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PROBLEMS CAUSED BY HYPROMELLOSE DURING STERILE FILTRATION OF EYE DROP PRODUCTS IN PHARMACEUTICAL INDUSTRY

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Tiivistelmä/Referat – Abstract

The problems caused by hypromellose in sterile filtration of ophthalmic products in the pharmaceutical industry were investigated. The research project was performed at NextPharma Oy's ophthalmics manufacturing facility in Tampere during the autumn of 2020. Hypromellose is an excipient commonly used in ophthalmic products as a viscosity enhancer to prolong the contact time of the preparation on the eye surface. In the ophthalmics compounding process, hypromellose is first dispersed by slowly sprinkling it into a hot solution and thoroughly mixing, after which the solution is cooled to room temperature. During cooling, the hypromellose dissolves and gels, increasing the viscosity of the solution. Incomplete dispersion or dissolution of hypromellose during the manufacturing process can slow down the filtration rate or even clog the filter completely due to undissolved hypromellose polymer material. Hypromellose is an industrially produced cellulose derivative that often contains some amounts of unreacted cellulose and other sparingly soluble polymer particles as impurities, which can also cause problems in filtration processes. Sterile filtration is a commonly used sterilization method for ophthalmic products, in which the prepared bulk solution is filtered through a 0.1 to 0.2 µm pore size filter membrane into a sterile receiving vessel. Due to the very small pore size, sterile filters are easily clogged if the solution contains poorly dissolved material. The purpose of this work was to collect additional information on the possible causes of clogging caused by hypromellose and to determine whether the filterability of a solution containing hypromellose can be improved by optimizing the manufacturing process parameters.

The design of experiments was prepared, creating a two-level full-factorial test matrix without replicates and with three centre points. Four different process parameters were used (mixing time, mixing speed, dispersion temperature, and cooling temperature). Minimum and maximum levels for the parameters were obtained in the initial tests, after which the test solutions were prepared and filtered in a randomized order according to the test matrix. The aim of the screening was to find out which parameters were affecting the filterability and what would be their optimal combination that would maximize the filtration rate and the yield of filtration. Finally, the optimized parameters were used to test different batches of hypromellose, comparing the results to previous filtration tests. Additionally, an alternative hypromellose dispersion method was tested to minimize the amount of insoluble material remained during the dispersion and cooling steps.

Of the parameters tested, mixing speed was the least significant, while cooling temperature had the most effect on the filtration results. The solutions with lower cooling temperature had better filtration results, which may be due to reduced aggregation of hypromellose due to increased hydration of the polymer chains. The temperature behaviour of hypromellose solutions could be an interesting subject for further investigation. Longer mixing times and higher dispersion temperatures produced slightly better filtration results on average, but the differences were not statistically significant. Most challenging in the study was controlling the temperature and mixing of the solutions, and the retention of insoluble hypromellose material at the walls of the compounding vessel. The alternative dispersion method gave promising preliminary results, but the method still requires further testing. It would be important to also find the root cause of the filter clogging mechanism e.g., by further analysing the clogged filter membrane. The study provided additional useful information of the behaviour of hypromellose solutions in solution preparations and during sterile filtration, which has been helpful in solving production problems.

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Pharmaceutical industry, ophthalmics, sterile filtration, hypromellose, process parameters

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Tiivistelmä/Referat – Abstract

Tutkimusprojektissa tutkittiin hypromelloosin aiheuttamia ongelmia silmävalmisteiden steriilisuodatuksessa lääketeollisuudessa. Tutkimusprojekti suoritettiin NextPharma Oy:n silmälääketehtaalla Tampereella syksyllä 2020. Hypromelloosi on silmävalmisteissa yleisesti käytetty apuaine, jonka tarkoitus on nostaa liuoksen viskositeettia ja täten pidentää valmisteen kontaktiaikaa silmän pinnalla. Valmistusprosessissa hypromelloosi ensin dispergoidaan ripottelemalla hitaasti kuumaan liuokseen, jonka jälkeen liuos sekoitetaan huolellisesti ja lopuksi jäähdytetään huoneenlämpöön. Jäähdytyksen aikana hypromelloosi liukenee ja geeliytyy, jolloin liuoksen viskositeetti nousee. Hypromelloosin epätäydellinen dispersio tai liuotus valmistusprosessin aikana voi aiheuttaa suodatusnopeuden hidastumista tai jopa tukkia suodattimen kokonaan. Hypromelloosi on teollisesti valmistettu selluloosajohdannainen, joka usein sisältää epäpuhtautena reagoimatonta selluloosaa ja muita niukkaliukoisia polymeeripartikkeleita, mitkä voivat myös aiheuttaa ongelmia suodatusprosesseissa. Silmävalmisteissa yleisesti käytetty sterilointikeino on steriilisuodatus, jossa valmistettu liuos suodatetaan 0,1 – 0,2 µm huokoskoon suodatinmembraanin läpi steriiliin vastaanottoastiaan. Johtuen erittäin pienestä huokoskoosta, steriilisuodattimet tukkeutuvat herkästi, mikäli liuos sisältää liukenematonta ainesta. Työn tarkoituksena oli tutkia hypromelloosin aiheuttaman suodattimien tukkeutumisen mahdollisia syitä ja selvittää, voidaanko hypromelloosia sisältävän valmisteen suodattuvuutta parantaa optimoimalla valmistuksen prosessiparametreja.

Koesuunnittelu luotiin neljälle eri prosessiparametrille (sekoitusaika, sekoitusnopeus, dispersiolämpötila ja jäähdytyslämpötila) kaksitasoinena full factorial -koematriisina ilman toistoja, kolmella keskipisteellä. Prosessiparametreille haettiin alkutestauksissa sopivat minimi- ja maksimiarvot, jonka jälkeen koeliuokset valmistettiin ja suodatettiin matriisin mukaisessa satunnaistetussa järjestyksessä. Seulonnan perusteella pyrittiin selvittämään suodattuvuuteen vaikuttavat parametrit ja niiden optimaalinen kombinaatio, jolla suodatusnopeus ja suodattuneen liuoksen määrä saataisiin maksimoitua. Lopuksi optimoiduilla parametreilla testattiin eri hypromelloosieriä verraten tuloksia aikaisempiin suodattuvuustestituloksiin, sekä testattiin vaihtoehtoista hypromelloosin dispersiomenetelmää, jolla pyrittiin minimoimaan dispersio- ja jäähdytysvaiheessa valmistusastiaan/liuokseen jäävä liukenematon aines.

Testatuista parametreista sekoitusnopeudella oli pienin vaikutus suodattuvuuteen, ja jäähdytyslämpötilalla suurin. Kylmemmäksi jäähdytetyt liuokset suodattuivat paremmin, mikä voi johtua polymeeriketjujen lisääntyneestä hydraatiosta ja siitä aiheutuvasta hypromelloosin vähentyneestä aggregoitumisesta. Hypromelloosiliuosten lämpötilakäyttäytyminen voisikin olla hyödyllinen aihe tarkemmille jatkotutkimuksille. Pidempi sekoitusaika ja korkeampi dispersiolämpötila tuottivat keskimäärin hieman parempia suodatustuloksia, mutta erot eivät olleet tilastollisesti merkittäviä. Tutkimuksessa eniten haasteita aiheutti lämpötilan ja sekoituksen kontrollointi, sekä liukenemattoman hypromelloosiaineksen jääminen valmistusastian reunoille. Vaihtoehtoisella dispersiomenetelmällä saatiin alustavasti lupaavia tuloksia, mutta menetelmä vaatii vielä lisätutkimuksia. Tärkeää olisi myös löytää juurisyy suodattimen tukkeutumiselle, esim. analysoimalla tukkeutunutta suodatinmembraania tarkemmin. Tutkimuksesta saatiin hyödyllistä lisätietoa hypromelloosiliuosten käyttäytymisestä liuosvalmistuksessa sekä steriilisuodatuksessa, mistä on ollut apua tuotannon ongelmien ratkaisemisessa.

Avainsanat – Nyckelord – Keywords
Lääketeollisuus, silmälääkkeet, steriilisuodatus, hypromelloosi, prosessiparametrit

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1 INTRODUCTION

Topical ophthalmic formulations are important for treating many common eye diseases, such as dry eyes, eye infections/inflammations, and glaucoma (Kaur et al. 2004; Gibson 2009). This type of drug administration is the most practical way for treating the anterior part of the eye, as drug penetration into the eye via systemic circulation is quite poor and would likely cause systemic side effects. As these types of formulations are directly administered to the eye, it is essential that the product is sterile (Eudralex – Volume 4, Annex 1). This is often achieved by sterile filtration followed by aseptic filling into the primary container. Sterile filtration is commonly used practice in pharmaceutical industry, because often either the formulation and/or the primary packaging material cannot withstand other sterilization methods like heat or radiation.

Common problem with sterile filtration of ophthalmic products is filter clogging, where the filter starts to clog at some point after the start of filtration (Allmendinger et al. 2015; Coulais et al. 2015). This leads to a decrease in the filtration rate, which will slow down the manufacturing process. In some cases, the filter can get completely clogged, resulting in low batch yield, and causing a significant loss of time, money, and valuable resources for the company. This type of problem was investigated in this research project at NextPharma Oy, Tampere. Based on earlier studies at the site, the hypromellose raw material had been identified as root cause of the problem, possibly due to some undissolved fibres/unreacted material in this viscosity agent. There had been noticeable batch-to-batch variation in the raw material as well; some batches had caused no problems at all, while others were practically unusable. All the raw material batches used had met the requirements in compendial monographs, but there still existed variation between batches, which could not be predicted based on the certificates of analysis (CoA) of the batches.

2 OPHTHALMICS

2.1 Use of ophthalmics as pharmaceutical dosage form

Drugs are commonly applied to the eye to achieve a local action on the surface or in the interior of the eye (Kaur et al. 2004). Typical indications for the use of ophthalmics are dry eyes, allergic conjunctivitis, bacterial and viral eye infections, glaucoma, macular degeneration, and macular edema (Gibson 2009). There are three main routes for the delivery of drugs to the eye: topical, systemic, and intraocular injection. Systemic administration is generally not favoured, because of poor drug penetration into the eye via systemic circulation. Intraocular injections have poor patient compliance and cannot be administered by patients themselves, which is why they are usually reserved for more serious conditions where topical administration is ineffective, such as age-related macular degeneration (AMD), posterior uveitis, and persistent macular edema due to diabetic retinopathy (Gibson 2009). Most of the common eye diseases, such as dry eyes, eye infections/inflammations, and glaucoma can be treated with topically administered drugs, making it the most widely used delivery route. Topical ophthalmics are also easier to administer compared to injections and have fewer side effects compared to systemic administration. Topically administered conventional dosage forms include solutions, gels, emulsions, suspensions, and ointments. Various newer approaches for ophthalmic drug delivery have been developed, such as injectable implant systems for treating the posterior part of the eye, and ocular inserts for prolonged topical drug release. Novel formulation designs for topically administered drugs, such as liposomes, micro- and nanoparticles, and microspheres have also been implemented. The goal of these drug delivery methods is to enhance the bioavailability of a drug or to offer controlled drug release such as delayed or sustained drug delivery. Other methods to increase the bioavailability of ophthalmics are

penetration enhancers or absorption promoters, which are used to increase the permeability of cell membrane, loosen the tight junctions between the cells, or both.

2.2 Topical administration

A vast majority of marketed ophthalmic products are topical formulations due to their suitability for most common eye diseases, convenient application, and better patient compliance. Topical formulations are suitable for treating diseases in the anterior part of the eye, such as glaucoma, bacterial and viral infections, dry eyes, and allergic conjunctivitis (Gibson 2009). A major problem with topical administration is achieving optimal drug concentration at the site of action (Kaur and Kanwar 2002). The limited volume of conjunctival pocket, absorption to systemic circulation, low permeability of the cornea, and short residence time in the eye are obstacles, which significantly reduce the bioavailability of topical ophthalmics (Kaur et al. 2004). The structure of the eye is represented in Figure 1.

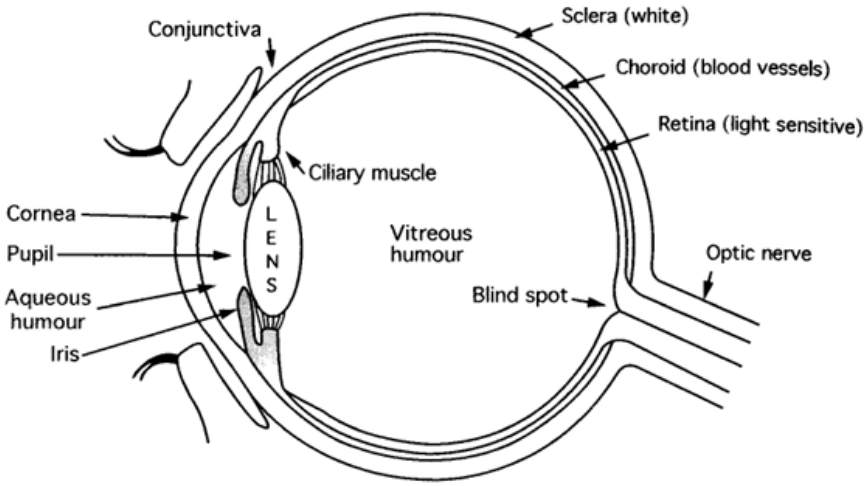


Figure 1. Structure of the eye (Gibson 2009)

To induce a response, a topically administered drug needs to reach the inner parts of the eye, usually by absorption through the cornea (Kaur and Kanwar 2002). A conventional drug product is administered to the conjunctival pocket, which can hold approximately 30 µl of solution. After administration, the product first encounters the tear film, cornea, and conjunctiva of the eye, which are the primary barriers preventing the drug from entering the eye (Kaur and Kanwar 2002; Gibson 2009). The cornea is a clear and colourless membrane, which consists of three layers: the outer epithelium, middle stroma, and inner endothelium. The epithelium and endothelium are lipophilic, preventing the permeation of polar, hydrophilic substances, while the stroma is hydrophilic, preventing the permeation of nonpolar, lipophilic substances. The sclera is a white and opaque membrane, which forms the outermost layer of the eye, covering the whole eye except the cornea. The anterior part of the sclera is covered by conjunctiva, which also covers the inner surface of the eyelids and the presents a permeability barrier to most drugs. The sclera contains a lot of blood vessels, which supply the anterior tissues of the eye, but also transports permeable drugs into systemic circulation.

In addition to the physical barriers of the eye such as the cornea and conjunctiva, blink reflex and tear production also hinder the bioavailability of the drug via multiple mechanisms (Gibson 2009). The surface of the eye is continuously lubricated by fluids produced by conjunctival and lacrimal glands, while blinking assists in spreading the fluids evenly and draining them via the nasolacrimal duct into the nose and throat. The combination of these effects causes a dilution of the drug dose and a rapid removal of the drug from the eye into the nasolacrimal duct. This can lead to significant amount of drug ending up in systemic circulation, resulting in systemic side effects. It takes an average of 5-6 minutes for a drop of aqueous solution to be completely eliminated from the eye, which is very short time for the drug to permeate the corneal barrier (Kaur and Kanwar 2002). In addition, the tear fluid can cause drug inactivation by binding and metabolism. Tear fluid consists of up to 2%

proteins, which can reduce the effective concentration of drugs by binding, and enzymes such as esterases, monoamine oxidases and aminopeptidases, which can metabolise some drugs (Gibson 2009). Poor permeability of the corneal epithelial membrane combined with rapid clearance and inactivation by the flow of tear fluid are causing poor bioavailability, which is why typically only less than 1% of the drug in conventional formulations is absorbed to the eye.

2.3 Formulation design of topical ophthalmics

The drug's permeation into the cornea is affected by its physicochemical properties, such as solubility, lipophilicity, molecular size and shape, charge, and degree of ionisation (Gibson 2009). The size of the drug molecule should be below 500 Da, as larger molecules are poorly absorbed. Most ocular drugs seem to penetrate the cornea by passive diffusion (Kaur and Kanwar 2002). Lipophilic drugs usually permeate transcellularly, while hydrophilic drugs favour paracellular route (Borchardt 1990). This is illustrated in Figure 2.

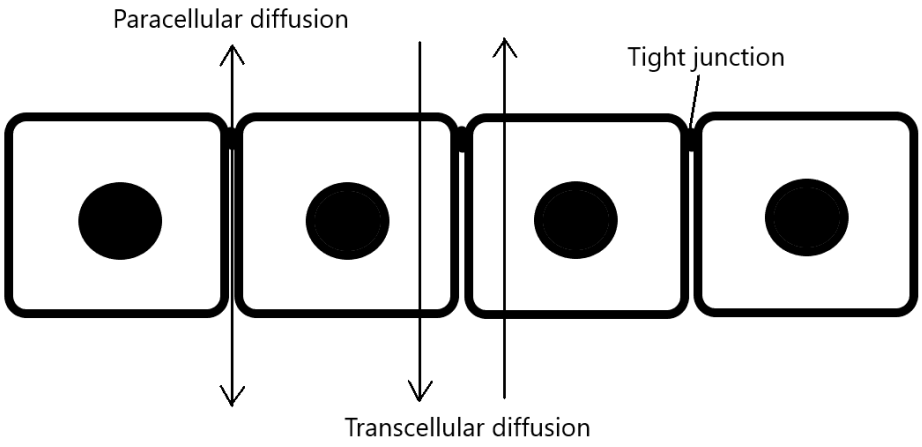


Figure 2. Diffusion pathways

Because of the structure of cornea, drugs that possess both lipophilic and hydrophilic properties are most effectively absorbed into the eye, and the optimal log P value for corneal penetration is 2-3 (Schoenwald and Ward 1978). Weak bases and acids are generally favoured for optimal permeation because of their capability to exist both in ionised and unionised form, though drugs in unionised form tend to have best permeation (Gibson 2009). The pH of the formulation should therefore be optimised with buffers, to maximise the amount of unionised drug without weakening its solubility too much. Of the ionised molecules, cationic drugs have better permeation due to the corneal epithelium being negatively charged (above pH 3.2).

In addition to optimising the product for maximal corneal permeation, osmolarity and stability are other important aspects to consider in the formulation. To avoid irritation and discomfort, which may induce tearing and therefore result in a rapid clearance of the product, the solution should ideally be isotonic with tear fluid, which is equivalent to 0.9% (w/v) solution of sodium chloride (Gibson 2009). The tonicity can be increased with excipients, such as sodium chloride or potassium chloride, or reduced by diluting the solution. Other common excipients used in ophthalmic solutions include antimicrobial preservatives and stabilising agents. Antimicrobial preservatives, such as benzalkonium chloride (BAC) are commonly used in multidose bottles, to ensure the sterility of the product during its period of use. Stabilisers, such as antioxidants (e.g., ascorbic acid) and/or chelating agents (e.g., disodium edetate (EDTA)) are sometimes needed to improve the shelf life of the product. This may be relevant especially if the active ingredient is susceptible to oxidative degradation.

