quite similar area. Also, the temperatures in those samples are similar. Samples from prerinses first (Sample 5 and 6) and second (Sample 7 and 8) rounds are in their own fingerprint areas. Although pH measurements in Table V shows, that Samples 5 and 6 from prerinse, Sample 13 from rinse after acidic wash and PW rinse samples 14 and 15 have quite similar pH value, Collo fingerprint area in terms of the CIV is completely different. CP on the other hand is pretty much in the same area in those samples, even though the different temperature affects to both CP and CIV. From this, it can be concluded, that pH measurement alone is not informative enough. Figure 26 shows CP and CIV separately presented in terms of temperature.

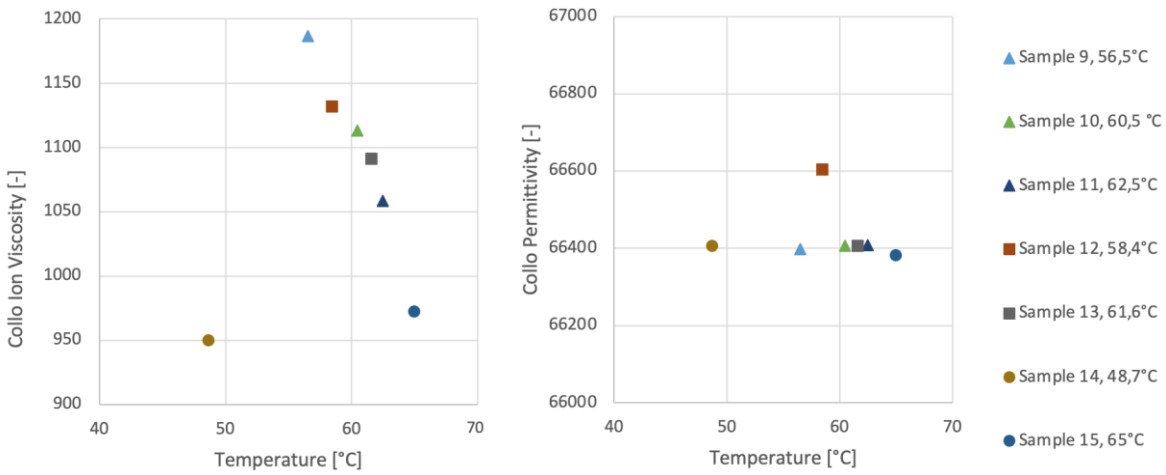

Figure 26 CIV and CP as a function of temperature for fingerprints from CIP process after production of product with API C

Figure 26 shows, that in rinses after alkaline (Samples 9-11), and acidic wash (Samples 12 and 13), when the temperature rises, the value of CIV decreases. The decreased CIV value in the figure 26 might be due to the fact that the alkaline and the acidic detergent are removed from the process as the CIP process progresses. In PW rinsing (Samples 14 and 15), CIV increases and CP decreases as the temperature rises, as also shown in Figure 24.

The moments from in-line data of Samples 5-8 from prerinse and fingerprints of detergent solutions are shown in more detail in Figure 27.

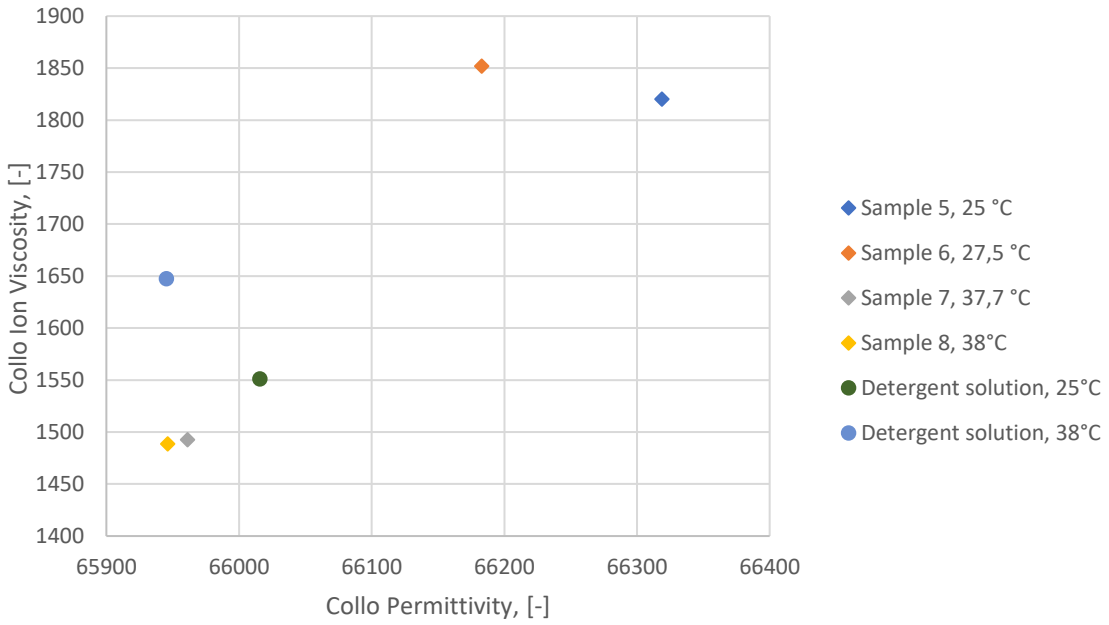

Figure 27 Collo fingerprints for prerinse samples 5-8 and detergent solutions in different temperatures. Fingerprint of detergent solution in 25°C is measured by ColloidTek Oy.

