PTR-115

Sterile Filtration of Sodium Carboxymethylcellulose Based Solutions—Factors Affecting Filterability

Daniel Sieber and Christian Muehlenfeld

Introduction

Sodium carboxymethylcellulose (Na-CMC) is often used by customers as a thickening agent in the preparation of parenteral liquid formulations, which are subject to subsequent sterilization after preparation of the solution. If the finished product or any process liquids are labile to gamma irradiation or heat sterilization, the sterilization method of choice is sterile filtration. In this case, sterility is achieved by filtering the material through a sterilizing grade filter with a pore size of 0.2 µm. The desired elevated viscosity from using Na-CMC often poses a problem to filterability during sterile filtration. This may result in poor filterability and long process times, up to complete blocking of the sterile filters. To counteract filter blocking and enable smooth sterile filtration processes, this study investigated factors in preparation of Na-CMC solutions that influence the filtration rate, as well as a sampling of regularly used molecular weight grades of Na-CMC.

Materials and Methods

Materials

The materials used in this study are summarized in Table 1 and Table 2. All types of Na-CMC were obtained from Ashland (Wilmington, DE, USA). Filters tested were provided by Pall Corporation (Basel, Switzerland) and were used as received.

Table 1: Na-CMC grades tested

Grade	Degree of Subsitution	Viscosity (mPa·s)	Weight Average Molecular Weight [Da]	Lots analyzed
Blanose™ 7M31F	0.7	1,500-3,100	395,000	33035
Blanose 7MF	0.7	400-600	250,000	C151723
Blanose 7LP EP	0.7	25-50	90,500	C170227
Blanose 7LF	0.7	25-50	90,500	43448
Aqualon™ 7L2P	0.7	50-200	49,000	73283

Note: This work is based on results that were published in *Bioprocess International 14* (3), 2016 ("Factors Affecting Sterile Filtration of Sodium-Carboxymethylcellulose–Based Solutions")
Parts of this work were presented as posters at UK PharmSci 2015 (Nottingham, UK), 10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 2016 (Glasgow, UK) and at 2017 AAPS Annual Meeting and Exposition (San Diego, USA). It also contains results from internal TSR 00153572.
Other parts will be presented as a poster at the 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 2018 (Granada, Spain).

Table 2: Sterile filters used for tested (0.2 µm rated sterilizing grade filters)

Filter name	Upstream/downstream membrane layers	Format tested	Graph reference
Supor® EX grade ECV	Asymmetric♦ Polyethersulfone (PES) / Symmetric PES	47mm disc	ECV

♦ Asymmetric filtration layers made to the same specification

Preparation of solutions
Na-CMC particles tend to agglomerate, or lump, when first added to water. To obtain good solutions easily, Na-CMC was added to a vortex of vigorously agitated water. Test solutions were prepared based on the dry mass of Na-CMC (w/w). The rate of addition was slow enough to permit the particles to separate and their surfaces to become individually wetted, but it was fast enough to minimize viscosity buildup of the aqueous phase while the gum is being added. The solutions were stirred vigorously for 1 hour until dissolution was complete using a laboratory propeller mixer. This mixer is a variable speed flow mixer. The rotational speed can be set from 0-2500 RPM. The direction of flow can either be clockwise or counter clockwise and various impellers can be used. For this project, a setting of 1500 RPM was used.

In some cases, the stirred solutions were then subjected to high mechanical shear forces by using a homogenizer (ultra-turrax T 50 digital disperser, IKA, Wilmington, NC, USA) as part of the solution preparation process. This system consists of a rotor within a stationary stator, and due to the high circumferential speed, the medium to be processed is drawn axially into the dispersion head and then forced radially through the slots in the rotor-stator arrangement. The high speed (up to 25,000 min^{-1}) and minimal gap between the rotor and stator produces extremely strong shear forces.

Determination of Viscosity—Na-CMC solutions
Test solutions for viscosity measurements were prepared as described in the Preparation of Solutions subsection. Brookfield viscosity was measured using a TDVII-LV viscometer (Brookfield Engineering, Middleboro, MA, USA) attached with spindle 61 at 60 rpm. Samples were equilibrated at 25 °C before viscosity testing. Each solution was measured three times and the average was calculated.