Frequent dosing using high concentrations of the drug is usually required, due to the short residence time and drug drainage caused by lacrimal fluid. This pulse-type dosing with high

drug concentrations can be irritating to the eyes and bothersome for the patient, which can cause reduced patient compliance. To combat these problems and increase the bioavailability in ocular drugs, many formulations are designed to increase the contact time between the drug and the cornea (Kaur and Kanwar 2002). Most common method to achieve this is utilisation of viscosity increasing agents, such as swellable polymers in the solution.

2.4 Viscosity enhancers in ophthalmics

Viscosity enhancers are used in ophthalmic formulations to increase the viscosity of the solution, which in turn increases the contact time between the drug and the surface of the eye, resulting in increased bioavailability (Kaur et al. 2004). Viscosity enhancers used in ophthalmics include cellulose derivatives such as hypromellose (HPMC, hydroxypropylmethylcellulose), hydroxyethylcellulose (HEC), and hydroxypropylcellulose (HPC), carbomer polymers, polyvinyl alcohol (PVA), povidone (PVP), hyaluronic acid (HA) and its derivatives, and dextran (Gibson 2009).

Some commercial ophthalmic products, such as Blocanol Depot by Santen Pharmaceutical Co. Ltd., contain polymers which start to gel on contact with the eye, due to ionisation in the tear film (Duodecim – Lääketietokanta 2020). While high viscosity products increase the residence time in the eye, they are usually less tolerated and can cause temporary blurring of vision (Gibson 2009). Most ophthalmic solutions are therefore formulated with a viscosity between 10 to 25 cP, to maintain an optimal level of tolerability while seeking to maximise the residence time in the eye. Examples of commercial products utilising viscosity enhancers are presented in Table 1.

Table 1. Examples of commercial ophthalmic products with viscosity enhancers (Johnson & Johnson 2016; Duodecim – Lääketietokanta 2020)

Product	Indication	Active pharmaceutical ingredient (API)	Viscosity enhancer	Marketing authorisation holder
Hyprosan	Dry eyes, keratoconjunctivitis sicca	Hypromellose	Hypromellose, sodium hyaluronate	Santen Pharmaceutical Co., Ltd.
Cosopt	Glaucoma	Dorzolamide, timolol	Hydroxyethylcellulose	Santen Pharmaceutical Co., Ltd.
Oftagel	Dry eyes, keratoconjunctivitis sicca	Carbomer	Carbomer, Polyvinyl alcohol	Santen Pharmaceutical Co., Ltd.
Yellox	Post-cataract surgery eye infection	Bromfenac	Povidone	PharmaSwiss Ceská republica s.r.o.
Visine Advanced Redness + Irritation Relief (not sold in Finland)	Redness of the eyes, irritated eyes	Dextran 70, PEG 400, povidone, tetrahydrozoline HCl	Dextran 70, PEG 400, povidone	Johnson & Johnson
Lacrisert (ophthalmic insert)	Severe dry eye syndromes, keratoconjunctivitis sicca	Hydroxypropylcellulose	Hydroxypropylcellulose	Bausch & Lomb

2.5 Manufacturing of ophthalmics

2.5.1 Overview

Manufacturing ophthalmic solutions resemble the manufacturing of parenterals, due to the strict sterility requirements (Eudralex – Volume 4, Annex 1). The industrial scale manufacturing process consists of several steps: liquid manufacturing, filtering, filling, sterilisation, quarantine, inspection, labelling, and packaging. Production process begins with analysing the incoming raw materials, which are released for production once their

quality has been approved. Required amount of approved raw materials (API and excipients) are weighed and transferred to solution manufacturing, where they are mixed with the solvent in sterilised or cleaned large compounding vessels, such as seamless steel tanks. After the solution contains all the components and is thoroughly homogenised, it is filtered before the filling step may begin. Ideally, the primary packages (blow-fill-seal (BFS) vial or multidose bottle) are filled with the product solution and terminally sterilised afterwards. However, the terminal sterilisation is not always possible, usually due to stability issues of the product or package material. In this case, either aseptic manufacturing or sterile filtration in combination with aseptic filling need to be used. After sterilisation, the filled containers containing the sterile product are put into quarantine before the quality of the product is inspected. Finally, the primary packages are labelled and encased with the package leaflet in the secondary package, which is sealed and serialised with a data matrix code. The process flow of the production at the NextPharma facility is presented in Figure 3. Throughout the process, GMP (Good Manufacturing Procedure) guidelines (Eudralex – Volume 4) need to be followed, and manufacturing must be done in compliance with the national authorities and legislation (Medicines Act 395/1987).

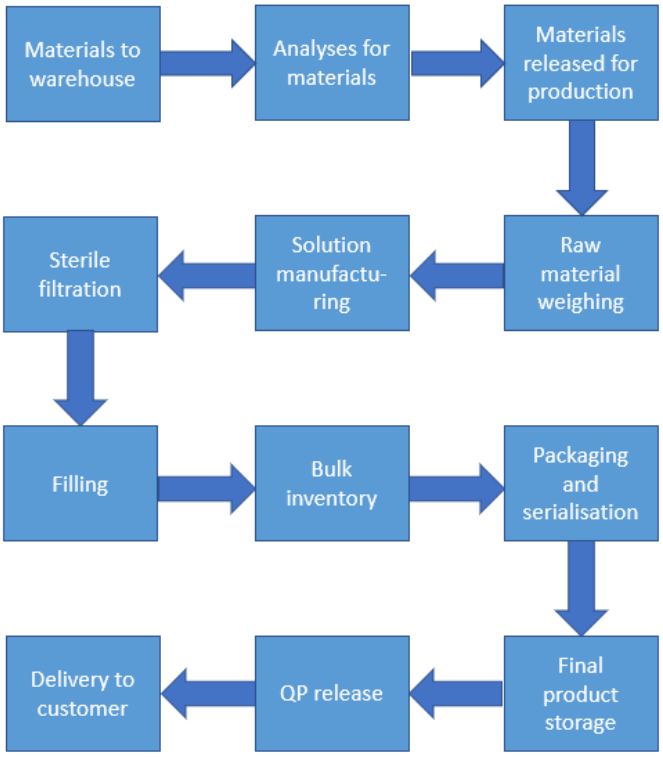


Figure 3. Production process flow

2.5.2 Solution compounding

The manufacturing of ophthalmic solutions can be as simple as a stepwise addition of excipients and API to the solution base in a compounding vessel and thoroughly mixing until homogeneous. However, usually the process is slightly more complicated, and some parameters need to be measured and adjusted during the compounding. Typical in-process controls for compounding are pH measurement and visual check to confirm the dissolution of raw materials. Sometimes using more than one vessel is required, if for example the formulation contains components which cannot withstand a heating process or heat sterilisation. These components then need to be compounded separately in another vessel and added to the main vessel afterwards through a sterile filter. Several process parameters

need to be validated for the solution manufacturing, such as temperature range and mixing speed in the compounding vessel(s), pressure during filtration etc. Process times should be minimised to avoid excessive bioburden before the sterilisation, and a low level of bioburden needs to be maintained throughout the manufacturing process (Eudralex – Volume 4, Annex 1). The clean areas need to be designed to minimise possibilities for contamination. The areas are maintained at positive pressure, working clothes need to shed no fibres or other particles, and environmental conditions are monitored with settle plates and particle counters throughout the process. After the compounding is finished, the solution is usually sterile filtered and aseptically filled to primary packages.

2.5.3 Sterile filtration

As mentioned earlier, quite often the formulation cannot withstand the conditions in the terminal sterilisation process, and chemical degradation or changes to the properties of the solution (e.g., viscosity) may occur. Additionally, ophthalmic solutions usually use LDPE bottles as a primary package, which cannot withstand the terminal heat sterilisation either (Gibson 2009). In this case, an alternative sterilisation method in the form of filtration and/or aseptic manufacturing needs to be chosen and justified to the regulatory authorities. Sterile filtration is a common method used in the manufacturing processes of ophthalmic solutions, due to its ease of use compared to aseptic manufacturing from sterile materials. A bioburden sample is taken from the solution before the filtration, and a validated filter integrity test is performed on the sterile filter before and after the filtration (Meltzer et al. 2008). The bioburden test and filter integrity tests need to be passed in routine production to confirm the microbial retention by the filter material. Critical parameters in this process step are filtration pressure and filtration time. Sterile filtration should be performed as close to the filling station as possible, however the time between the start of the preparation of a solution and the sterile filtration should be minimised (Eudralex – Volume 4, Annex 1).

The filling must be done aseptically in a grade A clean area, and every part or surface that is in contact with the product, such as the receiving vessel, tubing to the filling line, sterile filters, product contact parts of the filling equipment, and primary packaging materials need to be sterile. The principle of sterile filtration from compounding vessel to the holding vessel is presented in Figure 4.

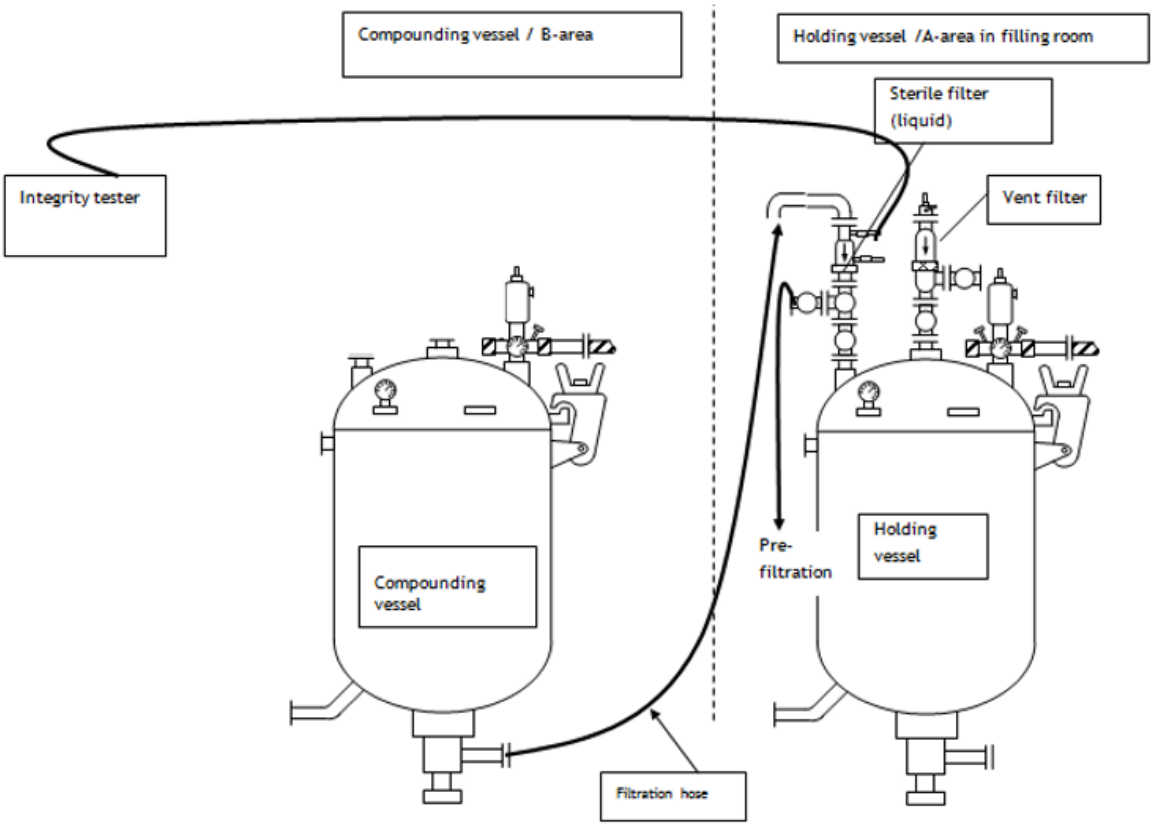


Figure 4. Sterile filtration principle (NextPharma Oy, unpublished presentation 2020)

While sterile filtration is often the preferred sterilisation method in ophthalmic solution manufacturing, problems may arise when using this method for viscous solutions, due to the clogging of the filters and slow filtration rates (Allmendinger et al. 2015; Coulais et al. 2015; Frei-Rutishauser et al. 2016). Hypromellose and other cellulose derivatives are also

known to contain impurities such as unreacted cellulose fibres and poorly soluble particles, which can cause problems during filtration (Porsch et al. 1997; Amouriq et al. 2002). To avoid these problems during solution manufacturing, specialised sterile filters or process optimisation is usually required.

3 HYPROMELLOSE

Hypromellose (hydroxypropylmethylcellulose, HPMC) is a partly O-methylated and O-(2-hydroxypropylated) cellulose, that is widely used in ophthalmic liquid formulations as an excipient (e.g., Fotil, Fotil forte, Alomide, Emadine, Isopto carpine, Livostin, Maxitrol, Pred Forte) or active ingredient (e.g., Artelac, Hyprosan) (Rogers 2009; Duodecim – lääketietokanta 2020). Hypromellose starts to gel on contact with water, increasing the viscosity of solutions, which can then be utilised to increase the contact time with the eye. Hypromellose can also be used as artificial tears as it increases the contact time and adhesiveness of lacrimal fluid and moisturises cornea and conjunctiva. Hypromellose is available in several grades that vary in molecular weight and degree of substitution (DS) i.e., the average number of substituted hydroxy groups per monomer unit. The structural formula of hypromellose is presented in Figure 5, where R is H, CH_3 or $CH_3CH(OH)CH_2$.

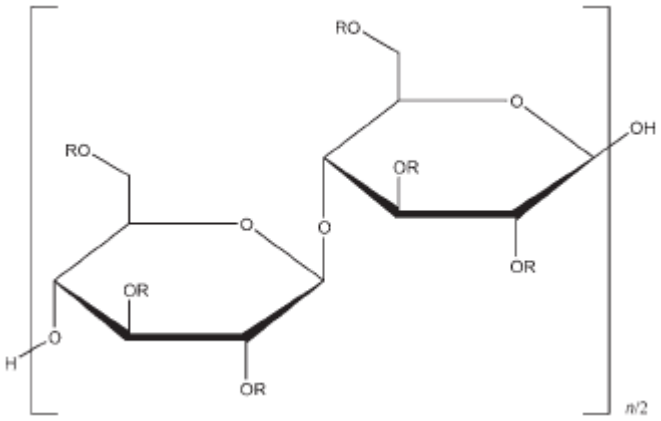


Figure 5. Structural formula of hypromellose (Rogers 2009)

Hypromellose is a derivative of cellulose, which is a natural substance and the principal structural material of all major plants, such as trees, cotton, seaweeds, and mosses (Richardson and Gorton 2003). Cellulose is a uniform, linear glucose polymer, consisting of anhydroglucose units (AGU) linked together by β(1⟶4)-D-glucosidic bonds. To produce hypromellose, the cellulose obtained from wood pulp or cotton is purified and reacted with NaOH solution (Richardson and Gorton 2003; Rogers 2009). Under alkaline conditions, the hydrogen bonds between the polymer chains are broken, which causes the cellulose to swell and absorb water. This opens the chains, making the hydroxyl groups within the AGU monomers more easily accessible, increasing the reactivity of the cellulose. The cellulose is then treated with chloromethane and propylene oxide, which react with the hydroxyl groups, producing methyl hydroxypropyl ethers of cellulose (Richardson and Gorton 2003; Rogers 2009). In this step of the process, a vast range of reactions are possible regarding the substitution of the hydroxy groups in cellulose (Zhou et al. 2014). The three free hydroxyl groups in the anhydroglucose units differ in reactivity, causing the substituents to distribute unevenly within monomers. The substituents may also distribute heterogeneously along the polymer chain, which may alter the behaviour of the final product (Richardson and Gorton 2003; Viridén et al. 2009a-c; Larsson 2010; Viridén et al.

2010a-b; Viridén et al. 2011a-b; Zhou et al. 2014). The product characteristics cannot be independently controlled during the manufacturing process of hypromellose, which is why the suppliers usually are not able to provide samples of hypromellose with fixed and desired characteristics (Košir et al. 2016). The properties of the hypromellose product are dependent on the quality of the wood pulp raw material and the parameters in the manufacturing process, and there can be major differences between suppliers, grades, and even different batches of hypromellose (Zhou et al. 2014).

Hypromellose powder is a stable material at room temperatures, although hygroscopic (Rogers 2009). Hypromellose molecules are non-ionic and generally stable in solutions over a pH range of 3-11 (Dow 2002; Rogers 2009). The non-ionic nature also renders hypromellose to be quite resistant to precipitation by metallic salts of ionic organics. However, if the amount of electrolytes in the solution exceeds certain limits, the competition of water molecules may result in reduced hydration and precipitation of hypromellose. Hypromellose solutions can be prepared by either: dispersing the raw material in cold water with vigorous stirring, dispersing the powder in hot water (above 90°C) with subsequent cooling, or dispersing in a non-solvent media such as vegetable oil, glycerin, corn syrup, polyethylene glycol (PEG), or concentrated salt solution (Dow 2002; DuPont 2020). Alternatively, the powder can be dry blended with other ingredients before adding to the solution. Hypromellose powders are soluble in cold water, but practically insoluble in hot water. The solution undergoes a reversible solution-gel transformation upon heating and cooling, and the gelation temperature varies between 50-90°C depending on the grade and concentration of the material. To prevent lumping during dispersion, either a high-shear mixer or similar is recommended or adding the powder to hot water (above 90°C) and cooling the solution after thorough dispersion.

4 PHYSICOCHEMICAL PROPERTIES OF HYPROMELLOSE

4.1 Specifications of Hypromellose grades

The critical properties of hypromellose are conventionally considered to be the levels of methoxy (MeO) and hydroxypropoxy (HP) substitution, viscosity, and molecular weight (Zhou et al. 2014). The European Pharmacopoeia (Ph.Eur 10.0) and the United States Pharmacopoeia (USP43-NF38) monographs define different hypromellose types based on their levels of methoxy and hydroxypropoxy substitution, which are presented in Table 2.

Table 2. Hypromellose types (Ph.Eur 10.0; USP43-NF38)

	Methoxy (%)		Hydroxypropoxy (%)	
Substitution type	Min.	Max.	Min.	Max.
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

Hypromellose manufacturers usually have also different grades within these types, based on their level of viscosity (mPa*s, 2% in water at 20°C). The compendial monographs of hypromellose (Ph. Eur 10.0; USP43-NF38) require the viscosity to be within 80-120% of the value stated on the label. As the acceptable ranges of these critical properties are quite broad, a noticeable amount of variation within grades exists (Dahl et al. 1990; Piriyaprasarth and Sriamornsak 2011). This batch-to-batch variation may cause material from separate

batches of the same grade to behave differently (Dahl et al. 1990; Viridén et al. 2009b; Larsson et al. 2010; Košir et al. 2016). Just specifying the levels of substitution may also be too simplistic in defining the characteristics of the polymer, as the substitution pattern may also have significant impact on the solubility and behaviour of hypromellose (Richardson and Gorton 2003; Viridén et al. 2009a-c; Zhou et al. 2014). Additionally, the specifications of the pharmaceutical grades of hypromellose do not provide information about its molecular weight or its distribution, although they influence the behaviour of the polymer (Larsson et al. 2010). Zhou et al. (2014) concluded that the current specifications are insufficient in ensuring comparable behaviour between batches, and new tests for characterising hypromellose need to be developed.