It can be seen from Figure 27 that Samples 5 and 6 have significantly higher CP value than the detergent solution or the Samples from the second round of the prerinse. CP reacts to physical changes, and as here the Samples contained solids, it can be seen here as increased CP value. Especially when the Sample 5 was taken from the beginning of the first round of the prerinse, it contained more solids than the Sample 6 taken from the end of the first round of the prerinse. This can be seen in the Figure 27 as decreased CP value. During the sampling of Samples 7 and 8, the process did not contain any solids, and therefore their CP value corresponds exactly to the detergent solutions' CP value in the same temperature.

The CIV value is also significantly higher in Samples 5 and 6 compared to the detergent solution. When there is a significant amount of product residue in solution in the form of solid, its CIV is also higher than if all residues were dissolved to liquid. This can be deduced from Figure 23 in which, dataset 5 contained 4 mg/ml of dissolved product residues. However, the change in CIV was small (only about 35 units) compared to smaller amounts of dissolved product residues.

Comparing the data obtained from the fingerprints of the samples in Figure 23 prepared in the laboratory, it can be seen that the Samples 7 & 8 are closer to detergent solution fingerprint point (measured by ColloidTek Oy) than the laboratory samples. It is possible that most of the excipients and APIs have already been removed at the beginning of the prerinse.

6.3.3 Results from in-line process data

The wanted sampling time points and the exact starting and ending points of each cleaning step was determined based on the set values of the circulation pumps controller. In addition, the delay caused by the transitional from pumps to the valves was taken into account. To make the figures as readable as possible, all the breaks in CIP process needed to be removed from the data. Breaks were caused for example as the cleaning tanks needed to be filled, and the breaks were removed based on the set values of the controller. The analyzing and plotting were done with Excel.

In this section, CP and CIV graphs are plotted from the data for each cleaning step of one CIP process. In reality, analyzes of cleaning process data are done from several different washes and their cleaning steps, which are compared to each other's. In this thesis, it was intended to demonstrate, what the data provided by the Collo Plate analyzer can illustrate about the CIP Process, and for simplification only the data of one CIP process is used. The results discussed in this section are from the same CIP process after the product with API C as the taken Samples 5-15 (Table IV). Figure 28 shows the CP and Figure 29 the CIV graphs from the data collected in one prerinse. In in-line process data figures, blue is the measuring results, orange is the temperature and black dashed line represents the CP / CIV value of the detergent solution used in cleaning step, compensated to the temperature of the graph. In all following CP and CIV figures, each process data point is median by the three preceding and three latter points of each point. The median has been used to reduce the interference caused by air in the data.

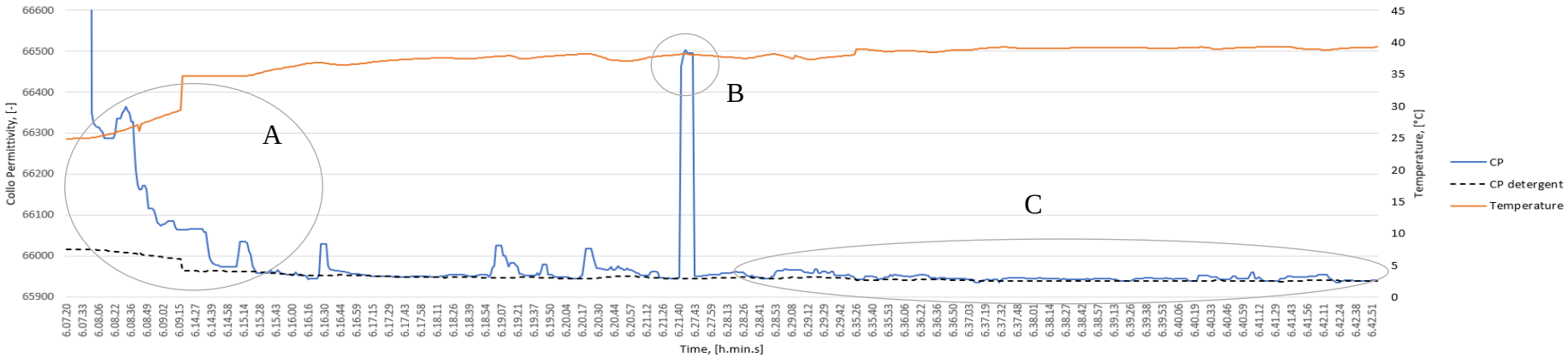

Figure 28 CP graph from the data collected from CIP process prerinse after production of product with API C. A is the point where there is a lot of product residues, B a point of air and C a second round of prerinse