Na-CMC solutions exhibit non-Newtonian behavior when mixing. This means that they will also exhibit the same behavior when taking viscosity measurements. As a result, it is important to define exactly the conditions under which viscosities were measured. This includes the temperature of measurement, spindle RPM, and duration of measurement. Because Na-CMC solutions are thixotropic, the relaxation time for higher concentration solutions is different than that for solutions of lower concentrations. That is, solutions of higher concentrations of Na-CMC require more time in the presence of a given shear rate to achieve a stable, minimum viscosity.

Filterability Testing
For filterability testing, sterilizing grade filters with 47 mm diameter were installed in a stainless steel filter housing (see Table 2). The inlet of the housing was connected to an upstream pressure vessel containing the Na-CMC solution to be filtered. The solution was passed through the filter by force of air pressure delivered from upstream of the pressure vessel. The filtrate was deposited in a vessel on a balance linked to a system that recorded changes in weight of the collection vessel over time. Tests were terminated either when blockage occurred or after filtration of 400 ml of test solution. All trials were carried out at 2 bar constant pressure after ramping up from 0.5 bar. If not written otherwise, tests were performed at room temperature.

Quantification of filtration performance
The filter capacity of a filter under constant pressure can be determined by a test methodology using a gradual pore clogging model. This law is characterized by the equation (Equation 1):

$$\frac{t}{V_m} = k \cdot t + \frac{1}{q_0}$$ **Equation 1**

Where t is the filtration time, V_m the volume (expressed as throughput in grams) filtered at time t, $1/q_0$ is the initial flux, and the slope, k, depends on the clogging potential of the filtered solution.
To test the filtration performance, time and corresponding filtrate throughput of a filtration test are recorded at constant pressure as described in the Filterability Testing subsection. The data is then plotted as time/throughput (t/V_m) versus time (t). Its representation is a straight line indicating filter plugging per the gradual pore plugging model. If the data do not plot as a straight line, this indicates the filter is plugging by some other model, such as cake formation. In these cases, a different method should be used to analyze the filter clogging behavior.

However, solving Equation 1 for V_m gives the following equation (Equation 2):

$$V_m = \frac{t}{k \cdot t} + \frac{1}{q_0} = \frac{1}{k + \left(\frac{1}{q_0 \cdot t}\right)}$$ **Equation 2**

Letting time go to infinity, $1/(q_0 \cdot t)$ becomes zero and $V_m = 1/k$ indicates the maximum throughput that can be filtered at time infinity. Thus, $1/k$ can serve as a measure of filter clogging. During filtration, the solution settles and particles reduce the pore size. Therefore, the lower the inverse of k, the more filter clogging occurs. Consequently, higher values for $1/k$ and thus, V_m signify less filter clogging and thus, a better filtration performance.

Design of experiments (DoE)
Design of experiments (DoE) was prepared and evaluated using the statistical software MODDE 11.0 (MKS Umetrics, Umeå, Sweden). All experiments were conducted in a randomized order. Center points were repeated three times to evaluate process reproducibility. Data were analyzed using multiple linear regression. A backward regression was performed eliminating nonsignificant factors or factor combinations ($p > 0.05$) starting with factors with the highest p-values. For all results, confidence levels were 95%.

Results and Discussion

We identified the following factors that might influence sterile filtration.
Sterile filtration process
- Filter material
- Temperature of the solution: The temperature of the solution is also of importance during the filtration process. Increasing the temperature can promote gelation of the solution that will lead to premature blinding of a filter. The heat generated on a pumped system can have significant impact of the volume that can be processed through the filter and lead to sudden blockage during the processing of a batch.

Solution preparation/mixing techniques
- The hot/cold technique is often used with cellulosic viscosity enhancers. The cellulose derivative is dispersed and thoroughly mixed at high temperature, at which point the cellulose is insoluble. This is followed by rapid cooling to a point where the cellulose becomes water soluble. If this is not controlled well premature blinding of filters can quickly occur.
- Mixing/stirring speed/shear and/or time
- Time between mixing and filtration

Material properties

The focus of this PTR is on the factor of solution preparation to provide guidance on how to properly prepare a solution used in sterile filtration to achieve the best possible filterability.