4.2 Substitution pattern

Batch-to-batch variation is a common problem with hypromellose products, not only due to the broad specifications of substitution levels and acceptable viscosity ranges in compendial monographs, but also due to the varying distribution of the substituents (Richardson and Gorton 2003; Viridén et al. 2009a-c; Larsson et al. 2010). The distribution of the substituents may vary within each glucose unit of the polymer, the hydroxypropoxy groups can propagate, and the substituent pattern can vary along the polymer chain (Larsson et al. 2010). Heterogeneous distribution of the substituents results in regions of unsubstituted glucose units and regions with more than average amount of substituents along the polymer chain. The substituent pattern has been shown in previous studies (Richardson and Gorton 2003; Viridén et al. 2009a-c) to affect the solution and gelling properties of cellulose derivatives. Heterogeneous substituent patterns in the polymer chain cause the polymer to form larger polymer structures in solution, increasing viscosity and causing significant loss in solubility (Richardson and Gorton 2003).

The distribution pattern of the substituents is affected by the parameters in the manufacturing process of hypromellose and the structure of the cellulose raw material. The cellulose polymer consists of semi-crystalline or microcrystalline areas, but also of significant amounts of disordered/amorphous regions (Zhou et al. 2014). When the cellulose swells in the manufacturing process, this causes the initial substitution to occur most likely on the crystal surface or in the amorphous regions, where the hydroxyl groups are more reactive and accessible. This may cause an effect, where the initial substitution promotes further reactions in the vicinity, due to the cellulose chain opening and creating more disordered cellulose. This may cause significant clustering of substituents, resulting in heterogeneity on substituent distribution along the polymer chain (Zhou et al. 2014).

The heterogeneity of hypromellose substitution can be investigated using enzymatic hydrolysis (Schagerlöf et al. 2006; Viridén et al. 2009a; Viridén et al. 2009c; Larsson et al. 2010; Zhou et al. 2014). The hydrolysis can be performed with endoglucanase enzymes, which can selectively break down the β(1⟶4)-D-glucosidic bonds in the cellulose chain. The substituents in the polymer chain present a steric hindrance, which prevents the hydrolysation of the enzymes, whereas the less substituted areas are more easily broken down. This causes a more randomly/homogeneously substituted hypromellose to be more robust against enzymatic hydrolysis than a heterogeneously substituted batch. The glucose released after the hydrolysis can then be detected using high-performance anion-exchange chromatography using pulsed amperometric detection (HPAEC-PAD) (Fitzpatrick et al. 2006; Viridén et al. 2009a). Alternatively, the enzymatic hydrolysates can be detected using size-exclusion chromatography (SEC) coupled with multi-angle-laser-light-scattering (MALLS) (Richardson and Gorton 2003). The characterisation of heterogeneity may provide valuable information about the properties of the polymer but can be analytically challenging and time-consuming.

4.3 Glass transition temperature

The glass transition temperature (T_g) is the temperature in which the interactions between the polymer chains start to break up due to the increased thermal motion of the main polymer chains (Larsson et al. 2010). At this temperature, the mechanical and mass transport properties of the polymer start to change, and the material transitions to a viscous or rubbery state. The T_g value of a polymer correlates to the interactions present in the material, thus giving valuable information about the behaviour of the polymer. The T_g value is affected by the molecular weight, interactions, flexibility, and bulkiness of the side groups in the polymer chain. Gómez-Carracedo et al. (2003) studied the influence of methoxy and hydroxypropoxy substitution on the T_g of cellulose ethers and concluded that the main factor influencing the T_g value is hydrogen bonding. Increased amount of hydrogen bonds strengthens the interactions between the polymer chains, thus requiring more energy to break and causing the T_g value to increase. Therefore, a high methoxy/hydroxypropoxy ratio should lower the T_g value, due to the methoxy groups blocking the hydrogen bonding. However, for samples with a different total degree of substitution, the methoxy/hydroxypropoxy ratio might not be as relevant (Larsson et al. 2010). The substituent ratios or the degree of substitution cannot explain all differences in the T_g values, which indicates that there are other batch specific interactions affecting the T_g.

Larsson et al. (2010) studied the differences in substituent patterns of two batches of hypromellose, which were of the same grade, but behaved differently. The batch specific differences were not detectable from the FT-IR spectrums nor the water vapour sorption analysis. However, the dynamic mechanical analysis (DMA) showed significant difference in T_g and onset temperatures between the batches. After further examination with four different batches, they concluded that the differences between the T_g values could not be

explained by differences in molecular weight, DS or the methoxy/hydroxypropoxy ratios of the polymers. After testing the heterogeneity in the distribution of the substituents using enzyme hydrolysis technique, they noticed a correlation with the T_g and the percent of glucose liberated after enzyme hydrolysis. Based on their findings, increasing heterogeneity seems to lead to increased interactions between the polymers, thus increasing the T_g value and influencing the behaviour of the material. In unsubstituted regions, the OH -groups of the glucose unit can form hydrogen bonds more freely, increasing the glass transition temperature (T_g) value of the polymer. It has also been hypothesised that the hydrophobic substituents have stronger interactions in heterogeneously substituted polymers, which would also increase the T_g value. Based on the results of their studies, Larsson et al. (2010) suggest the use of dynamic mechanic analysis (DMA) for the determination on the glass transition temperature as a good complement in the characterisation of hypromellose samples with heterogeneity of substituents.

4.4 Molecular weight distribution and average molecular weight

The molecular weight of a polymer usually correlates to the length of the chain, which can be expressed by the degree of polymerisation i.e., the number of monomers in the chain (Ravve 2012, p. 52). Longer chains behave differently in solutions, as they have decreased mobility, increased chain-to-chain interactions and entanglements, and increased glass-transition temperatures. Molecular weight affects many physical properties of the polymer, such as viscosity in a solution, and it can be calculated as a number-average molecular weight (M_n) or weight-average molecular weight (M_w). The M_n is the total mass of the sample divided by the number of molecules in the sample, whereas M_w has more emphasis on the weight of each individual molecule, usually being 3-10 times the M_n (Ravve 2012, p.52; DuPont 2020). The ratio of M_w to M_n gives an indication about the distribution of

molecular weight in the sample. Two polymer samples with equal M_w may have different physical properties, if their molecular weight distributions are different. Variations in molecular weight distribution and average molecular weight can affect key processes in the dissolution of the polymer, such as wetting, hydration, swelling, and gel formation (Levina and Rajabi-Siahboomi 2014; Mašková et al. 2020). Polymers with a higher molecular weight form a thicker gel layer and tend to swell faster compared to low molecular weight polymers (Tritt-Goc et al. 2005). The molecular weight of hypromellose typically varies from 10 to 1500 kDa (Rogers 2009). Hypromellose is known to be quite polydisperse material, partly due to the natural variety in the molecular weight of cellulose (Larsson et al. 2010; Levina and Rajabi-Siahboomi 2014). However, the specifications of the pharmaceutical grades of hypromellose usually present only solution viscosity values instead of providing information about the molecular weight or its distribution. To better predict the behaviour of the polymer material, determining these values as well is usually recommended.

Size-exclusion chromatography (SEC) coupled with multi-angle-laser-light-scattering (MALLS) is a useful technique for determining molecular weight distribution and average molecular weight of polymers (Richardson and Gorton 2003; DuPont 2020). In this technique, the analytes are separated based on their hydrodynamic volume and eluted in order of decreasing size. The stationary phase in the column is composed of a porous, three-dimensional network, which can be composed of e.g., silica, polystyrene-divinylbenzene or dextran (Hansen et al. 2012, p. 158-160). Separation occurs when the analytes penetrate into the pores of the stationary phase. The larger particles cannot enter the pores, so they travel through the column at the same pace as the moving phase and elute first. Smaller particles enter the pores of the stationary phase, which increases their travel time. At the detector, the particles pass through a laser source, which causes the light to scatter in multiple directions, which is then detected at different angles. The intensity of the light

scattered by the molecule is directly proportional to its molecular weight, and the angle gives information of the size and conformation of the molecule.

4.5 Solution behaviour of hypromellose

When exposed to aqueous media, hypromellose quickly starts to hydrate, resulting in a lowering of T_g below ambient temperature and the polymer transitioning into an amorphous/rubbery state (Viridén et al. 2009b; Viridén et al. 2011b; Ford 2014). The hydration rate of the polymer may be affected by the particle size of the material and the different ratios of MeO/HP substitution. Since the methoxy group is more hydrophobic than the hydroxypropoxy group, the solubility of hypromellose may also vary depending on the ratio of these substituents (Larsson et al. 2010). After initial hydration and progressive plasticisation of the polymer, a swelling process starts, uncoiling and extending the polymer chains (Ford 2014). As the uncoiling progresses, more locations become available for hydrogen bonding and further molecular interactions. This process results in the gelling of the solution, increasing its viscosity. The swellability of the hypromellose polymer is affected by the degree of substitution and the ratio of the substituents (Košir et al. 2016). The average particle size and particle size distribution influence the swelling as well; small particles swell faster, resulting in faster and more uniform gel formation. However, this effect diminishes after the hypromellose chains have been fully hydrated. Hypromellose solutions exhibit pseudoplastic viscosity behaviour, which results in a decrease of viscosity as the shear rate is increased (DuPont 2020). This shear thinning behaviour needs to be considered when performing viscosity measurements with a rotational viscometer, as the shear rate is dependent on the rotational speed, size and shape of the spindle, and the size and shape of the container used (Brookfield Manual No. M13-2100).

The European Pharmacopoeia (Ph.Eur 10.0) classifies viscosity and degree of substitution as the two relevant characteristics for hypromellose, when it is used as a viscosity enhancer. However, many studies (Richardson and Gorton 2003; Viridén et al. 2009a-c) have indicated that the reality is more complicated, and there are a lot of other characteristics that may have a significant effect on the behaviour of hypromellose. In addition to DS, the solution properties of cellulose derivatives are affected by the type of substituent groups, their position, ratios, and distribution along the polymer chain (Richardson and Gorton 2003; Akinosho et al. 2013). The ratio of methoxy/hydroxypropoxy -substituents and the molecular weight of the polymer affect the thermal gelation temperature and swelling properties of hypromellose (Sarkar 1979; Viridén et al. 2009b; Mašková et al. 2020). In aqueous solutions, hypromellose exhibits reversible solution-gel transition at temperatures between 50 to 90°C, depending on the polymer grade and concentration. The gelation point depends on the levels of methoxy and hydroxypropoxy substitution and the ionic strength of the solution. At low temperatures, the polymer is fully hydrated, while at high temperatures, the polymer starts to dehydrate, resulting in more hydrophobic interactions between methoxy groups. This causes the gelling of the solution and is often accompanied by clouding of the solution. The cloud point is the temperature at which the polymer molecules start to phase separate from the solution, forming large aggregates (Akinosho et al. 2013). Presence of electrolytes may lower the cloud and gelation points of the solution due to reduced hydration, which increases the tendency for the polymers to form aggregates (Mitchell et al. 1990). The clouding behaviour is dependent on several factors, including molecular size and structure, sample concentration, solvent, rate of heating, etc, but when experimental factors are kept constant, differences in clouding can be related to molecular differences (Fitzpatrick et al. 2006).

On aqueous solutions of methylcellulose, (MC), it has been shown that there exists reversible temperature-dependent aggregation (Porsch et al. 1997). The solutions

deaggregate when refrigerated but start to aggregate when kept at room temperature for periods of time. This is likely caused by the decrease of hydration of polymer chains as the temperature is increased, which in turn increases the hydrophobic interactions between the chains. The increase in molecular size may contribute to an increase in aggregate formation, which have been shown to increase the flow resistance and result in blockage of filters (Porsch et al. 1997). This kind of aggregation is not as pronounced in hypromellose, as the hydroxypropyl groups reduce the hydrophobic interactions due to steric and hydrophilic effects. However, heterogeneous distribution of substituents could theoretically cause forming of similar aggregations even with hypromellose.

4.6 Effect of hydroxypropyl substitution

Increasing the hydroxypropyl substitution increases the initial gelation temperature in 2% hypromellose solutions and decreases the gel strength (Zhou et al. 2014). Akinosho et al. (2013) also found that higher amounts of HP substituents lower the crystallinity of hypromellose. These effects are probably caused by the steric and entropic effects caused by the bulky HP groups, which disrupts the hydrophobic interactions between methoxy groups and therefore reduces the interactions between polymer chains (Akinosho et al. 2013). This opens possibilities for hydrogen bonding between water molecules and the polymer, increasing the hydration of the polymer (Košir et al. 2016). In other studies, HP groups have been shown to decrease the elastic character of viscoelastic gels (Bodvik et al. 2010). The gel properties may also be influenced by structural differences in the distribution of the HP groups. In the manufacturing process of hypromellose, the hydroxypropyl group may theoretically substitute further with either a methoxy group or another HP group, even creating long HP oligomer side chains. The increased steric hindrance caused by this effect

may cause a decrease in the gel strength. However, unlike in some other cellulose ethers, there is little evidence for this kind of additive substitution in hypromellose (Ford 2014).

4.7 Analytical methods for characterising hypromellose

To gain more information about the differences between hypromellose batches, properties such as molecular weight, molecular structure, morphology, thermal behaviour, and rheology should be investigated. As described earlier in this text, SEC-MALLS is a useful technique for determining the average molecular weight and its distribution for hypromellose. SEC can be used to separate the molecules based on their hydrodynamic volume, and the masses of said molecules can be determined by a light scattering detector such as MALLS or LALLS (low angle laser light scattering) (Wittgren and Porsch 2002). These can be used in combination with a concentration-based refractive index (RI) detector. Coupling MALLS to RI detection gives signals proportional to the molar mass and concentration, allowing direct determination of molar mass without the need of calibration standards (Richardson and Gorton 2003). The advantage of MALLS compared to LALLS is the ability to also determine the size of the molecules. The average molecular weight can also be calculated by measuring the intrinsic viscosity with a capillary viscometer. However, this method does not provide information about the molecular weight distribution.

Structural characterisation for polymers is usually performed after degradation, as the intact polymer can be too large and complex for most analytical techniques (Richardson and Gorton 2003). The degradation can be done via partial or complete hydrolysis of the polymer and a subsequent analysis the hydrolysis products. With this method, information about the distribution of substituents along the polymer chain, or within monomer units

can be extracted. For characterising the hydrolysis products, HPAEC-PAD is an alternative to SEC-MALLS (Viridén et al. 2009a). HPAEC-PAD quantifies the amount of unsubstituted glucose and oligosaccharides liberated in the enzymatic hydrolysis, while SEC-MALLS measures the molar mass of the hydrolysates. However, these techniques do not measure changes in the substituent distribution level. For this purpose, techniques that are sensitive to chemical differences, such as nuclear magnetic resonance (^{13}C NMR or ^{1}H NMR), infrared (IR) spectroscopy, or mass spectrometry (MS) can be used (Fitzpatrick et al. 2006). Viridén et al. (2009a) used proton nuclear magnetic resonance (^{1}H NMR) for determining the degree of substitution of hypromellose. NMR can also be coupled with SEC, to gain more information about the relationship between molecular size and level of substitution (Fitzpatrick et al. 2006). The degradation products can also be analysed using different mass spectrometric techniques, such as electrospray ionisation ion trap (ESI-IT), ESI-triple stage quadrupole (ESI-QqQ), and matrix assisted laser desorption ionisation time-of-flight (MALDI-TOF) (Adden et al. 2009).

Other ways of characterising polymers, such as hypromellose, include studying its morphology, thermal properties, mechanical properties, and rheology. Morphology i.e., the overall form of the polymer structure can be studied using X-ray diffraction (XRD) if the polymer is solid and crystalline (Kljun et al. 2011), or different microscopic methods, such as transmission electron microscopy (TEM) (Bodvik et al. 2010) or scanning electron microscopy (SEM) (Amouriq et al. 2002). Semicrystalline polymers can be analysed using small-angle X-ray scattering (SAXS) (Bodvik et al. 2010), or differential scanning calorimetry (DSC) (Akinosho et al. 2013), which also provides information about the polymer's thermal behaviour. Thermal properties, such as glass transitions and other phase changes can be analysed also with thermogravimetric analysis (TGA), differential thermal analysis (DTA), thermomechanical analysis (TMA), and dynamic mechanical analysis (DMA) (Manley 1989).

DMA and TMA are also useful techniques in characterising the viscoelastic behaviour of solid polymers.

Rheology i.e., the study of the flow of a material in solution, directly affects the filterability of the solution. Rheological properties of a solution, such as viscosity can be analysed using a rotational or extensional rheometer. As hypromellose is a pseudoplastic material, its viscosity is affected by the measuring parameters: shear rate, temperature, size and shape of the container, and size and shape of the spindle (AMETEK Brookfield 2017). To gain a thorough understanding of the material's behaviour in a solution, its viscosity should be measured as a function of temperature on both heating and cooling, and at different shear rates. As viscosity affects filterability, finding the temperature where the viscosity is at its minimum would be beneficial. Additional important parameters to investigate are the shear rate and how much it influences the viscosity of our sample solutions, and how rapidly the viscosity returns to normal level after shearing.

5 POSSIBLE REASONS FOR FILTER CLOGGING

There are several different mechanisms, which may contribute to the filterability issues of hypromellose solutions. The properties of the hypromellose product are dependent on the quality of the wood pulp raw material and the parameters in the manufacturing process. Due to the natural variability of the cellulose raw material, the molecular weight distribution, and the average molecular weight of hypromellose may vary between batches, possibly affecting the gelling properties of the solution. Polymer chains with higher molecular weight

may also contribute to clogging of filters due to slower disentanglement, lower solubility, and higher tendency of aggregation. Due to the broad specifications of acceptable viscosity ranges and substitution levels in compendial monographs, different batches of the same grade may have vastly different chemical properties, even though they fulfil the specifications. Different batches may have different ratios of methoxy/hydroxypropoxy substitution and different total degree of substitution, which affect the solution behaviour of hypromellose such as gelation temperature, gel strength, and aggregation of the polymers. As described earlier, the substituents may also distribute heterogeneously along the polymer chains during the manufacturing process, which can cause unpredictable behaviour in a solution. Heterogeneous substitution can affect the solubility of the raw material, viscosity of the solution, and may contribute to the aggregation, especially in the areas or chains which have a lower presence of HP groups. These kinds of aggregates tend to dissolve more slowly than individual polymer chains, which may contribute to the clogging of the filters if the mixing time before filtration is not adequate. As cooling of the solution has been shown to decrease aggregation due to increased hydration, it could have a positive effect on filterability (Porsch et al. 1997). A previous laboratory test at the NextPharma Oy site found an improvement in filterability with samples which were refrigerated and filtered few days later. Other studies (Coulais et al. 2015) have also found, that the filterability is improved if the solution is allowed to stand for some time (few days) before filtration. This may be due to improved dissolution of less soluble particles, more time for the aggregates and longer polymer chains to hydrate and disentangle, and/or the change in viscosity over time. The lower temperature may also have an effect due to increased hydration of the polymer chains. However, cooling the solution below room temperature in production scale is challenging, and the microbiological purity requirements of the solution also limit the storage time prior filtration.