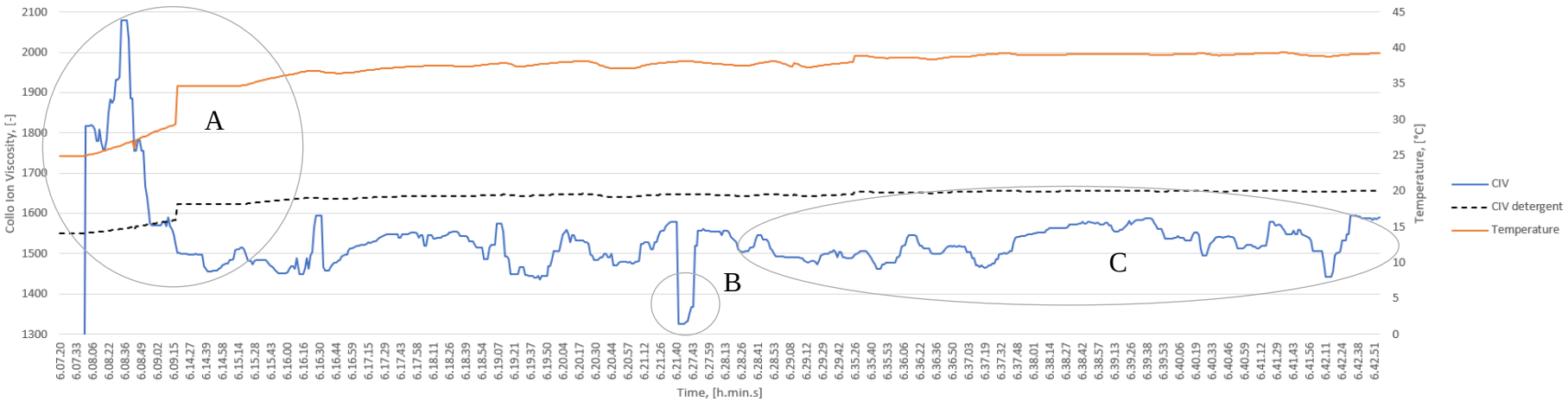

Figure 29 CIV graph from the data collected from CIP process prerinse after production of product with API C. A is the point where there is a lot of product residues, B a point of air and C a second round of prerinse

Figure 28 shows, that most of the API and excipients residues are removed quickly after the start of the initial rinse. As CP respond with size and phase changes of solution, it can be seen from Figure 28, that at the beginning of the prerinse (point A), the phase changes a lot, so the residues are mostly in solid phase. Sample 5 from the same CIP process also showed visually, that there is a lot of solids at the beginning of the prerinse (point A). In the middle of the Figure 28, the odd higher point (point B) is probably air. Most of the individual peaks of the air have been smoothed out by calculating the median for each point. In solution at the point B, the solution contained so much air that the median could not smooth it. As the prerinse progresses, the CP values stabilize close to the CP values of detergent solution at the same temperature.

Even though CP figure from same cleaning step (Area C in the Figures) seems to be close to the detergent solutions, the CIV, that responds to chemical concentration, still have variability to detergent solution CIV. In the prerinse, the same routes are processed twice, and the shapes of the graph's endings resemble slightly each other. The changes in the Area C in the Figure 29 might be caused because of the air released in to process when the cleaning routes are changed. When compared to CIP process of clean equipment, this assumption made sense.

Figure 30 and Figure 31 show the CP and CIV of the alkaline (cleaning step 2) and acid wash (cleaning step 4) from the draining stage after the recirculation washes. Major of the detergent solution removed in the recirculation phase are drained to different piping than where the Collo inline sensor is. A small portion of the detergent solution are, however, drained through the Collo Plate analyzer. The obtained data is presented in Figure 30 and 31. Figure 30 shows alkaline wash yellow and acid wash blue. Temperatures and the theoretical CP values of pure the detergent solution (calculated with Equations 1 and 2) is also shown in the Figure 30.