Influence of solution preparation on sterile filtration performance
Given the similarity in the formulation composition (Na-CMC grade, lot number, and concentration/viscosity) and the target filtration process, the influence of solution preparation on sterile filtration performance was further investigated. The preparation of Na-CMC solutions can be crucial in obtaining stable solutions, by promoting the degree of disaggregation of the polymer. In this approach, extensive assessments and evaluations were performed to identify a solution preparation process that can be commonly adopted for all Na-CMCs for sterile filtration purposes. Variables of the solution

preparation process that might influence the sterile filtration performance include the temperature of the solution preparation, different mixing/stirring times, and the use of additional high mechanical shear on disaggregation (e.g. ultra turrax) to further improve the dispersion and disaggregation of Na-CMC particles by breaking internal associations of gel centers (microgels). Under certain conditions, solutions of Na-CMC are susceptible to degradation of the polymer. Permanent loss in viscosity can occur, resulting from scission of the long-chain molecules. To exclude the possibility that use of high mechanical shear leads to degradation, and thus, a permanent loss of viscosity of the solution, a solution of 0.4% (w/w) Blanose™ 7M31F Na-CMC was subjected to different times of high mechanical shear forces as part of the solution preparation process. The Ultra turrax rotor/stator stirrer used in this process was operating at 20,000 min^{-1}. Figure 1 shows the effect of increasing mechanical shear on the filtration performance, while Figure 2 shows the effect of increasing shear on solution viscosity. Generally, the use of mechanical high shear results in better filtration performance, however, there is a loss in viscosity with increasing time of applied mechanical shear, indicating possible degradation of the polymer long chains.

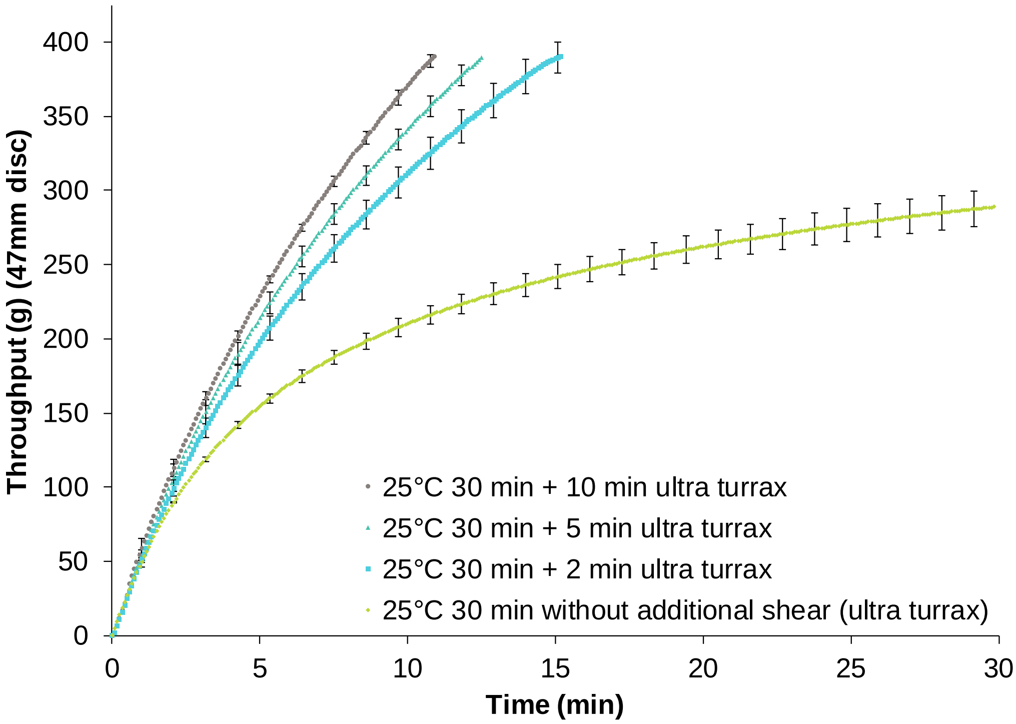

Figure 1: Filtration performance curve of 0.4% Blanose™ 7M31F solutions prepared with increasing shear times. The ultra turrax rotor/stator stirrer was operating at 20,000 min^{-1}. (AV±s, n=3).