The viscosity of the solution is also an important factor affecting the filtration; more viscous solutions tend to have slower filtration rates and higher probability of clogging the filters (Coulais et al. 2015; Frei-Rutishauser et al. 2016). As the viscosity of the hypromellose solution is affected by temperature, it might be interesting to test the effect of different solution temperatures on filterability. However, this is not easily implemented at production scale, because the filter integrity test methods have been validated for temperatures of 20 ± 5°C (Pall Corporation 2005). Allmendinger et al. (2015) studied factors affecting the sterile filtration of protein formulations and discovered that the presence of surfactant can have an impact on the filtration performance. In their study they found that addition of polysorbate 20 increased filtration resistance due to the surfactant adsorbing to the surface of the polyether sulfone (PES) -filter. Some ophthalmic products contain benzalkonium chloride (BAC) as an antibacterial preservative, which also has surface-active properties. Although the amounts used are quite small, investigating its effect on filterability could provide useful information. Allmendinger et al. (2015) also noticed, that the applied shear rate defined by filtration pressure can affect filterability. On shear thinning materials such as hypromellose, increased filtration pressure or mixing speed should theoretically improve filterability due to increased shear rate. Coulais et al. (2015) investigated the effect of operating parameters on sterile filtration of hyaluronic acid, and suggested using constant, high pressure during filtration, preferably with a pressurised vessel instead of a pump. Other sources (Frei-Rutishauser et al. 2016) suggest starting the filtration at low pressure, and gradually increasing the pressure to operating level. Based on these studies, testing the filterability at different pressure levels might be worth investigating. Finally, there is usually always some amount of fragments of fibres, gel particles, and other insoluble matter present in solutions of cellulose ethers, which may cause the clogging of the filters (Porsch et al. 1997). The insoluble impurities are a more difficult problem to solve because they are most likely to be unaffected by the adjustment of process parameters. Investigating the insoluble matter on the filters could give valuable

information on whether the problem can be solved by simply process optimisation or if some other solutions need to be considered.

6 AIM OF THE STUDY

The focus of this project was the investigation of filterability issues during the manufacturing of ophthalmic solutions containing hypromellose (Methocel E4M, manufactured by DuPont) at NextPharma Oy, Tampere. The primary goal was to solve the filterability problem, and additional goals included getting more information about the characteristics and behaviour of hypromellose, finding out what causes the clogging of the filters, what causes the batch-to-batch variation, and how to improve lab-scale filterability testing. In previous studies at NextPharma Oy, the filter clogging problem has been mitigated by using an asymmetric dual layer polyether sulfone (PES) membrane filter, which is intended for filtration of viscous solutions. The filter currently used consists of an asymmetric PES pre-filter layer and a hydrophilic PVDF final filter layer. However, the sterile filtration is defined as a critical process and switching the filter material would require a new marketing authorisation application for the customer company, which is a time and money consuming process.

The plan for the research project was to test if filterability of hypromellose containing solutions could be improved by adjusting different process parameters, such as mixing speed and -time, solution temperatures and dissolution times. Also, to be determined was the critical concentration of hypromellose i.e., the concentration where filtration problems begin to occur. Additional experiments of interest were the particle size distribution analysis

and molecular size distribution analysis of the raw material and their effect on filterability, analysing the undissolved content, and testing different filter materials. The aims of the study were investigating the variability in hypromellose raw materials, i.e., why some batches cause the clogging of the sterile filters and finding the optimal process parameters to achieve better filtration results.

7 MATERIALS AND METHODS

7.1 Compounding process

A screening of process parameters was performed in laboratory scale, simulating production scale conditions to test if the filterability of hypromellose containing solutions could be improved by adjusting different process parameters. The process flowchart is illustrated in Figure 6.

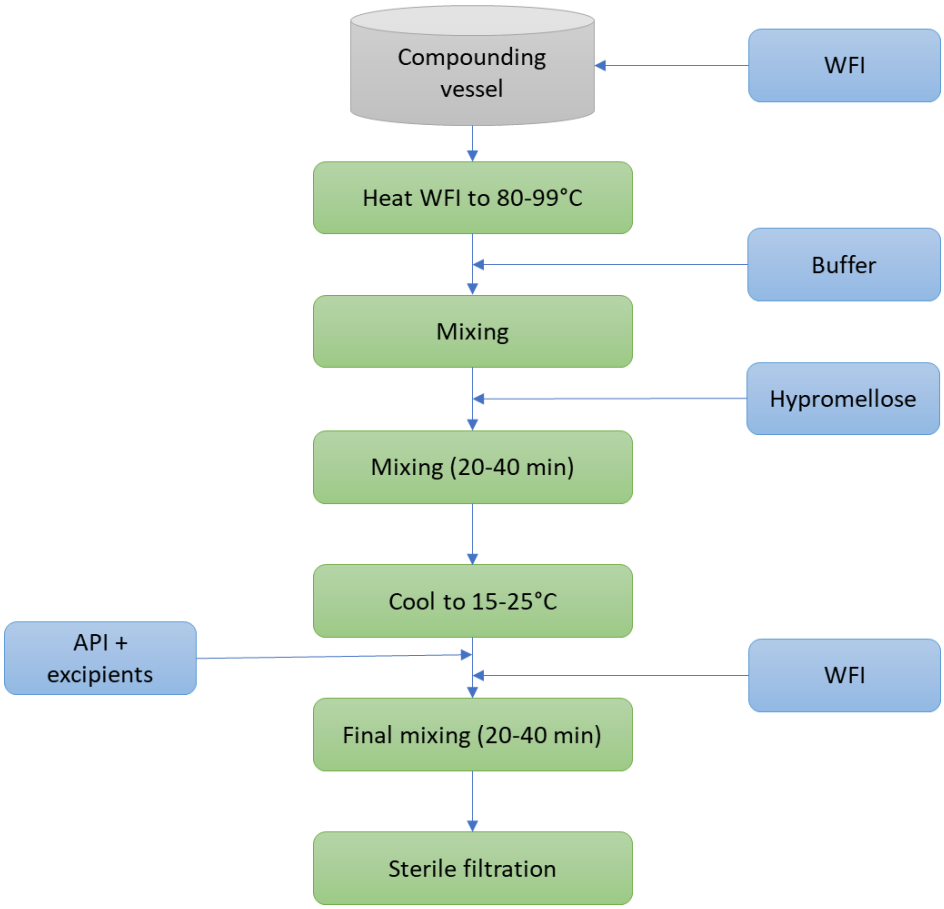


Figure 6. Process flowchart

The solutions were prepared by heating approximately 85% of the total volume of WFI to 80-99°C, slowly adding the hypromellose under mixing, dispersing the hypromellose for 20-40 minutes, and cooling the solution under mixing to 15-25°C. The cooling was performed by placing the compounding beaker in a basin filled with cold water and ice. After cooling, WFI was added up to final volume and the solution was mixed for another 20-40 minutes. The mixing speeds used for the screening were 250-350 rpm. After inspecting the solution for undissolved content and clarity, sterile filtration was performed. The filtrate was collected to a tared laboratory beaker on a top loading balance, and during filtration the amount of filtrate was recorded in one-minute intervals for up to 30 minutes, after which

the filtration was stopped. Viscosity of all filtered solutions was measured, and pH was measured of the solutions in the preliminary testing for comparison to the buffered solutions.

7.2 Instruments and reagents

The test solutions (0.5% (m/V) hypromellose in water) were prepared using WFI -grade water, and all the reagents used were Ph.Eur. quality. For the heating and mixing of the solution, laboratory heaters with magnetic stirrers were used, and a paddle stirrer was also tested. Reagents were weighed using analytical laboratory balance, and the solution and beaker were weighed using a larger top loading balance. Sterile filtration was performed using a peristaltic pump, silicone tubing, and a capsule sterile filter. During preliminary testing, a pressure tank was also tested for the filtration. The viscosity measurements were performed using a rotational rheometer, using same parameters as for the actual product: 8 ml sample volume, 25°C solution temperature, 60.6 rpm spindle speed, and 1 min measuring time. Same spindle and parameters were used for each measurement. The instruments used are presented in Table 3, and the reagents used are presented in Table 4.

Table 3. Instruments used in the experiments

Instrument	Model
Analytical balance	Mettler XP205 Delta Range
Magnetic stirrer/heater	Framo Geratetechnik M21/1
Magnetic stirrer/heater	VWR VMS-C10
Paddle	Heidolph PR 30 Pitched-Blade Impeller
Paddle stirrer	Heidolph RZR 2052 Control
Peristaltic pump	Cole-Parmer Masterflex L/S Digital Drive
pH meter	Mettler Toledo Sevenmulti S40
pH probe	Mettler Toledo InLab Routine Pro
Pressure tank	Merck Millipore 5 l XX6700P05
Pump tubing	Masterflex L/S silicone tube
Rheometer	Brookfield DV3T-LV
Rheometer spindle	Brookfield Ametek SC4-18
Sterile filter	Mini Kleenpak 20 capsule with Fluorodyne EX EDF membrane (by Pall Corporation, UK)
Sterile filter	Mini Kleenpak capsule with Fluorodyne EX EDF membrane (by Pall Corporation, UK)
Tachometer	Testo 470
Thermometer (solution preparation)	Testo 110 probe thermometer
Thermometer (viscosity measurement)	ETI ltd. 222-055 reference calibration thermometer
Toploading balance	Mettler Toledo XP32001L
Waterbath (viscosity measurement)	Brookfield TC-202

Table 4. Reagents used in the experiments

Reagent	Manufacturer	Grade
Hypromellose	DuPont	Methocel E4M
Citric acid monohydrate	Merck KGaA	Ph.Eur
Sodium citrate	Merck KGaA	Ph.Eur

7.3 Design of experiments

During the screening and initial testing, only one batch of hypromellose was used due to possible batch-to-batch variation, which might skew the results. Additional testing was performed after the screening, which included two other hypromellose batches to see if the optimised parameters would provide consistent results regardless of the hypromellose batch used. The effect of four different input parameters were tested in the screening: mixing time, mixing speed, dispersion temperature and cooling temperature. The measured output parameters were filtration rate, viscosity, and total amount of filtered solution. The screening of experiments was designed using Modde Pro 12.1 software (by Sartorius AG, Germany). For the screening, a full factorial design using 2 levels with no replicates and 3 centre point measurements was used, for a total of 19 runs. The order of the test runs was randomised to reduce the effect of outside factors that might affect the results. Statistical models were created using PLS (partial least squares) method and the results were analysed using Modde Pro 12.1 analysis wizard.

7.4 Preliminary testing and method development

7.4.1 Testing pressure tank and paddle stirrer

First solution was prepared for the testing of a pressure tank for filtration and a paddle stirrer for mixing. The pressure tank would simulate the conditions in the production more closely than a peristaltic pump since the pressure stays constant during the filtration. Paddle stirrer was tested to adjust the rpm levels more accurately, and to get more repeatable experiments. For filtration, Fluorodyne EX EDF Mini Kleenpak 0.2 µm filter capsules (by Pall Corporation, UK) were used, which have 20 cm^2 nominal effective filter area (EFA). According to sources from Pall, the EFA value contributes to the total amount of solution which can be filtered, and this should scale linearly. For this reason, the appropriate volume of solution to be prepared for this filtration area was calculated, so that the ratio would resemble the ratio used in production scale. The EFA per litre ratio in production is approximately 55 cm^2/l, which would be approximately 0.4 l of solution for the filters used in lab scale testing. To challenge the filter and induce clogging of the filter, the sample size was increased to 1 l (2 l for the first solution). The hypromellose raw material was weighed into 5 container jars, which were then combined in the laboratory and mixed thoroughly in a large beaker and stored in two sealed plastic container bottles. The sample bottles were stored in a dark and dry place at room temperature.

The mixing speed used in production scale manufacturing is 250 rpm, which was decided to be tested as a minimum level for the screening. The maximum was set to 350 rpm, which is close to the upper limit of production scale mixers. For this solution, the maximum heating temperature that could be used during the screening before the solution starts to boil, was tested. The cooling temperature was 20-25°C, as instructed in the standard filterability test

used in the laboratory. The mixing speed was set to 250 rpm for this solution, and the solution was heated up to 98-99°C. Hypromellose was added to the heated solution slowly during 2-3 minutes without rinsing the beaker. It was observed that the mixing speed with the selected paddle was not sufficient, as it did not create a vortex and the hypromellose remained on the surface of the solution for some time after addition. The mixing was continued for 20 minutes, during which the solution started boiling at one point due to low mixing speed and high temperature (99.4°C at maximum). After 20 minutes, the solution was cooled in a water bath to 21.9°C while mixing at 250 rpm. Ice was added to the water bath to speed up the cooling process. The beaker was then removed from the water bath, and final water was added on a top loading balance up to 2018.0 g. The density of the placebo solution had been determined to be 1.009 g/cm^3, although that solution also contains citrate buffer. The density of the hypromellose solution without the citrate buffer was not measured in this study, but the same density value was used as it would not impact the results. Finally, the solution was mixed for 20 minutes with mixing speed of 250 rpm, and afterwards the solution was visually inspected for impurities, clarity, and undissolved content. One brownish fleck was observed at the bottom of the beaker and one also floating on the surface of the solution. Inspection of the paddle stirrer revealed that it was rusty on the inside. Most likely some rust fell off into the solution as the paddle was moved, attached, and detached during the experiment. Some transparent particles were also observed floating on the surface, which were probably undissolved hypromellose fibres. Additionally, above the water level on the beaker walls, some undissolved hypromellose powder and thicker gel were observed, which were likely not properly dissolved due to inefficient mixing.

The solution was poured into a pressure tank, which was sealed and connected to a gas line and a sterile filter capsule using pressure tubing. Filtration was performed to a tared glass container bottle on a top loading balance, starting with venting air out of the filter capsule

and filling the capsule with the solution using a low pressure (0.5 bar). 21.1 g of filter flush was collected to a graduated cylinder using 0.5 bar pressure, after which the main pressure valve was closed. It was observed that the solution dripped through the filter very slowly during filter flush. The filtration was then started by opening the pressure valve and increasing the pressure to 2.0 bars. The amount of filtrate (grams) was recorded in one-minute intervals and stopped after 30 minutes. Viscosity and pH were measured from the solution left in the pressure tank; the results are presented in Chapter 8.1.

7.4.2 Testing the effect of citrate buffer on filterability

In the large-scale manufacturing process, citrate buffering agents are added to the solution before addition of Methocel. Before starting the screening, the effect of citrate buffer on filterability was tested by comparing the results to a placebo without the buffer. A total of 7 solutions were prepared, 4 containing the buffer and 3 without the buffer, and their filterability results were compared. The fourth buffer solution was prepared due to one of the filtration results deviating from the other two in the first three measurements. The paddle stirrer was considered too rusty to be used so it was replaced with a magnetic stirrer and a 70 mm magnetic stir bar. The pressure tank was also switched to a peristaltic pump due to ease of use and cleaning. For this buffer testing procedure, 1 l solutions were prepared, and Pall Mini Kleenpak 20 capsules were used for filtration. In the standard NextPharma filterability tests, 5 l batches are prepared, and larger Pall Mini Kleenpak capsules are used. Otherwise, standard NextPharma filterability test guidelines for this product were followed: solutions were heated between 90-95°C and cooled to 15-25°C. Mixing times used were 20 mins, but mixing speeds were not accurately determined at this point. For the filtration, approximately 230 cm piece of pump tubing (single use) was used for each solution. Pump speed during filter flush and air venting was 50 rpm instead of the

100 rpm used in the standard NextPharma Oy filtration test. The actual filtration was carried out with 400 rpm pump speed. The results are presented in Chapter 8.1.

7.4.3 Preliminary tests for the conditions of screening tests

For the screening, at first the mixing speeds needed to be optimized. For this purpose, two smaller Framo M21/1 magnetic stirrers/heaters were chosen (coded PD34 and PD35) with more accurate dials than the previously used, larger VWR VMS-C10 plates (PD136 and CRA163). First task was to find a suitable minimum and maximum levels for the mixing speeds, but at this point of the study there was no method to accurately measure the rpms of the magnetic stirrers. Therefore, this was performed by comparing the vortex sizes at different mixing speeds using 840 ml of water in a beaker, which was the initial water volume before addition of hypromellose. The maximum mixing speed was set so that the vortex almost reached the magnet bar, and higher speeds would have caused splashing. At first, PD35 was used for heating step and PD34 was used for cooling and final mixing steps. However, it was soon after noticed that these heaters were not efficient enough to heat the solutions to 98-99°C, so the larger VWR plate (PD136) was brought back for the heating step. The minimum mixing speed was set for the PD34 and PD136 to a level which was just sufficient to mix the hypromellose solution after cooling (creating a small visible vortex in the solution).

Next objective was to find suitable minimum and maximum temperatures during the heating/dispersion step. According to literature, the dispersion temperature should be at least 80°C (Rogers 2009) or 90°C (Dow 2002; DuPont 2020). It was decided to test 80°C as the minimum temperature and 99°C for the maximum temperature. 80°C seemed suitable, as the hypromellose did not start gelling prematurely and there were no insoluble gel particles or clumps to be seen after the final mixing. For the maximum temperature, 99°C caused some problems as sometimes the solution started boiling, which is something that should be avoided. For this reason, 98°C was set as

the maximum temperature for the heating step. Another problem was evaporation of water during the heating step, especially with solutions that had longer mixing times and higher heating temperatures. The compounding beaker was covered with aluminium foil, but as it was not a fully closed system, some evaporation was bound to happen during the heating. This can cause some of the hypromellose to stick to the walls of the beaker as the solution evaporates and the solution surface level lowers, which causes problems during the filtration. An Erlenmeyer flask was tested instead of beaker to counter the excessive evaporation, but the idea was soon abandoned after one flask broke during the mixing. To counter the evaporation, small amounts of hot WFI was added instead during the heating step.

7.4.4 Additional testing for mixing speeds

After these preliminary tests, a tachometer was obtained from the validation department. With this device, testing and setting the rotation speeds of the magnet bars more accurately was possible. Four different magnet bars, four different beakers and four different magnetic stirrers (PD35 & PD36 (Framo M21/1) and PD136 & CRA163 (VWR VMS-C10)) were tested. It was already known that there are some differences between the magnetic stirrers, but surprisingly there were significant differences in the rotation speeds between different beakers and magnet bars as well. Some beakers had more convex bottom surfaces, and some magnet bars rotated more unsteadily than others, regardless of the beaker or stirrer chosen. As the differences were significant (up to 100 rpm), the best rotating magnet bar and beaker pair and was chosen, and it was decided to use only this pair for the screening. With this magnet bar/beaker pair, the rpms for the four stirrers were set: PD136 and PD35 to 250 rpm, CRA163 and PD36 to 350 rpm. However, after a few days of testing it was observed, that the rotation speeds had not stayed at the same level on the mixers. The smaller PD35 and PD36 mixers had had a significant drop in their mixing speeds, while the larger PD136 and CRA163 mixers rpms had stayed pretty much at the same level, with only

a minor drop in their mixing speeds. Therefore, the PD35 and PD36 plates were abandoned, and the screening was started over by using just the larger mixer plates. Due to the mixing speeds not staying consistent, it was decided to set and test the mixing speeds with a tachometer before every solution preparation from this point forward.