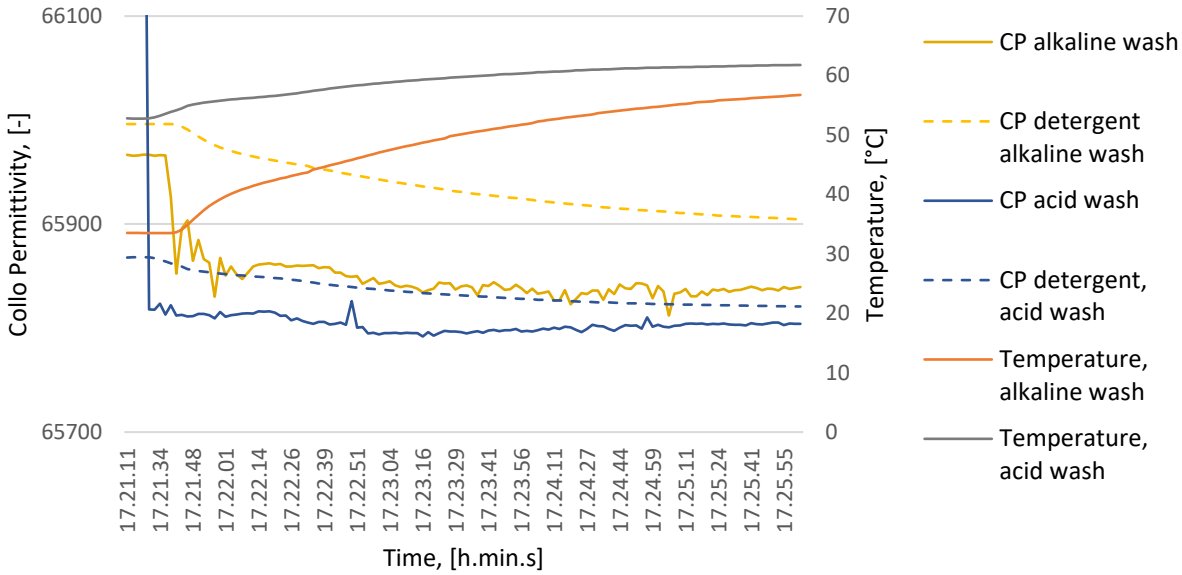

Figure 30 CP graphs of acid and alkaline wash from the process and "theoretical" detergent CP in same temperature

From the Figure 30 it can be seen that the CP value of the acid wash in high temperature is lower than the CP value of the alkaline wash in lower temperature. However, when comparing the theoretical results to the data obtained from the measurements, it can be noted that the measured and theoretical values in the acid wash have smaller ratio when compared to the ratio of the theoretical and measured CP values of the alkaline wash. In addition, when comparing the ratio of theoretical values and measured values, it can be noted that the ratio of theoretical values is remarkably bigger. It would appear, that at the same temperature, the CP level of the acid wash would be slightly higher than the alkaline wash level, but the differences are small. In Figure 31 for CIV graphs, yellow is alkaline wash and blue acid wash. Temperatures and the theoretical concentrations of pure the detergent solution is also shown in the Figure 31.

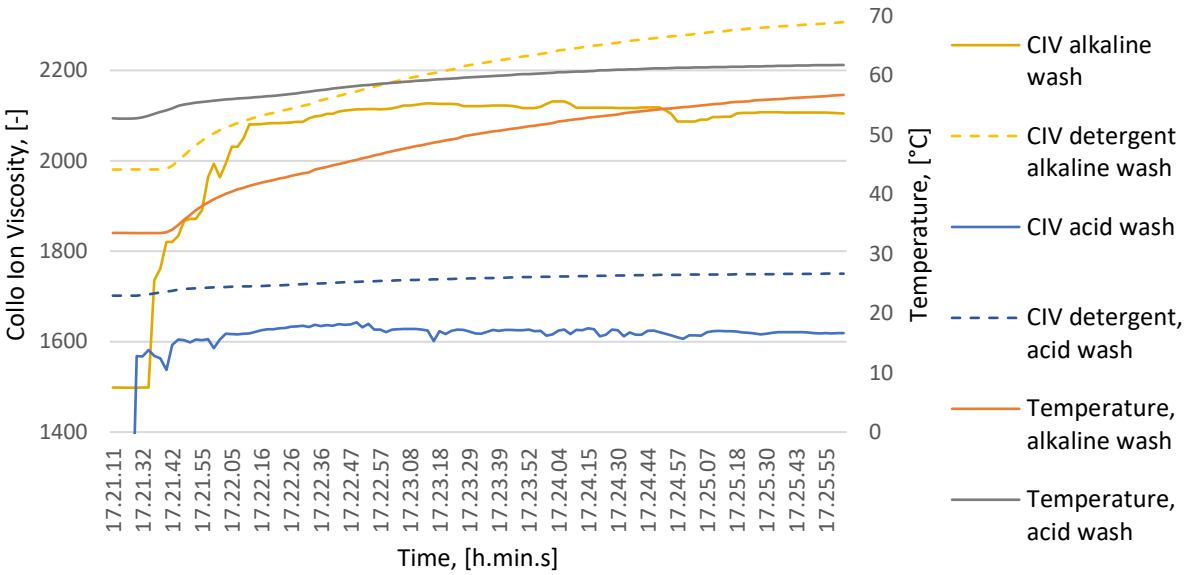

Figure 31 CIV graphs of acid and alkaline wash from the process and "theoretical" detergent CIV in same temperature

The difference in temperatures, in the beginning of the timespan presented in Figure 30 and 31, is rather big, 25 degrees. However, when comparing the ratio of the theoretical detergent solutions of alkaline and acid solution, it can be seen that the closer the temperatures get to each other, the bigger the CIV ratio increases. Also, the CIV value after alkaline wash would be clearly higher if the measurements were at the same temperature. From the data obtained from the sensor, the ratio does not appear to increase in the same way as in the theoretical graphs. Figure 32 and 33 shows the CP and CIV for rinse steps after alkaline and acid wash.