7.5 First screening and subsequent tests

7.5.1 Screening with Mini Kleenpak 20 -capsules

Now that the minimum and maximum levels for the input parameters had been set, and the test conditions adjusted for better repeatability, it was time to begin the screening process. For this screening, 1 l test solutions were prepared and Pall Mini Kleenpak 20 -capsules with Fluorodyne EX EDF membrane were used for filtration. However, the filter capsules ran out during the screening and only 13 out of 19 test solutions were analysed for this screening. The results are presented Chapter 8.2.

7.5.2 Viscosity measurements

After the screening, additional viscosity tests for the solutions that had been prepared earlier were conducted. The purpose of these tests was to see if the viscosity is affected by storage time, and how much it is affected by temperature. For this testing, three different solutions were used: S1401 (prepared 1 day before testing), S701 (prepared 8 days before testing) and S103 (prepared 15 days before testing). The solutions were measured at temperatures of 16, 20, 25, 30 and 40°C. The results are presented in Chapter 8.2.

7.5.3 Testing larger batch size

As the filter capsules ran out of stock and the screening could not be finished as originally planned, a decision was made to redo the screening using larger filter capsules with larger batch sizes. The plan was to use Pall Fluorodyne EX EDF Mini Kleenpak capsules for filtration, which have 240 cm^2 nominal effective filter area (EFA). To match the EFA/Volume -ratio in production, approximately 4.5 l batch size was calculated to be sufficient. To match the ratio used in previous screening (20 cm^2/l), 12 l batch size would be required, which would be too large for lab scale equipment. It was decided to test 7 l batches using 10 l beakers. First problem was scaling up the mixing efficiency, as the magnet bar dimensions could not be scaled up at the same ratio as the beaker size and solution volume. Two larger magnet bars were tested for these larger batches: one with similar diameter but slightly longer (80 mm length) (magnet bar B), and one with a larger diameter as well (magnet bar C). At first, the rpms were set to 300 using the beaker and magnet bar that had been used in the earlier screening. After that, the rpm was checked with a 10 l beaker and magnet bar C, and the result was around 330-340 rpm. It was also noted that the magnet bar C does not rotate steadily and has higher oscillation in the rpm levels while measuring with a tachometer. Nevertheless, it was decided to run the first test solution with this magnet bar as its dimensions were more appropriate for this batch size. First test solution was prepared using centre point parameters, with the exception that the mixing speed was slightly higher (330-340 rpm).

A problem was encountered at the beginning of cooling step of the first test solution, as the weight of the water bath combined with the larger beaker and batch size was too much for the CRA163 mixer, resulting in an error code and the mixer halting completely. The water bath was removed, and the mixer was attempted to start with just the beaker containing

the solution. The mixing still seemed laborious for the mixer and cooling the solution with a water bath was attempted again with the other mixer (PD136) after it had cooled. This mixer managed to stir the solution even with all the weight on top of it, although the speed seemed to slow down as the solution started to cool and form a viscous gel. The hypromellose had formed large “rags” in the solution during the time it was not mixed, but they dissolved during the cooling and final mixing steps and the solution appeared clear before starting the filtration. 200 ml of filter flush was taken with these larger filter capsules, but the pump speeds were kept the same as in the previous screening.

Before preparing the second test solution, three different beakers and magnet bars (size B) were tested with a tachometer, and the best rotating pair was chosen. The mixing speed was set to 300 rpm for CRA163 -mixer with the chosen magnet bar and beaker but was forgotten to set for PD136. This time the CRA163 -plate was used for heating step, as it cannot be used for the cooling step due to the excessive weight of the water bath. During the final mixing step, it was noted that the mixing speed was inadequate: no vortex was produced, and the solution seemed stagnant at surface level. Therefore, the mixing speed was increased during this step to achieve adequate mixing of the solution.

For the third test solution, magnet bar C was tested again due to inadequate mixing observed with magnet bar B in the previous solution. For this solution, the parameters which produced best results in previous screening were tested to see if the filtration result would improve in a similar fashion as with the smaller filter capsules. The best result in the earlier screening was with the solution S103, with a filtration rate of 20.44 g/min, so the same parameters were chosen for this test solution. The mixing speed was set to 250 rpm with the larger magnet bar C. It was quickly observed that once again the magnet bar C rotated unsteadily, causing the solution to form waves in the beaker during the heating

step. During the final mixing, again the mixing seemed inadequate as with the previous solution. The mixer also appeared to be at its maximum capacity, as no visual speed increase was observed when it was attempted to increase the mixing speed with the dial. The results for the test solutions are presented in Chapter 8.4.

7.6 Second screening

7.6.1 Preparations and testing

After testing the method with 7 l batch sizes, larger filter capsules, and different magnet bars, the next steps to be taken were discussed. As the mixing was problematic with the test solutions, some modifications needed to be made for the screening. It was decided to reduce the batch size to 5 l and abandon the larger magnet bar C, as it rotated poorly. To produce sufficient mixing with magnet bar B, the mixing speed would need to be increased. Paddle stirrer was also considered, but because at this point it was necessary to speed up the process due to timing constraints and start producing two solutions per day, the idea was abandoned as only one usable paddle stirrer was available for this study. A 10 l beaker was chosen instead of a 5 l beaker as a compounding vessel to get a better diameter/height relationship and therefore better mixing efficiency. Third VWR VMS-C10 mixer (coded PD135) was acquired to start producing two solutions per day. CRA163 was used for heating step, PD136 for cooling step, and PD135 for final mixing. As two beakers and magnet bars were also required to produce two solutions per day, several other 10 l beakers and magnet bars were tested for the screening, and the best pair of the tested ones was chosen.

For the first test solution (V101), mixing speeds were initially set to 250 rpm for all three mixers. This speed proved to be too low to produce a vortex during final mixing, so higher speeds with PD135 and PD136 were tested during the final mixing step. A maximum mixing speed, after which the speed did not seem to increase further when turning the dial, was found for both mixers. A minimum mixing speed was also determined for both mixers, where a small vortex was observed. Due to the testing of mixing speeds, the final mixing time was probably slightly longer than 20 minutes for this solution. Temperature of the solution was measured also before start of filtration: 16.8°C. The solution practically filtered completely, with only some left in the tubing and filter capsule as the 30-minute mark was reached and filtration was ended. During next day, the mixing speeds using the same mixer dial settings were measured with a tachometer: 340 rpm and 500 rpm were set as minimum and maximum mixing speed parameters for the screening, respectively. This solution ended up having the best filtration result of all the solutions that were tested with this hypromellose batch, but it was not included in the screening results due to longer mixing time during final mixing and testing of different mixing speeds.

7.6.2 Screening with Mini Kleenpak -capsules

Test solution V101 was not included in the screening, so screening was initiated with solutions V301 and V401, which were prepared during the same day. V301 was prepared using the same magnet bar and beaker as the previous solutions. As compounding of the second solution (V401) was started, a problem with the second beaker and magnet bar that had been chosen was observed. The magnet bar rotated unsteadily during heating step, which caused waves and quite a lot of hypromellose to stick on the walls of the beaker. The solution compounding and filtration was carried out, but the result was quite poor compared to the two previous solutions. The poor filterability result was assumed to be because of this unsteady mixing. During next day, three new (size B) magnet bars and three

new 10 l beakers were tested, to find a pair better suited for steady mixing. One of the beakers was found to be decent for mixing, so it was chosen with a magnet bar which had best rotation properties (out of the three tested) for the screening. It was decided to leave solution V401 out of the screening, due to the mixing problems with the beaker and magnet bar used in this solution. This solution was later redone using the newly selected beaker and magnet bar (V402). The results of the screening are presented in Chapter 8.5.

8 RESULTS AND DISCUSSION

8.1 Results of preliminary testing

For the first solution prepared, a paddle stirrer was tested for mixing and pressure tank was used for the filtration step. Only a total of 58.6 g of filtrate was collected during the 30-minute filtration time. Viscosity and pH were measured from the solution left in the pressure tank instead of the filtrate. Viscosity was measured after 3 minutes of spindle rotation for this solution, but after 1 minute for later measurements, as it was noticed that the spindle oscillation stabilised quite rapidly. The pH result was 5.54 and the viscosity result was 39.6 cP. The expected viscosity for this hypromellose content (0.5%) is 20-25 cP, so the result was quite interesting. The higher viscosity could be due to inadequate mixing during the solution preparation, which could have caused the hypromellose to not disperse evenly, resulting in formation of larger/longer aggregates and crosslinking polymer chains (Dow 2002; DuPont 2020).

The effect of citrate buffer on filterability was tested by comparing the results to a placebo without the buffer. The results for the buffer testing are presented in Table 5, and the filtration curves are presented in Figure 7.

Table 5. Results of buffer testing

Sample	B1	B2	B3	B4*	UB1	UB2	UB3
Buffer (y/n)	Y	Y	Y	Y	N	N	N
Filtration rate (initial, g/min)	27.3	32.0	36.7	29.5	28.5	29.3	30.0
Filtration rate (midpoint, g/min)	8.6	12.5	6.9	8.2	13.0	12.3	13.5
Filtration rate (end, g/min)	5.3	3.4	3.0	7.2	4.1	4.2	3.3
Filtered amount (g)	292.1	370.5	300.6	326.8	398.1	361.8	402.0
Filtration time (min)	28	25	30	30	30	26	30
pH	5.54	5.73	5.78	-	5.67	5.84	5.45
Viscosity (cP)	23.6	23.8	23.2	23.9	23.2	23.3	22.6

*An extra solution with buffer was prepared because of variation in the previous three solutions (solution B2 deviated from the other two results). pH was not measured for B4.

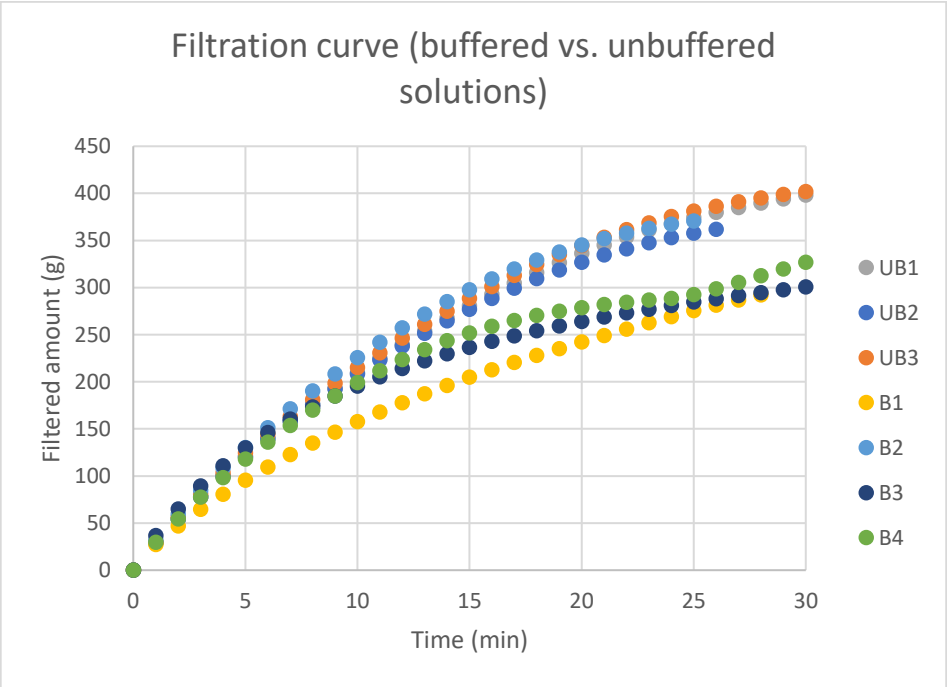


Figure 7. Filtration results - buffered (B1-4) vs. unbuffered (UB1-3) solutions

According to the buffer test results, the citrate buffer appears to have some diminishing effect on the filterability of hypromellose solutions, and this would require further investigation. Three of the four solutions containing the citrate buffer had noticeably worse filtration result when compared to the unbuffered solutions. There was also some variation in the results; one buffered solution (B2) deviated from the other three, having similar filterability as the unbuffered solutions. The B4 solution had an odd increase in the filtration rate towards the end of the filtration, which could be caused by some blockage opening due to increased pressure build-up in the capsule. As the results using the unbuffered solutions were more consistent and appeared to be more repeatable, it was decided to perform the screening without the citrate buffer. The Methocel solutions are not affected by small pH changes (Dow 2002; DuPont 2020), so the citrate buffer was not deemed critical for the study.

8.2 First screening results

The results of the first screening, performed with 1 l batch size and Pall Mini Kleenpak 20 -capsules with Fluorodyne EX EDF membrane is presented in Figure 8 below. The test parameters and results for each solution in table format is presented in Table 6.

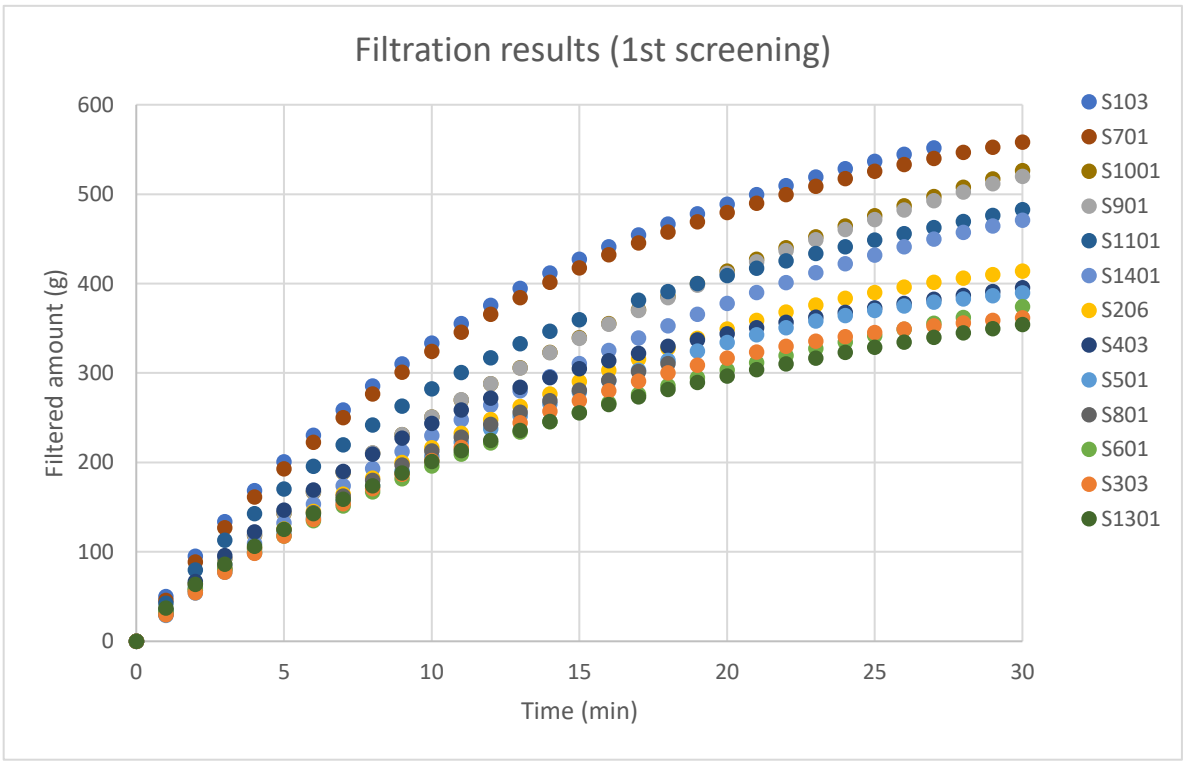


Figure 8. Filtration results of first screening

Table 6. First screening parameters and results

Sample	S501	S601	S103	S303	S901	S206	S403	S701	S801	S1001	S1101	S1301	S1401
T(addition, °C)	90	90	80	98.2	98	98	80	98.2	80	98	80	80	80.1
T(cooled, °C)	20	20	15	25	15	25	25	15	25	15	15	25	15
Mixing speed (rpm)	300	300	250	250	250	350	250	250	350	350	350	350	350
Mixing time (min)	30	30	20	20	20	40	40	40	40	20	40	20	20
Cooling time (min)	76	56	90	33	73	51	36	89	48	95	74	47	69
Filtration rate (initial, g/min)	29.1	34.4	50.1	29.5	34.8	31.2	35.4	45.7	30	34.4	42.5	37.1	32.5
Filtration rate (midpoint, g/min)	13.2	10.8	15.6	12	16.1	13.8	9.8	15.8	11.8	16.7	12.7	9.6	15
Filtration rate (end, g/min)	3.2	-	7.4	2.7	8.3	3.7	4.1	5.6	8.9	9.2	6.5	4.5	6.3
Filtered amount (g)	389.6	374.4	551.8	361.7	519.8	413.9	395.2	558.1	310.6	526.4	482.8	354.1	470.7
Filtration time (min)	30	30	27	30	30	30	30	30	18	30	30	30	30
Filtration rate (g/min)	12.99	12.48	20.44	12.06	17.33	13.8	13.17	18.6	17.26	17.55	16.09	11.8	15.69
Viscosity (cP)	23.0	22.5	23.3	23.4	22.7	22.4	23.1	22.6	23.3	23.1	22.4	22.7	23.2
Notes	1)	2)							3)				

1) Centre point. Rpms were checked with a tachometer and set to 300 before starting the work.
2) Centre point. The reading on 29-minute mark was not taken, and therefore end filtration rate was not calculated.
3) After 18 minutes the tubing came off the capsule and filtration was stopped.

Although the screening could not be finished due to the capsules running out, some important data was gathered from the results. After analysis of the results with Modde Pro 12.1, it was observed that the cooling temperature appeared to have an impact on the filterability and filtration rate of the solutions. Comparison of the results of different cooling temperatures is presented in Figure 9.

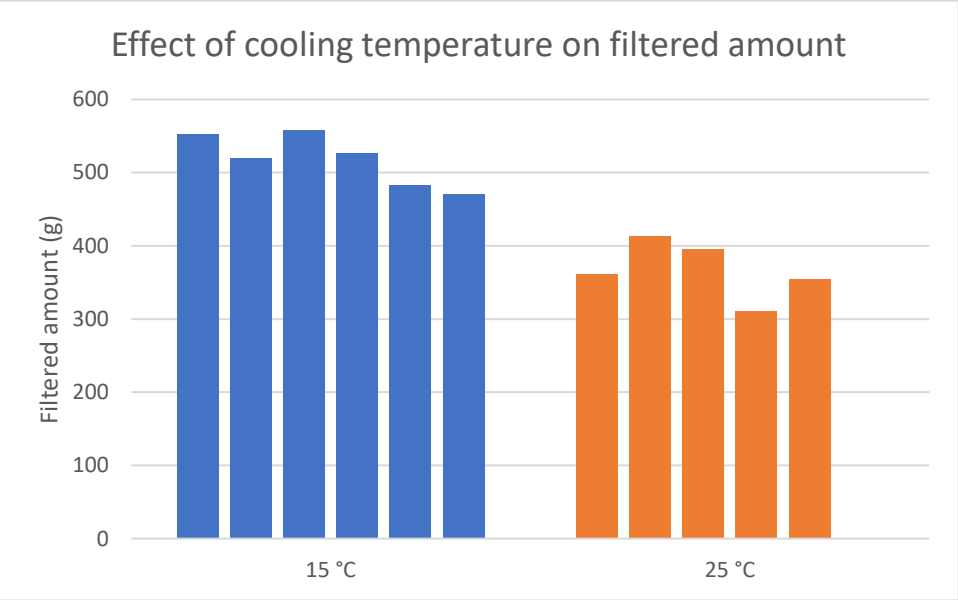
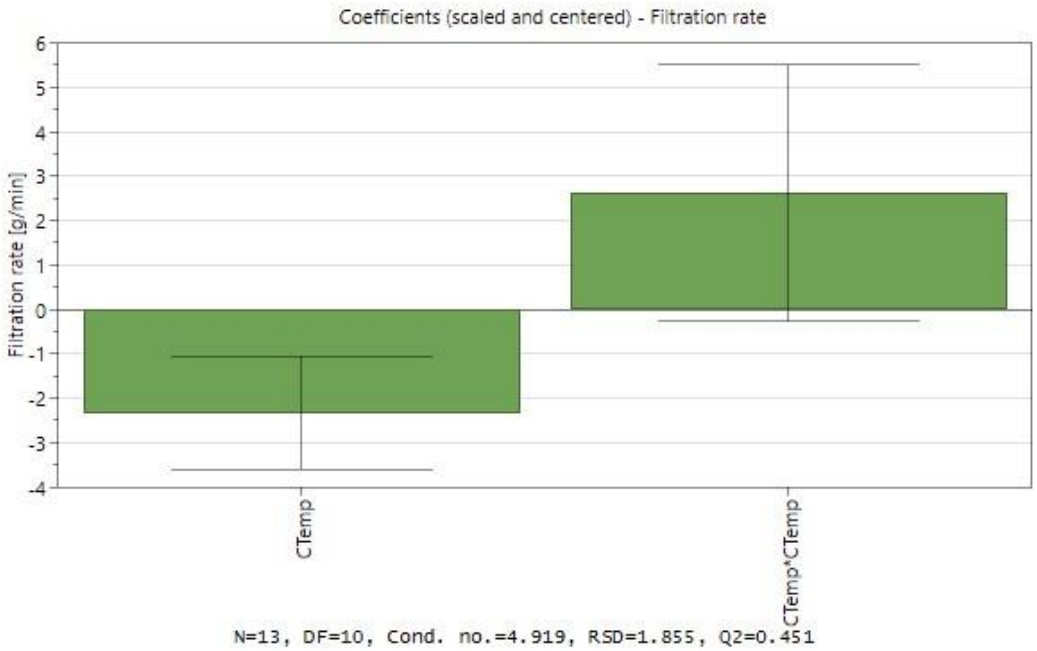


Figure 9. Effect of cooling temperature on filtered amount

Modde coefficients and summaries of statistical models for filtration rate is presented in Figure 10 and for filtered amount in Figure 11. The only statistically significant parameter affecting the filtration rate and filtered amount was the cooling temperature, and viscosity of the solution was unaffected by any of the parameters tested.



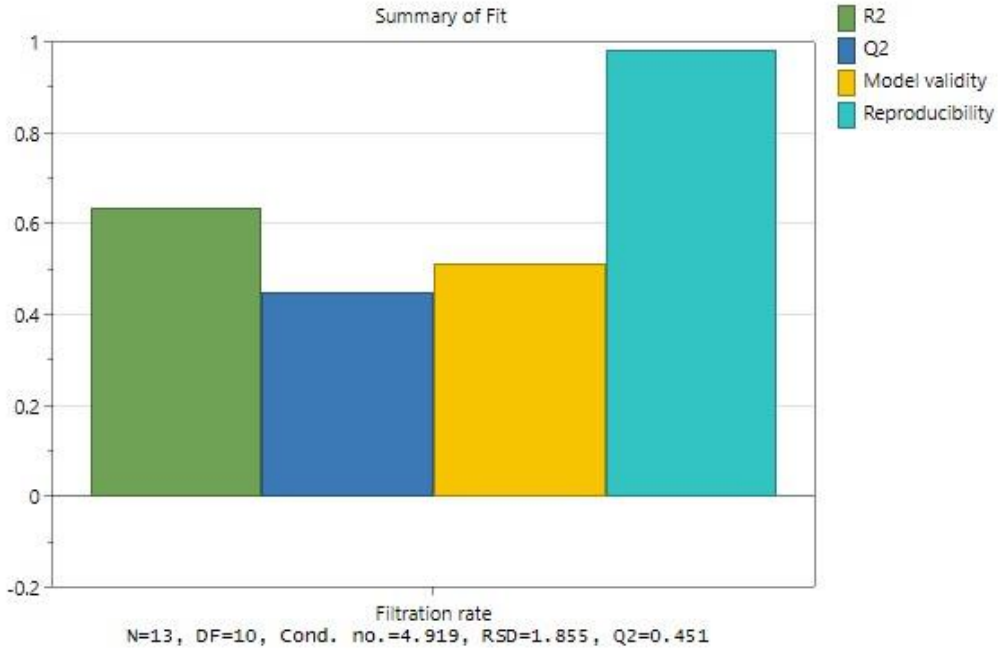


Figure 10. Filtration rate - coefficients and summary of statistical model (PLS)

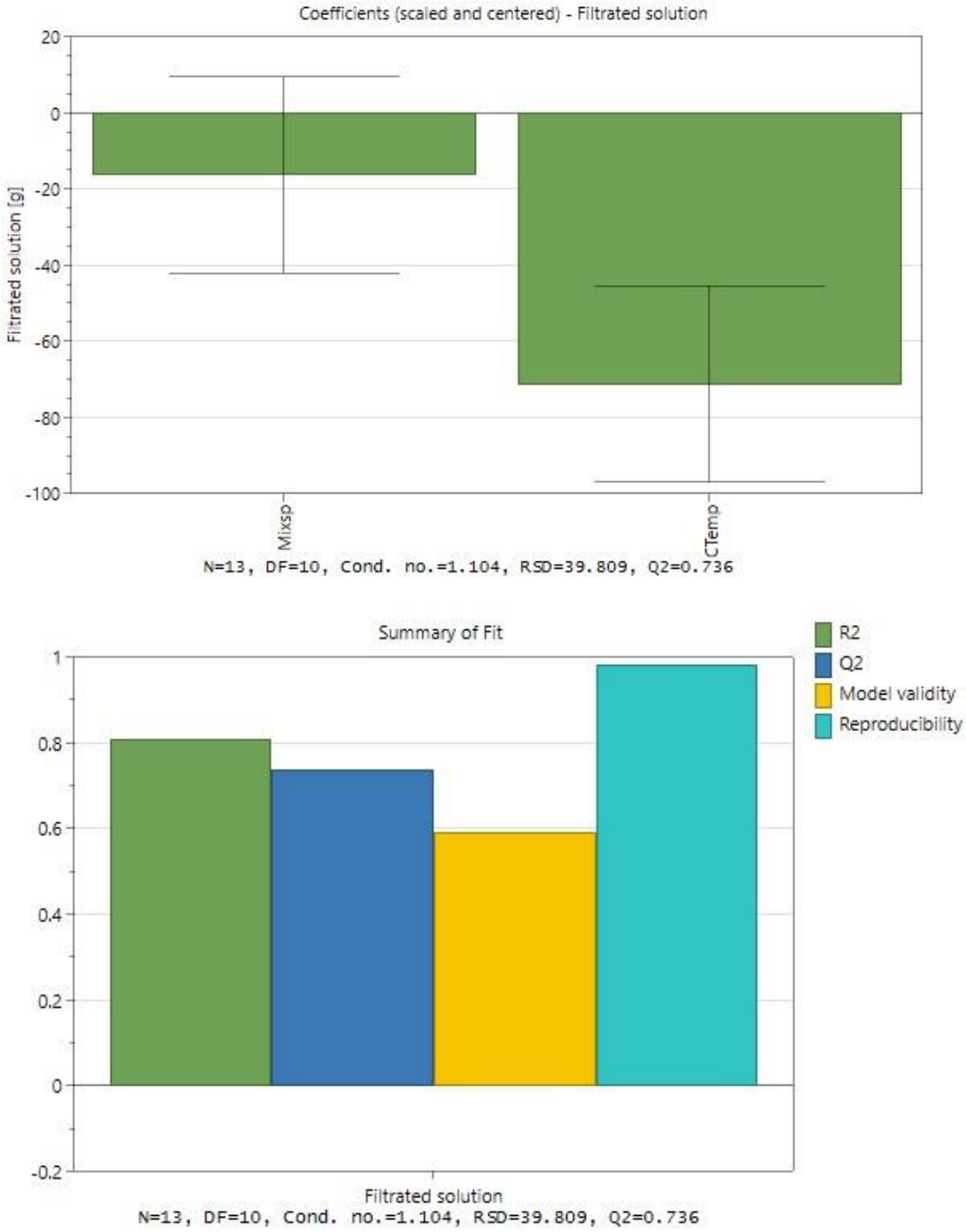


Figure 11. Filtered solution amount - coefficients and summary of statistical model (PLS)

R2 value greater than 0.5 shows that the model has significance and Q2 value greater than 0.1 shows that the model has significant future prediction precision. Q2 value greater than 0.5 is a sign of a good model. Model validity value greater than 0.25 indicates that there are no significant problems with the model, and reproducibility greater than 0.5 indicates that replicate tests would produce similar results.

8.3 Viscosity measurements

After the first screening, additional viscosity tests for the solutions that had been prepared earlier were conducted. The results are presented in Figure 12 below.

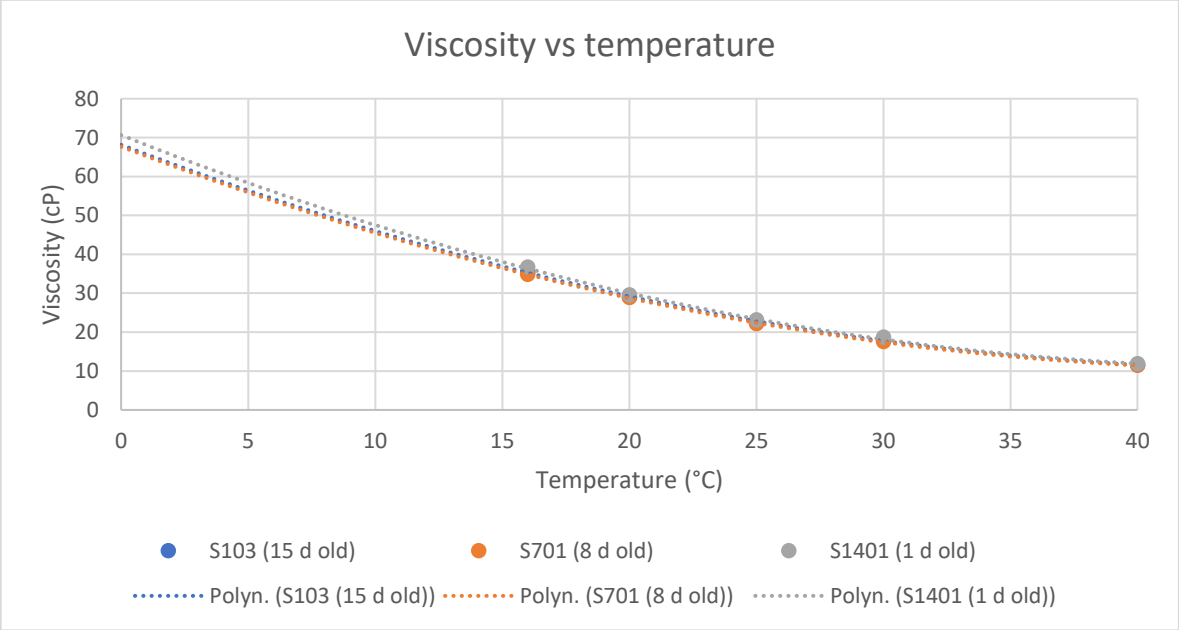


Figure 12. Effect of temperature and storage time on viscosity

As can be seen from the results, the storage time does not appear to have a significant effect on the viscosity of the solution. Additionally, the effect of temperature on the viscosity was confirmed with these tests; the viscosity decreases as temperature is increased (Rogers 2009). For the first 29 solutions prepared, the mean viscosity was 23.0 cP with a standard deviation of 0.5 cP. Minimum viscosity was 22.2 cP and maximum 23.9 cP. Based on the results, the solution manufacturing process parameters do not appear to have an impact on the viscosity of the filtered solution.

8.4 Results of larger batch size testing

After the first screening, three test solutions were prepared with 7 l batch size and using larger Pall Mini Kleenpak -capsules with Fluorodyne EX EDF membrane for filtration. The filtration results were compared to the two centre point measurements (S501 and S601) from the previous screening. As the EFA value is 12 times larger than with the Mini Kleenpak 20 -capsules, it was expected that the total amount filtered would be approximately 12 times higher as well. When calculating the total amount filtered including the filter flush (approx. 200 g), the result was 11.1 – 11.6 times larger than in the smaller batch, which is quite well in line with the previous results. The test parameters and results in table format for each test solution is presented in Table 7.

Table 7. Parameters and results of 7 l batch test solutions

Sample	U501	U601	U101
T (addition, °C)	90	90	80
T (cooled, °C)	20	20	15
Mixing speed (rpm)	330	300/-	250
Mixing time (min)	30	30	20
Cooling time (min)	129	110	165
Filtration rate (initial, g/min)	335.8	307.9	302.5
Filtration rate (midpoint, g/min)	129.5	126.8	128.4
Filtration rate (end, g/min)	58.7	48	65.7
Filtered amount (g)	4370.2	4165.9	4233.7
Filtration time (min)	30	30	30
Filtration rate (g/min)	145.7	138.9	141.1
Viscosity (cP)	22.1	23.3	23.5
Notes	1)	2)	3)

1) Problems with mixer turning off due to excessive weight.
2) Mixing speed not set for the cooling plate. Mixing speed increased for final mixing step
3) Different filter lot. Unsteady mixing.

Filtration result for second test solution (U601) was slightly lower than with the previous solution (U501). The filtration result for the third test solution (U101) was similar with the previous two test solutions, even though the results were expected to be better using these parameters, based on analysis of earlier results. It is possible, that the temperature does not play as big of a role with the larger capsules/batch sizes. Inefficient/unsteady mixing may have also caused the result to be lower than expected. It is also worth mentioning, that the filter capsule for the third solution was from a different lot than the two previous solutions, as there can be some variation between filter lots as well.

8.5 Second screening results

The results of the second screening, performed with 5 l batch size and Pall Mini Kleenpak -capsules with Fluorodyne EX EDF membrane is presented in this Chapter. Some problems were encountered during this screening, as sometimes the hypromellose would stick to the beaker walls due to unsteady mixing. This often caused problems during the filtration step, as the solid hypromellose would then be submerged in the solution only after addition of final water, leaving it less time to properly dissolve. It is suspected that this poorly dissolved or undissolved hypromellose can contribute to the clogging of the filters. Another problem was with solutions V501-V901, which all had exceptionally poor filtration results, and some of the solutions clogged the filter almost immediately. All these solutions were filtered with a filter lot that was not used in any of the other solutions, which would indicate a possible problem with the said filter lot. A curious observation was also made when measuring the viscosities of these solutions. Filtrates of solutions V801 and V601, which clogged the filter immediately, had low viscosities compared to all the other solutions. The viscosity of the unfiltered solution and the filter flush of these solutions were within a normal range. Apparently, the filter lets some water or diluted solution through even after it is clogged, which could explain these results.

In addition to the test solution V101 and the solution V401 which had mixing problems (described in Chapter 7.6.2), solutions V501-V901 were excluded from the Modde analysis due to probable issue with the filter lot used for these solutions. The test parameters and results in table format for the solutions excluded from the screening are presented in Table 8. The test parameters and results for the solutions included in second screening are presented in Table 9.

Table 8. Parameters and results for solutions excluded from second screening

Sample	V101	V401	V801	V501	V601	V701	V901
T(addition, °C)	80	80	80.1	89.3	89	98	98
T(cooled, °C)	15	25	25	20	20	15	15
Mixing speed (rpm)	250++	340	500	420	420	340	340
Mixing time (min)	20	40	40	30	30	40	20
Cooling time (min)	94	56	88	76	71	123	110
Filtration rate (initial, g/min)	354	275	9.5	117.3	11.3	171.8	222.8
Filtration rate (midpoint, g/min)	143	62	4.6	18.6	4.2	65	85.6
Filtration rate (end, g/min)	63	3.4	3.3	3.2	2.9	4.1	4.1
Filtered amount (g)	4748	2403	40.1	569.3	50.4	1203	2011
Filtration time (min)	30	28	8	15	10	17	23
Filtration rate (g/min)	158.3	85.8	5	38	5	70.8	87.4
Viscosity (cP)	23.5	22.8	7.6	20.8	8.0	21.4	22.4
Notes			1)	2)	3)		

1) Mixing problems during cooling, filter started clogging during filter flush.
2) The filter started clogging almost immediately.
3) Filter started clogging during filter flush.

Table 9. Second screening parameters and results

Sample	V301	V102	V201	V1201	V402	V1501	V1101	V1001	V1601	V1401	V1301	V1701	V1801	V1901	V502
T(addition, °C)	98	80.2	98	89.1	80.3	98	80.5	98	98	80.1	80.4	80.5	80	98.2	89.1
T(cooled, °C)	25	15	25	20	25	24.8	15	15	15	15	25	15	25	25	20
Mixing speed (rpm)	340	340	500	420	340	340	500	500	500	500	500	340	340	500	420
Mixing time (min)	20	20	40	30	40	40	40	20	40	20	20	40	20	20	30
Cooling time (min)	103	114	66	71	55	88	90	130	110	94	68	91	67	75	68
Filtration rate (initial, g/min)	326	272	278	202	281.5	319.8	295.6	322.5	339.5	273.4	245.9	330.6	224.6	249.5	276.9
Filtration rate (midpoint, g/min)	98	116	-	76.3	100.8	99.8	122.6	128.6	133	109.1	99.2	126.9	93.4	98.4	97.1
Filtration rate (end, g/min)	4	49.2	3.6	4.2	4.5	4.2	39.5	44.7	53.3	11	4.6	38.9	4.6	4.3	3.5
Filtered amount (g)	3104.7	3786.9	2737.3	1355.4	2210.7	3133.7	3913.6	4174.4	4388.9	3371.5	2119.1	4153.1	1595.1	2308.2	2891.6
Filtration time (min)	30	30	30	17	22	30	30	30	30	30	22	30	18	24	30
Filtration rate (g/min)	103.5	126.2	91.2	79.7	100.5	104.5	130.5	139.1	146.3	112.4	96.3	138.4	88.6	96.2	96.4
Viscosity (cP)	22.9	23.4	22.4	22.5	22.4	21.9	23.4	22.4	22.8	23.1	23.1	23.5	22.6	22.4	22.4
Notes			1)	2)				3)	3)				3)		4)

1) Some problems with mixing during cooling step were encountered, the mixing had to be turned off and restarted with lower speed. During filtration, 15 min result was not recorded.
2) New filter lot
3) Some mixing problems
4) Measuring centre point again, because all three earlier ones had differing results. PD135 mixer shut down before the end of final mixing, possibly due to some connection issue with the wiring.