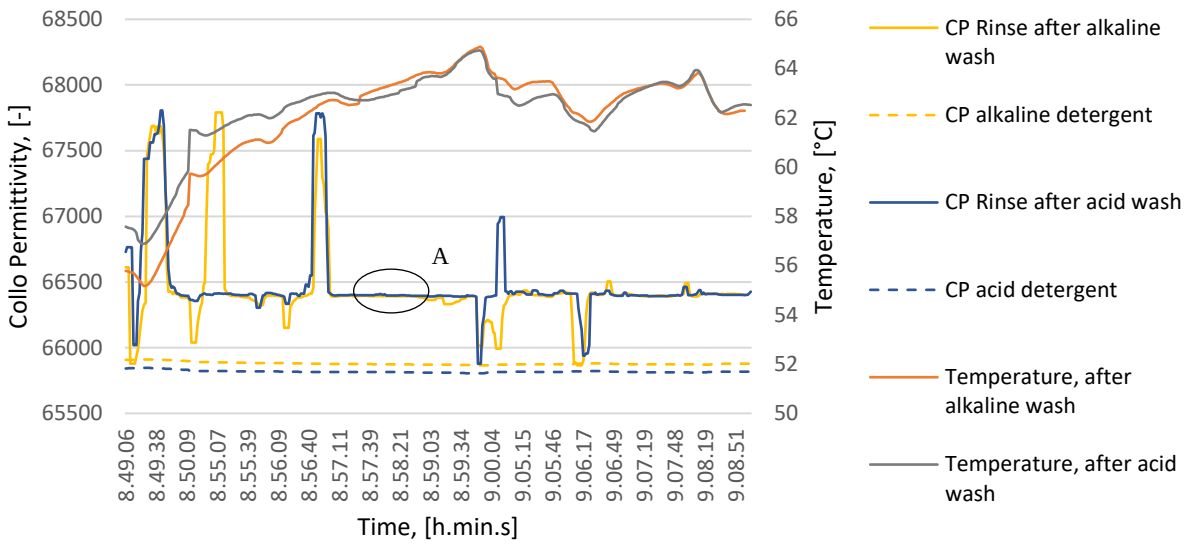

Figure 32 CP of the rinse steps after alkaline and acid wash. Point A indicates a point that has remained flat for a long time, where the CP remains at the level of tap water.

Figure 32 shows the rinsing step after alkaline and acid washes. Their basic level is very much the same which reflects the CP level of tap water. The high peaks upwards are air in the CP graph but the peaks going downwards (toward detergent CP levels) are detergent residues that are rinsed off after recirculation. It can also be seen from Figure 32 that the detergent residues after both, alkaline and acid washing, come from the same routes at several points. This shows that the rinsing step after circular washes is important in removing any detergent solutions. Point A indicates a point that has remained flat for a long time, where the CP remains at level of tap water.

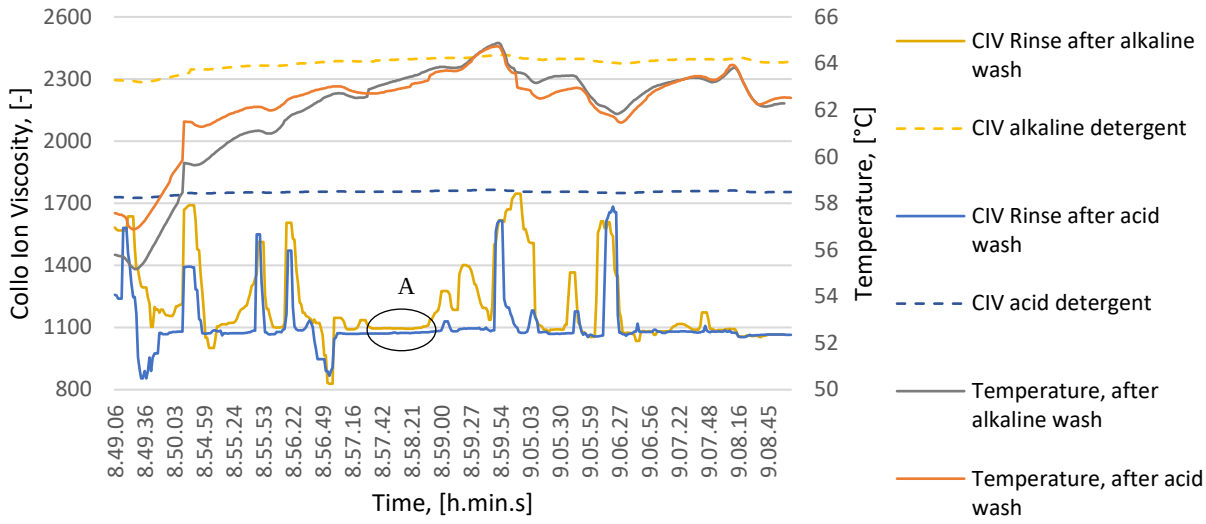

Figure 33 CIV of the rinse step after alkaline and acid wash. Point A indicates a point that has remained flat for a long time, where the CIV remains at the level of tap water.

As in the CP plot in Figure 33, the removal of detergent residues is can also be noted in the CIV graph. The basic level of tap water at this temperature (CIV approx. 1100) is also well reflected in the level to which the concentrations always return after detergent residues. At the same points, where the air made a peak in Figure 32 upwards, they go downwards in the CIV graphs. The CIV figure also shows variations in concentration, and more clearly certain routes from which detergent residues come during rinsing. Detergent residues come from several routes and the results show that rinsing is necessary for those routes and their duration cannot be shortened. In both, Figure 32 and Figure 33, the point A indicates the point of tap water. In both CP and CIV graphs, that is the point, during which no detergent residues are removed. Thus, from the point of view of the CIP process, if the process is wanted to shorten in some washing route, it should be the route in point A.

Figure 34 and Figure 35 show the CP and CIV graphs of the final step of CIP process, the final rinse. In Figure 34 and 35, blue line represents the measurement results (after calculating median for each point), black dash is the theoretical level for the tap water, yellow line is CP/CIV level of air and range line is the temperature.

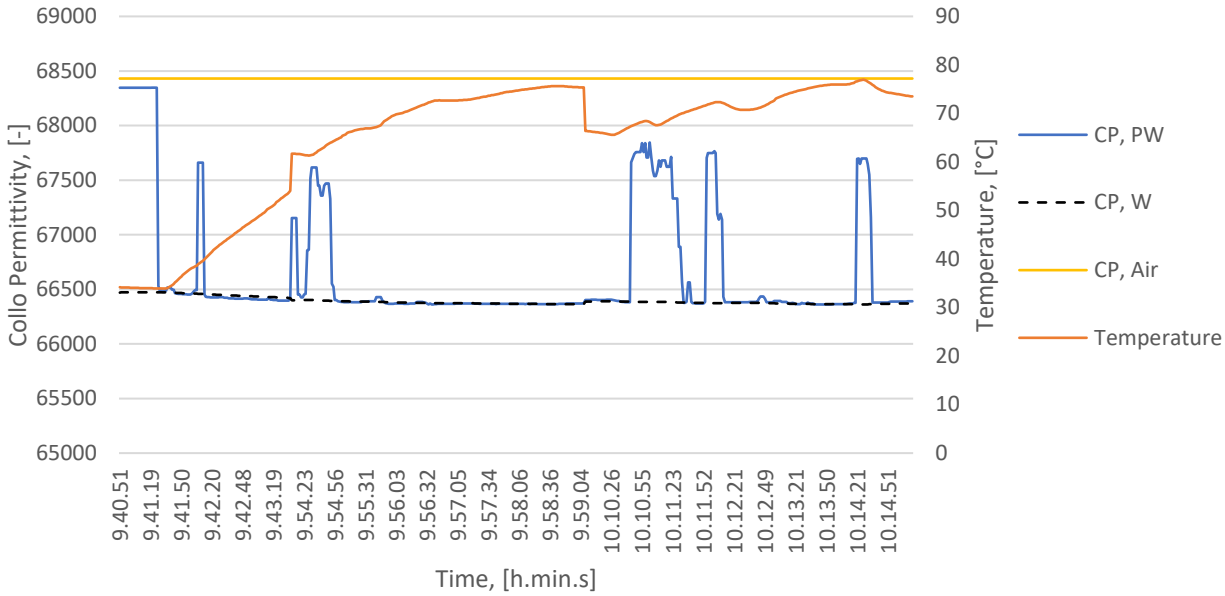

Figure 34 Blue CP graph of the final rinse with purified water

In Figure 34, black dash is the theoretical level for the tap water, yellow line is CP level of air and red line the temperature. Figure 34 shows, that the CP level of purified water is almost the same than the level of tap water in same temperature. Similarity in CP level is assumed, as there should not be physical differences between PW and W. Clear peaks on the CP graph of the cleaning steps are caused by air. There is more air in PW rinse solution than in previous rinses. This may be due to the fact, that before PW rinsing, the equipment is dried after the rinse with tap water. As noted earlier, there is no difference in the CP graph with purified and tap water at the same temperature.