Because of time constraints, there was no time to redo all the solutions from V501-V901, but centre point V5 was redone (V502) to get at least two centre points for the final analysis. However, the results for these two centre points (V502 and V1201) also had significant differences in their filtration rates and filtered amounts. After analysis of the results with Modde Pro 12.1, it was once again observed that the cooling temperature appeared to have an impact on the filterability and filtration rate of the solutions, while none of the other parameters tested were statistically significant. The effect of cooling temperature on filtered amount is presented in Figure 13 and on filtration rate in Figure 14. The bars marked with red colour are from the "poor" filter lot.

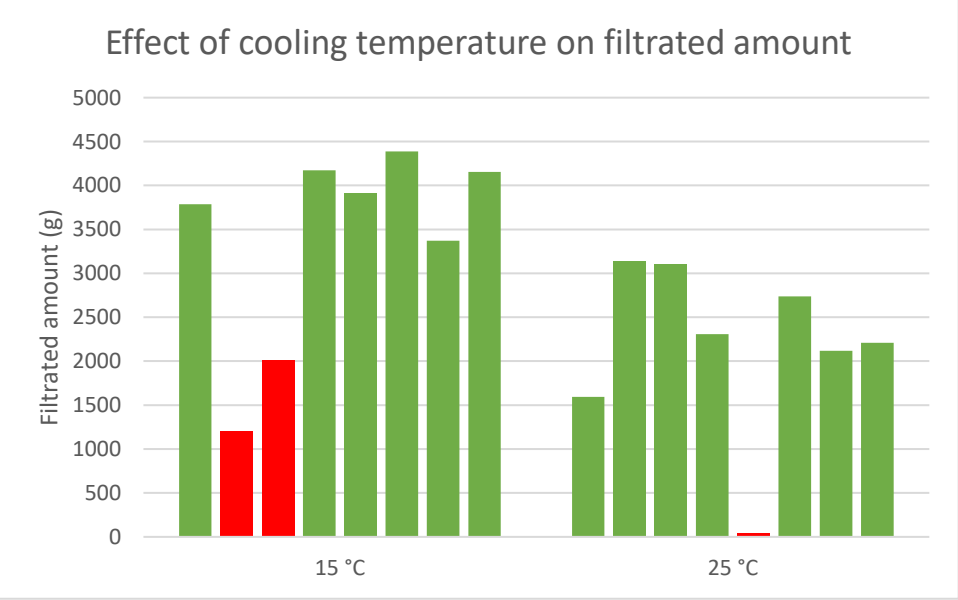


Figure 13. Effect of cooling temperature on filtered amount

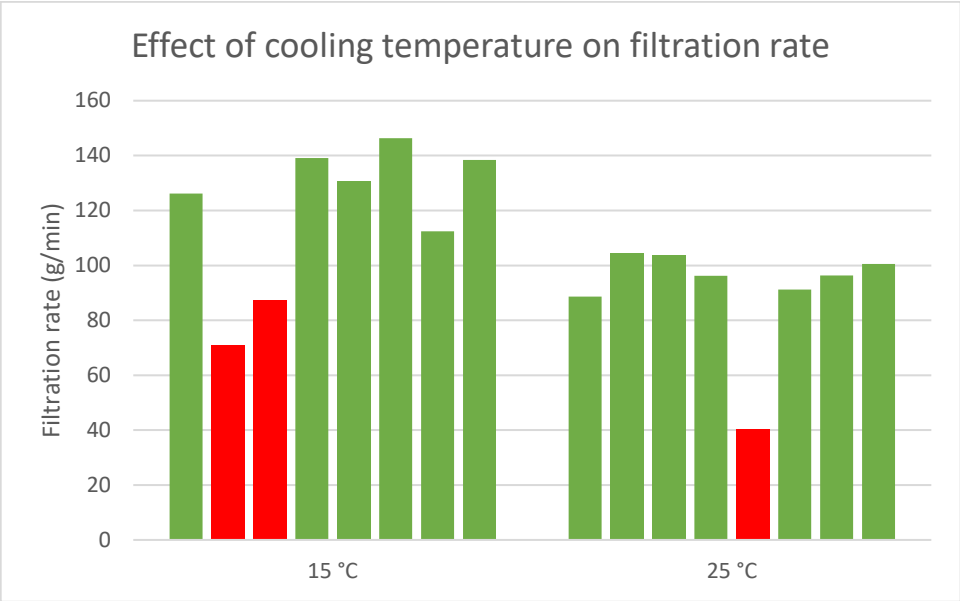


Figure 14. Effect of cooling temperature on filtration rate

Modde coefficients and summaries of statistical models for filtration rate is presented in Figure 15 and for filtered amount in Figure 16. The summary of the model fit for filtration rate was overall quite good, and it shows that the model was significant. For the filtered amount, the overall reproducibility of the model was quite poor as there was a lot of variation with the centre points. However, the R2 and Q2 values greater than 0.5 shows that the model is significant and has future prediction precision.

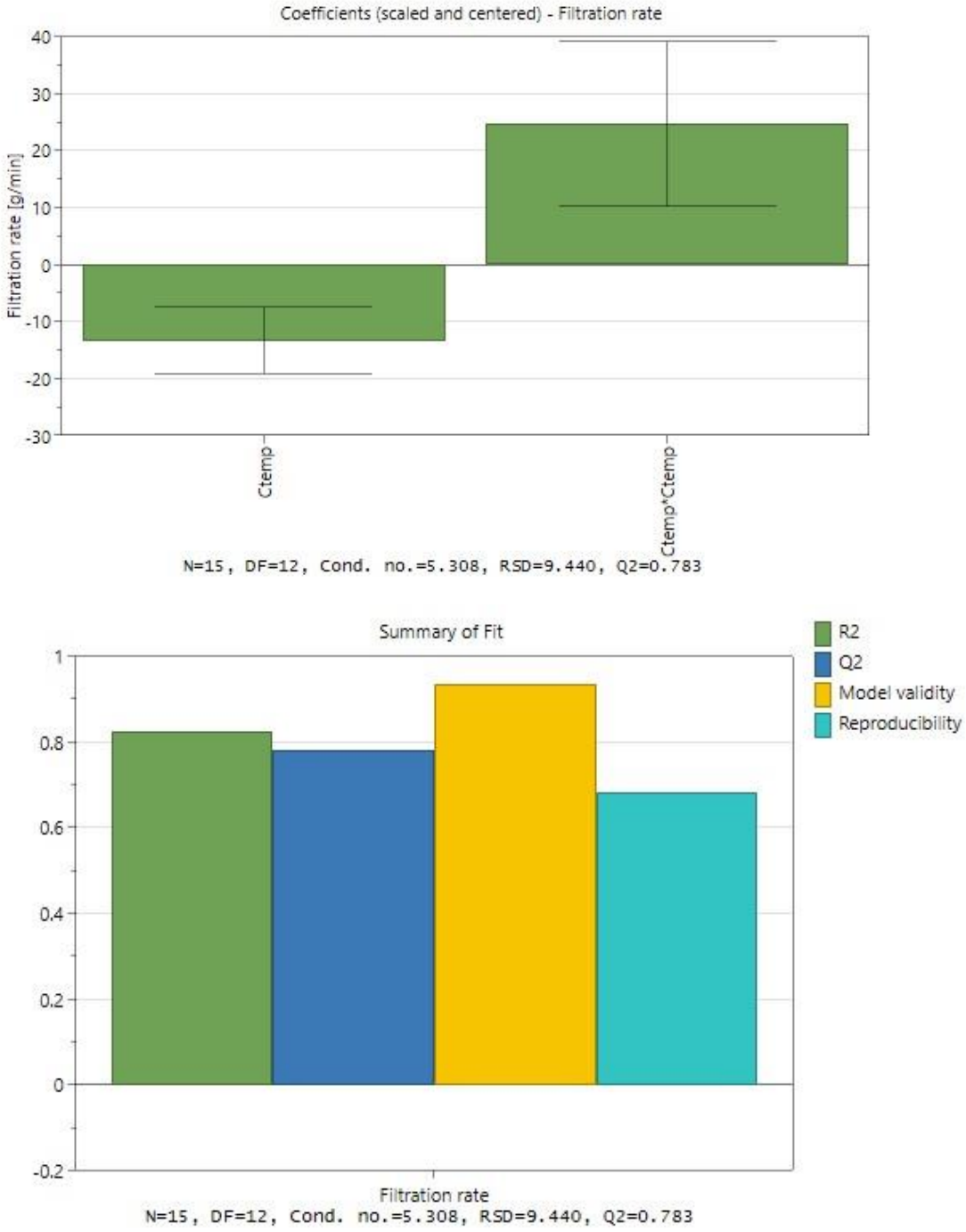


Figure 15. Filtration rate - coefficients and summary of statistical model (PLS)

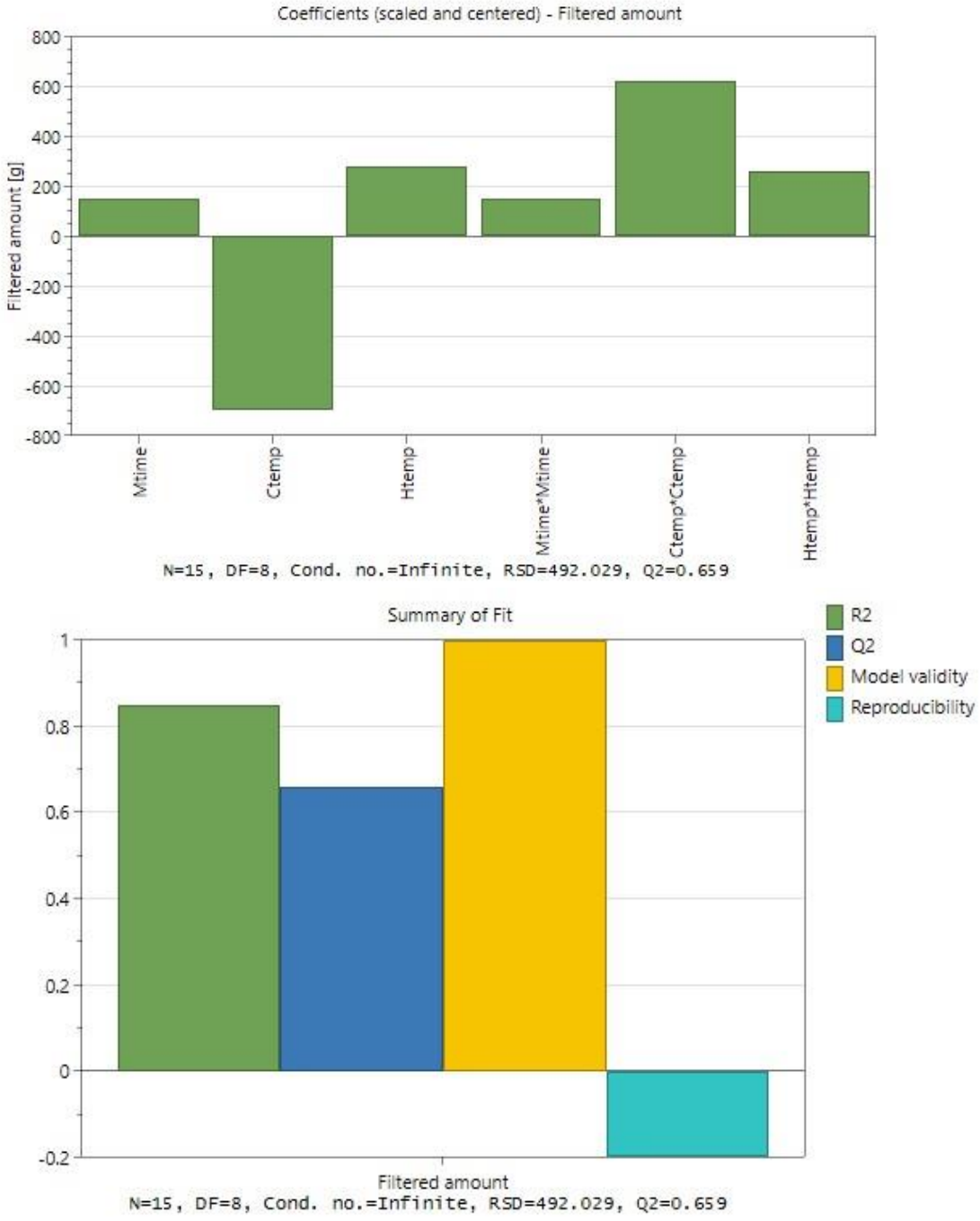


Figure 16. Filtered solution amount - coefficients and summary of statistical model (PLS)

8.6 Additional testing

After analysis of the screening results, it was apparent that the results from solutions V501-V901 were deviating from the normal trend. All these solutions were from the same filter lot, and this filter lot was not used in any of the other solutions. This would indicate that the poor filterability results from these solutions may be due to the different filter lot. For this reason, the two centre points here (V5 and V6) were redone hoping to achieve some consistency in the results. V502 was included in the screening and Modde analysis, but solution V602 clogged the filter almost immediately. This was possibly caused by unsteady mixing during the heating step, which might have caused more hypromellose than usual to stick to the walls of the beaker after heating. An attempt was made to rinse the beaker walls with small amounts of water during the cooling step, but apparently it was not sufficient.

To prevent the hypromellose sticking to the walls during heating, the mixing should be as steady as possible, and the evaporation during heating needs to be minimal. For this reason, an alternative dispersion technique was also tested. With NextPharma's method, the initial water volume used in the dispersion of Methocel is roughly 80% of the total water volume, while DuPont (2020) recommends dispersing the Methocel in 1/5 – 1/3 of the total volume of water. Using the DuPont's method, the cooling begins by adding cold water almost to the final volume. This would raise the solution surface level and help all the hypromellose on the beaker walls to dissolve during the cooling/final mixing steps. With this method, a faster cooling is achieved which might affect the gel formation (Košir et al. 2016). During testing of the DuPont method, the initial water volume had to be increased to 2000 – 3000 ml to prevent splashing due to high mixing speed. The final six solutions (V603, V702-V705, and V706B) were prepared with the DuPont dispersion method, and finally the "optimal"

parameters were tested on three different hypromellose batches. The parameters and results of these additional test solutions are presented in Table 10.

Table 10. Parameters and results of additional testing

Sample	V602	V603	V702	V703	V704	V705	V706B
T(addition, °C)	88.9	89.5	98.2	98	98	98.6	98
T(cooled, °C)	20	20	15	15	15	15	15
Mixing speed (rpm)	420	420	340	340	340	340	340
Mixing time (min)	30	30	40	40	40	40	40
Cooling time (min)	69	87	80	61	56	87	80
Filtration rate (initial, g/min)	26	328	328.5	274.5	270.4	279.7	317.6
Filtration rate (midpoint, g/min)	13.8	119.3	129.5	113.6	151.1	128.3	112.6
Filtration rate (end, g/min)	4.3	14.5	43.4	46.6	105.3	76.2	8.3
Filtered amount (g)	110	3817.2	4209.6	3711.2	4753.5	4131.6	3502.7
Filtration time (min)	8	30	30	30	30	30	30
Filtration rate (g/min)	13.8	127.2	140.3	123.7	158.5	137.7	116.8
Viscosity (cP)	14.5	22.6	22.9	22.4	20.9	22.3	23.2
Notes	1)	2)	3)		4)	5)	6)

1) Centre point replicate. The filter got clogged almost immediately, possibly due to unsteady mixing. Viscosity was measured for filter flush: 19.8 cP, and unfiltered solution: 22.7 cP.
2) Redoing the previous centre point solution using a DuPont dispersion method.
3) Testing optimal parameters using a DuPont dispersion method. 2000 ml of initial water was added. The rotation was unsteady again, causing a lot of hypromellose to stick on the walls. At the start of cooling, 3000 ml of cool water was added, rinsing the walls while pouring from the beaker.
4) Testing the optimised results with a good hypromellose batch (181613). The DuPont dispersion and optimal parameters were used. Results were compared to older test.
5) Testing a bad hypromellose batch (182754) using DuPont dispersion and optimal parameters. Results were compared to older test.
6) Testing same hypromellose batch 180813 which was used in the screening with buffer, using DuPont dispersion method.

For V603, 3000 ml of initial water was added, but the mixing was unsteady, and the water formed large waves around the beaker. The unsteady mixing continued even after adding

the hypromellose, which caused a lot of undissolved material to stick to the walls. 1500 g of cold water was slowly added to the solution while also rinsing the walls in the process during the start of the cooling step. An additional 500 g was added, which barely covered all the hypromellose on the walls. After the cooling step, there was quite a lot of gelled hypromellose on the walls at the solution surface level, which was scraped off with a spatula into the solution before adding the final water. Some gel was still left on the solution surface level, as not all had dissolved during the final mixing. Nevertheless, the filtration results were good for solution V603, but it was not included in the screening results or Modde analysis because a different dispersion method was used.

Based on the data acquired from the screening, the optimal process parameters were determined using the Modde Pro -optimiser. Modde optimiser results are presented in Figure 17.

Objective | Setpoint (#15) | Alternative setpoints

	Response	Criterion	Value	Graph	log(D)	Prob. of failure	Cpk
1	Filtration rate	Maximize	133.765		0.647497		
2	Viscosity	Predicted	22.8882				
3	Filtered amount	Maximize	4376.08		-0.481108	39%	0.0859526

	Factor	Role	Value	Graph	Factor contribution
1	Mixing speed	Free	415.616		0
2	Mixing time	Free	39.9996		6.92653
3	Cooling temperature	Free	15.0002		71.2275
4	Heating temperature	Free	97.9998		21.846

Figure 17. Modde optimiser results

As can be seen from the optimiser, to achieve maximum filtration rate and filtered amount, mixing time of 40 minutes, cooling temperature of 15°C and heating temperature of 98°C

would be optimal. Solutions V702 and V703 were prepared for testing the optimal parameters obtained from the screening process and Modde analysis: 15°C cooling temperature, 98°C heating temperature, 40 min mixing time, and 340 rpm mixing speed. As the mixing speeds did not have much of an impact on filtration results, any speed between 340 and 500 rpm could have been used for these solutions. The same parameters were also used for test solutions V704 and V705, where a "bad" hypromellose batch and a "good" batch were tested and compared to earlier laboratory test results performed by a laboratory technician. The comparison between different hypromellose batches performed by a laboratory technician earlier at NextPharma Oy with the standard filterability test method is presented in Figure 18.