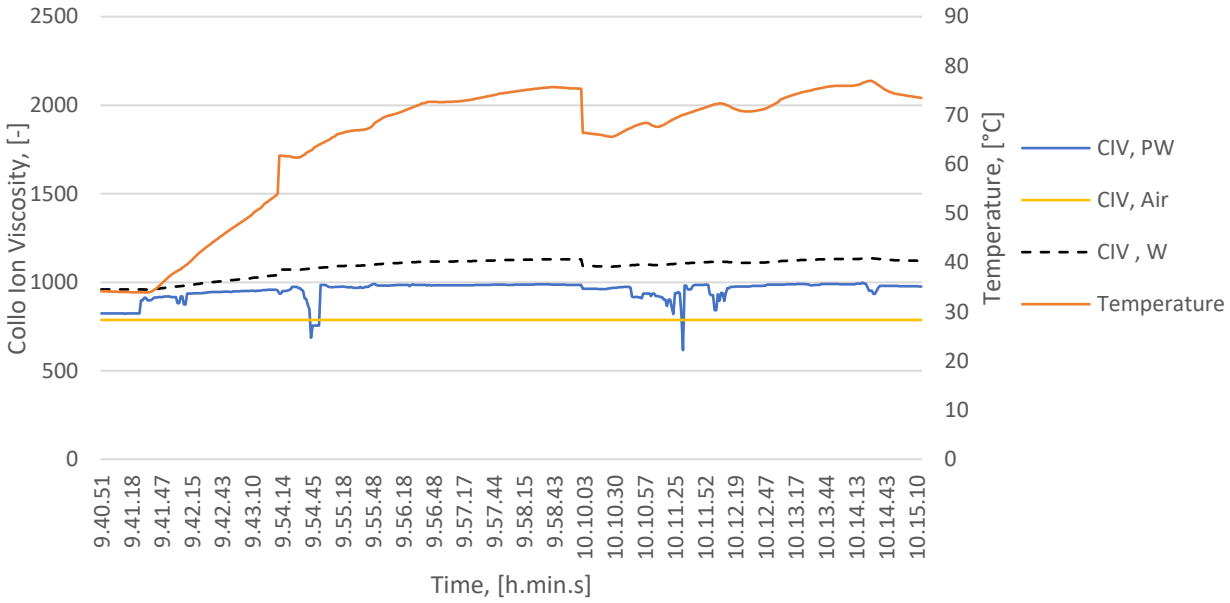

Figure 35 Blue CIV graph of the final rinse with purified water

As the CIV reacts to free ions, difference between purified water and tap water can be seen clearly in final rinse. In purified water, several purifying operations has been used to remove impurities that are always found in tap water, and therefore PW contains less free ions than the tap water. Therefore, the CIV level of PW in Figure 35 is considerably lower than the CIV level of tap water at the same temperature. The CIV figure also presents the downward peaks in the same points as the upward peaks are in Figure 34. It can be deduced from the Figure 35, that all tap water residues have left the process as expected, and the outcoming water is clean.

6.4 Comparison of selected methods

In Table VI is presented matrix following the logic of the matrix presented in the Table II. The Table VI shows the potential of the method based on the results obtained in the experimental part. Green indicates the high potential of that method in that region, yellow the weaker potential and red the weakest.

Table VI Matrix of selected methods potential for CIP process based on experimental part

	Raman		Collo	
Type of measuring	By taking samples		In-line	
Potential in CIP	Raman is potential method for analyzing the prerinse of the CIP process. In correct wavelength range, the Raman might be potential.		Really potential but more comparable information would be provided if effect of temperature and air were considered.	
Sensitivity	Raman sensitivity depends on the measuring device and the wavenumber range		Collo is really sensitive and might react to unwanted changes, like air and foaming.	
Detects solids	Detect solids		Detects solids	
Detects API's + excipients	Detects API and can identify it Detects excipient and can identify it.		Detects API but cannot distinguish it	
Detects detergents	Does not detect detergents at this wavelength range.		Detects detergents but differences between used acid and alkaline solutions is small	
Detects water quality	Does not detect difference between purified and tap water at this wavelength range.		Detects differences between tap and purified water	

7. CONCLUSIONS

The aim of this thesis was to examine measuring and analyzing methods that could be utilized in development and optimization of Clean in Place processes in pharmaceutical industry. The purpose of the measurement method is to obtain more information about the process and if the cleaning steps work as expected. At best, with the information provided by the measurement method, unnecessary and ineffective cleaning steps could be reoptimized. By this, the CIP process could be changed to more effective and efficient manners, to avoid the need for re-washing.

The aim of the literature review was to create an overall picture of CIP processes and to find out what kind of measuring and analyzing methods have been used for Clean in Place processes in various industries, and which could possibly be suitable in developing the CIP process. The purpose was also to find out if there are some methods that could possibly be suitable but are not yet used in CIP processes. The results obtained from literature reviews were summarized in the form of a matrix, from which the most potential methods could be easily deduced.

In the experimental part the most potential technologies based on the literature review were challenged and their applicability to the pharmaceutical industry's CIP processes were tested in practice. The experimental part was done by analyzing the laboratory and process samples as well as the process in real time. The experiments were done with Raman spectroscopy and Collo Plate analyzer. The aim was to find out whether the changes in product, detergents or water concentrations and stages can be seen from CIP process rinses. The selected methods were also tested at very low concentrations. In addition, the samples were measured with a conductivity and pH meters, to gather additional information about the samples.

The thesis results show that both Raman spectroscopy and Collo Plate analyzer have potential to be used in the following and analyzing the cleaning process. Collo Plate analyzer provides the possibility to follow the process in real-time by using an in-line sensor, which provides information from each cleaning step separately. Sensor defects solid materials, phases (CP; Collo Permittivity) and concentrations (CIV; Collo Ion Viscosity) in each

cleaning step. Collo-technology is able to detect both acidic and alkaline detergents, detect production residues (API and excipients) and make a difference between purified water (PW) and tap water. In addition, a difference in between products containing active pharmaceutical ingredients (API) and products containing API + excipients was observed on a small fingerprint area. The results obtained from Collo indicate, that the detergent residues from the recirculation's are removed in the rinsings, from the process as expected, and that the PW coming out from the process in the end of the last cleaning step does not contain any residues from the tap water. When the Collo analyzer is located in the drain line of the CIP process, the progress of the cleaning process can be monitored. In addition, in the rinses following the acidic or alkaline wash, those cleaning routes can be inspected where the detergent residues no longer occur. When inspecting the obtained data from several different CIP processes, it can be reasoned that their rinsing time could be shortened without affecting to the outcome of the cleaning process.

The results obtained from the Raman spectroscopy indicates, that it is an effective method to follow the prerinse of the CIP process. In the prerinse the product residues (API + excipients), and their concentration changes, can be detected and identified. In addition, the differences in between the products containing API and the products containing API + excipients could be detected by using PLS model. If there were an in-line version of Raman spectroscopy in use, it might also have been possible to find the point in the prerinse, where all the residues have been removed. Also, if there were a residue, it could have been possible to tell what ingredient is slower to rinse off in the prerinse. The used wavelength range of the Raman device in this study was not wide enough to analyze the changes in the detergents or in the qualities of the water. However, according to the literature review, it could be possible to identify those changes with a wider wavelength range. However, with the equipment used in this study, the Raman spectroscopy does not appear to be suitable for analyzing the whole CIP process.

The conductivity and pH measurements from the process samples appeared to be a suitable method in gathering general information from every cleaning step. The conductivity measurement proved, that at least in the CIP processes from where the samples were taken, the rinses after the circulations were effective and the detergent residues were removed from the

process as expected. The results obtained from the conductivity meter data also indicated that the rinse water coming out from the last final rinse is clean enough. Therefore, can be assumed, that the PW rinse works as expected. The pH meter was able to detect the removal of the product residues in the prerinse through the change in pH. In the first round of the prerinse, the samples were neutral, as the process contained much acidic product residues, which neutralized the alkaline detergent solution. In the second round of the prerinse, pH was strongly alkaline, from where can be assumed that the product residues were already removed from the process.

While comparing the results obtained from Collo and Raman, the technologies confirm findings from each other, that the first round of the prerinse of the CIP process removes efficiently the major of the product residues from the process. pH measurements also support these results. According to the data obtained from Raman measurement, the second round of the prerinse did not include any peaks caused by the products or excipients in neither of the studied CIP processes. According to the data obtained from Collo measurement, in the second round of the prerinse, the phase does not change remarkably, and the CIV figure was similar to CIV figure of cleaning of clean equipment. According to the results, it appears that only one round of prerinse is enough to remove the product residues.

Despite the fact, that both of the selected technologies have potential to be implemented in the CIP process of the pharmaceutical industry, they both have still some challenges. In Collo Plate analyzer, the biggest challenges are the air bubbles and temperature change, which both caused problems to interpretation of the measurements. When observing the obtained results, the data was modified by using a median. The median was done by using 3 previous and 3 following values as the set of values. However, using the median it also weakens the genuine changes in the results. The rising temperature in turn affects directly to the results as decreased CP values and increased CIV values. The analysing and comparing of the results could be improved by making temperature compensation for each of the cleaning steps. The total removal of the air might be difficult, but it can be minimized by changing the sensor installation setup, and therefore the unwanted measurements of air can be reduced. In Raman technology, the biggest issues are concerning the wavelength range, which should be wide to be able to detect all the changes in the process. In this work, Raman technology

is mainly able to detect the changes only in the prerinse phase, while Collo is able to provide data from the changes during the whole process. However, Raman technology is able to identify the source of the change, as Collo is only able to detect changes in the concentrations and amount of solid material.

The next step could be trying to solve the challenges concerning Collo. After solving the issues, further investigation towards the capability of Collo in telling additional information from the recirculation phase of the CIP process can be considered. Even though Collo is able to detect changes, it might be more interesting to be able to tell the reason for the change. Therefore, testing Raman with different wavelength areas with process samples could be interesting and progressive. If it appears to be potential, it could possibly be considered to implement an in-line version of Raman, as an alternative for Collo, for experimenting the monitoring of the CIP process.

After comparing all the results from studies between these selected methods, the better information of the CIP process is provided by Collo Plate analyzer. In order for Orion Corporation to be able to utilize the information Collo Plate analyzer provides, it would be important to have an efficient software for processing a large amount of data. In Orion Corporation, the sensor could also be utilized in some other applications, if the sensor was able to show only genuine changes without unwanted measurement data.

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