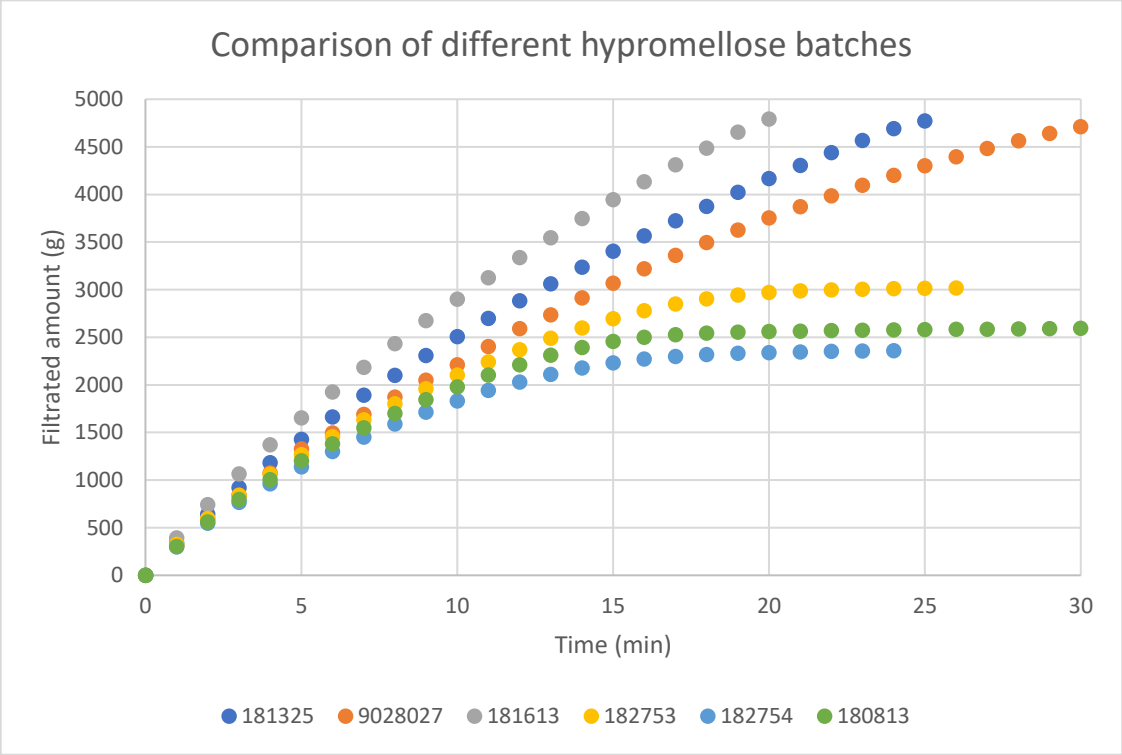


Figure 18. Comparison of different hypromellose batches

Of these hypromellose batches, 182754 was chosen for testing as a “bad” hypromellose batch, and 181613 was chosen as a “good” batch. For these two selected hypromellose batches, the new filtration results were compared to the old results, presented in Figure 19.

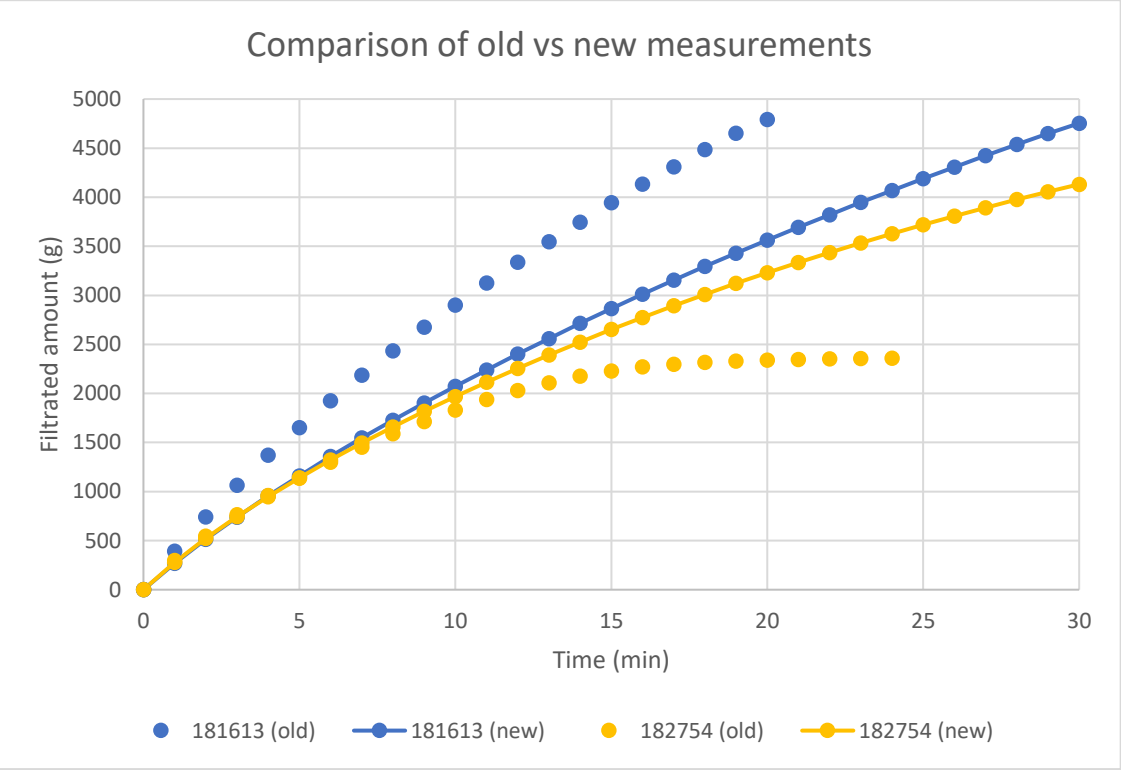


Figure 19. Comparison of old vs new measurements

The sample size for these tests was quite small due to time constraints, but the results from testing with DuPont dispersion method and optimised manufacturing parameters were quite similar for both batches. The good hypromellose batch filtered completely, but the filtration time took 10 minutes longer than in the older test. The results of the bad hypromellose batch were an improvement over the older test, as previously the filter got clogged at 24 mins, with a result of 2359.5 g.

The final solution (V706B) was prepared using the same hypromellose batch (180813) which had been used in the screenings, using DuPont dispersion method and optimised parameters, but with a citrate buffer included. This was done to see if the results would be affected by the inclusion of the buffer, as was seen in the first tests of this thesis project. The filtered amount for the buffered solution was lower than with the other solutions prepared in these final tests, which was somewhat expected. Unfortunately, there was time to only prepare one solution, so no clear conclusions can be drawn from this test. The comparison of the solutions prepared using the DuPont dispersion method are presented in Figure 20.

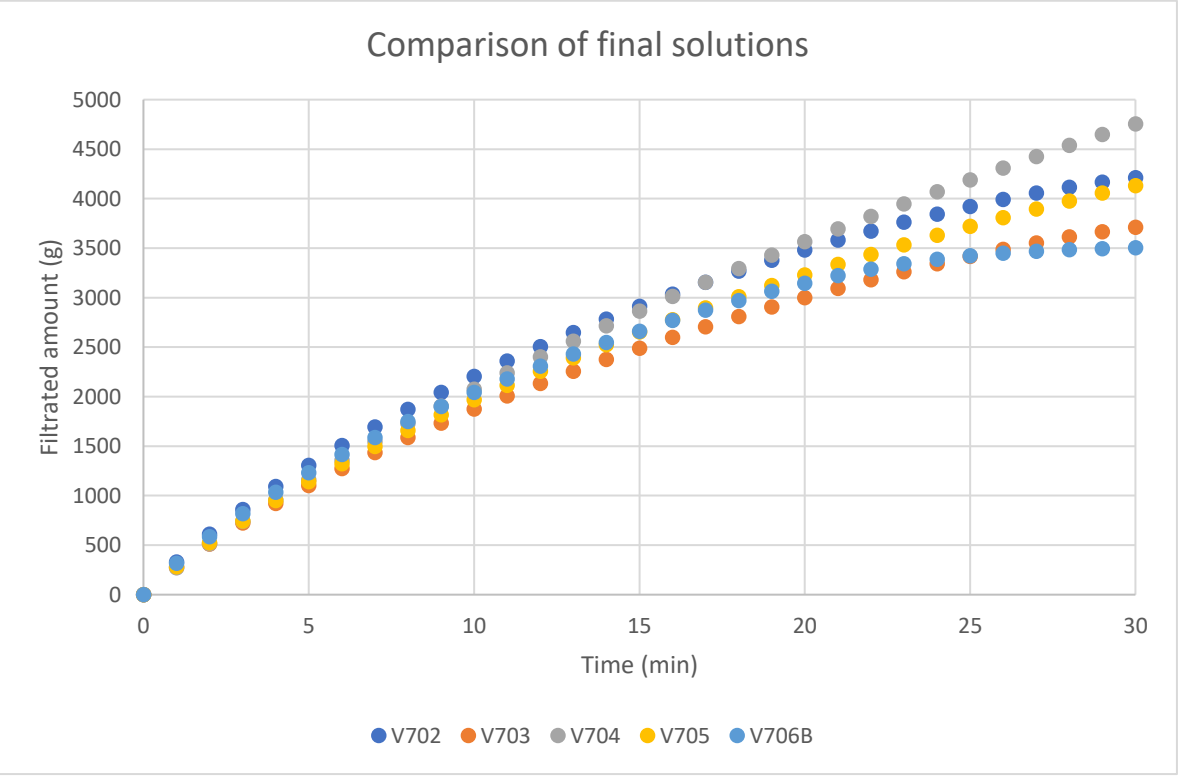


Figure 20. Comparison of final solutions (DuPont dispersion)

Of these final solutions, V704 had the best result, which was probably due to a better hypromellose batch used (181613), and the buffered solution V706B had the worst result. However, the results of these final measurements were quite similar even though different hypromellose batches were used.

8.7 Discussion

Developing a robust analytical method for assessing the filterability of hypromellose proved to be quite challenging, as solutions prepared in seemingly identical way sometimes provided totally different results. This can also be seen in Modde analysis results, presented in Figure 16. It is still unknown if this is a result of the heterogeneity of the hypromellose raw material (Dahl et al. 1990; Viridén et al. 2009b; Larsson et al. 2010; Košir et al. 2016) or if the solution manufacturing is extremely sensitive to small variations in the manufacturing process. Proper dispersion of hypromellose is critical for the process, as poor dispersion can result in the hypromellose forming aggregates/clumps in the solution (Dow 2002; DuPont 2020), which are likely to clog the filter. Slow addition of hypromellose is important, and the water needs to be hot enough for the hypromellose to not start gelling prematurely. Based on the test results, 80°C seemed to be sufficient temperature for dispersion, but 98°C provided better results on average. However, the difference was not statistically significant. Dispersion temperature over 90°C would be recommended as instructed by the hypromellose supplier, to ensure proper dispersion before gelling begins.

Mixing speed was the least significant parameter of the ones tested; 500 rpm results were slightly better compared to 340 rpm, but again the difference was statistically insignificant. If the mixing is unsteady however, problems can arise during the filtration. Undissolved

hypromellose can easily stick to the walls of the beaker, especially if the solution forms waves due to unsteady mixing, or if solution evaporates excessively during the heating step. This undissolved hypromellose can then detach from the beaker back into the solution after water is added before final mixing. If the final mixing time is not sufficient, this material can remain undissolved and subsequently clog the filter. This is likely not a problem with the large-scale manufacturing but proved to be problematic using the laboratory equipment. For laboratory scale testing, a paddle stirrer would be more suitable for this kind of study, as it would eliminate the problems encountered with the magnet bars and magnetic stirrers, and the mixing would be more consistent. The DuPont dispersion method appeared to provide more consistent results, but the sample size was quite small, and more testing would be required. The DuPont dispersion method uses a smaller initial water volume, which often formed large waves with the high mixing speed used in the tests and resulted in a lot of hypromellose sticking on the beaker walls. This was not a problem however, because more water is added at the start of the cooling phase which submerges the hypromellose on the beaker walls, leaving enough time for it to properly dissolve.

40-minute mixing time provided better results on average than 20-minute mixing, but once again statistical significance could not be achieved with these tests. Longer mixing time will be safer to ensure proper dissolution of the hypromellose but will increase manufacturing costs and is likely not worth the investment if the yield is only slightly better. In large batch manufacturing, the solution is often filtered the next day after it has been manufactured, which should leave plenty of time for the hypromellose to dissolve properly. However, the solution is not mixed after the manufacturing is finished on the first day, and the filtration is usually started straight away on the second day without mixing the solution first. Based on the manufacturing batch records, no clear differences have been found between filtration results of solutions filtered the same day, and solutions filtered during next day. Leaving the mixer on after finishing the manufacturing, and possibly even during filtration

could theoretically improve the filtration results due to increased shear rate (Allmendinger et al. 2015), but this would need to be tested with laboratory scale batches first, and unfortunately there was no time left to conduct these tests in this project.

The parameter in this project that provided statistically significant results was the cooling temperature; solutions cooled to 15°C provided better results than solutions cooled to 20 or 25°C. However, the cause for this is currently unknown, and would require more research. It is known that viscous solutions can cause problems during sterile filtration (Coulais et al. 2015), which is why it is curious that a cooler hypromellose solution has better filterability even though it is more viscous than a room temperature solution. Possible reason for this is, that the aggregation of hypromellose is decreased in lower temperature due to increased hydration of the polymer chains (Porsch et al. 1997). This would result in better dissolution of the raw material and therefore reduce the possibility of filter clogging due to undissolved content in the solution. There could also be some effect on the filter materials by this lower temperature, but this is unlikely as the temperature difference is not that significant and the filter materials should be robust enough for this operating temperature. The cooling time was not controlled, as it was difficult to repeatably cool the solution to a desired temperature within a same timeframe. The cooling times were usually longer when cooling the solutions to a lower temperature, and it may have some minor contribution to the results as well. However, as can be seen in Figure 21 below, the impact of cooling time on the results was marginal compared to the effect of cooling temperature. The red dots represent solutions that were cooled to 15°C, yellow dots for 20°C, and blue dots for 25°C. This graph contains solutions from the V-series, except solutions V501-V901 and V602, as all of these had severe filtration issues which were likely a result of other factors, as described earlier.

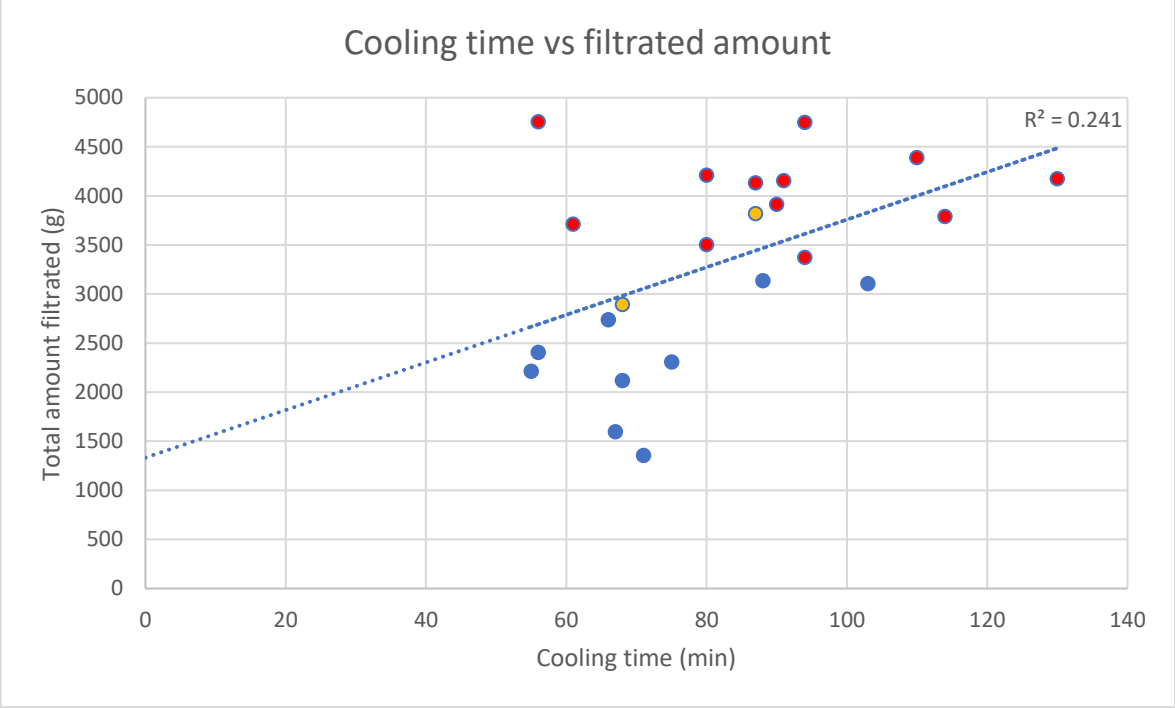


Figure 21. Effect of cooling time on filtered amount

The low R^2 value (0.241) shows that there is no significant correlation between the cooling time and the total filtered amount. However, the solutions cooled to a lower temperature (red dots) clearly show better filtration results on average when compared to the solutions cooled only to 25°C (blue dots).

Based on the results of this screening, studying the behaviour of hypromellose solutions cooled to a lower temperature would be beneficial for tackling these filtration problems. It would be interesting to test whether the filterability improves further if the cooling temperature is lowered below 15°C. It should also be investigated which is more critical for the filtration, the temperature of the solution during filtration or the temperature to which the solution was cooled. In other words, would the filtration results be as good if the

solution was cooled down to 15°C and then left to warm back to room temperature or above, before filtration. Practical implementation of cooling the solution to 15°C with production scale batches is difficult however, as the cooling capacity of the water circulating in the tank jacket is limited.

9 CONCLUSIONS

Setting up a method for testing the filterability of different hypromellose batches that is robust and scales well to production scale is challenging. The hypromellose material appears to be either very heterogeneous, the solutions are overly sensitive to minor deviations in the manufacturing process, or both. This has also been observed in the production scale batches, where the results can vary widely even with the same hypromellose batch used and with experienced operators preparing the solutions. Several problems were encountered while setting up the screening method for this study, most of them related to mixing and temperature control. A paddle stirrer with a proper paddle could solve many problems encountered with the use of magnet bars and magnetic stirrers and would be recommended for future testing. A pressure tank, despite being more laborious to set up and use, would be recommended over peristaltic pump for the filtration tests, due to constant pressure during filtration and closer resemblance to production filtration conditions. The DuPont dispersion method could be used to minimise the risk of undissolved hypromellose remaining on the beaker walls, but this should be further investigated and compared to the standard NextPharma procedure. The effect of citrate buffer should also be further investigated, as the results indicated that it possibly has some diminishing effect on the filtration results.

Based on the screening results, higher temperature during dispersion, longer mixing time, and lower cooling temperature provided better filtration results on average, while mixing speed was the least significant parameter, having little to no impact on the filtration results. However, the effect of cooling temperature was statistically significant; solutions cooled to 15°C provided better filtration results than solutions cooled to 20 or 25°C. The reason for this is currently unknown and would require further research. Possible reason for this is increased dissolution of the material at lower temperature, caused by decreased aggregation of hypromellose polymer chains. Due to time constraints, some of the planned additional testing could not be carried out, such as testing different hypromellose grades and concentrations, studying the clogged filter membrane, and trying to isolate and analyse the undissolved content. For future studies, it would be recommended to investigate what is the material (impurities in the raw material such as unreacted cellulose, poorly dissolved hypromellose, or something else) and mechanism that causes the clogging of the filters. Knowing this, it could then be possible to develop an analytical method for determining the amount of impurities in hypromellose batches, or to find conditions which would minimise the risk of filter clogging.

10 REFERENCES

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