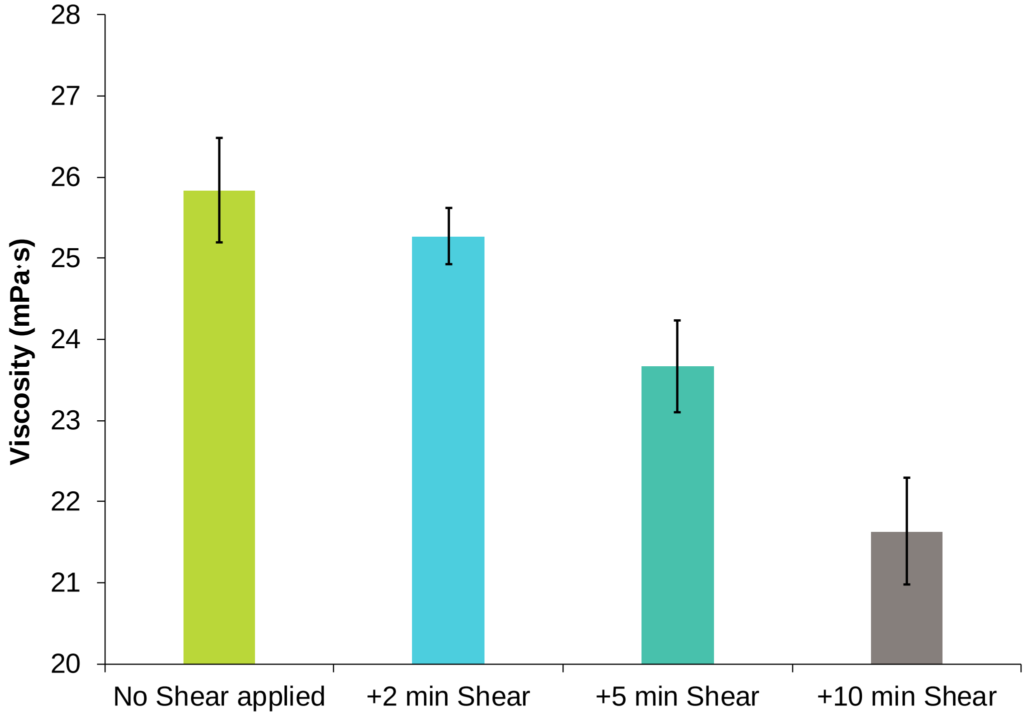

Figure 2: Resulting viscosities of 0.4% Blanose™ 7M31F solutions prepared with different shear times (average ± confidence interval, AV±CI; n=3).

A Student's t-test was performed on the viscosity data to determine if the resulting viscosities after additional shearing are significantly different from the viscosity of the Na-CMC solution without use of additional mechanical high shear. Viscosities after 5 min and 10 min of additional high shear stirring showed statistically significant (α=0.05) differences compared with the run without any additional shear. The trial with only 2 minutes of additional shear, on the other hand, resulted in a statistically identical (α=0.05) viscosity.

Data from the filtration runs of the prepared solutions of Blanose™ 7M31F Na-CMC (Figure 1) were further analyzed in terms of the time/throughput (min/g) versus time (min) curves (Figure 3). The resulting straight lines for all solutions indicate filter plugging per the gradual pore plugging model. These curves are shown for the entire run as well. For this period the comparative slopes of the time/throughput (min/g) versus time (min) curves (or the plugging constant *k*) indicate directly the plugging rates expressed as the ratio of the packed volume of solids removed per gram of filtrate to the clogging value of pores within the filter medium. These values of *k*, are all for very nearly the same conditions of pressure drop, solution viscosity, and initial solids concentration. Although *k* was originally intended to indicate the rate of plugging during the standard blocking period (second fouling regime), the present data indicate that *k* is also directly related to the filtration performance of the different solutions of Blanose™ 7M31F. These values of *k*, are all for the same conditions of pressure drop, initial solids concentration, and show only small differences in solution viscosity. Thus, it appears that *k* does not only characterize the rate of plugging of a medium for the standard blocking period, but also the filtrate capacity of the medium before or until plugging, irrespective of the construction of the medium.

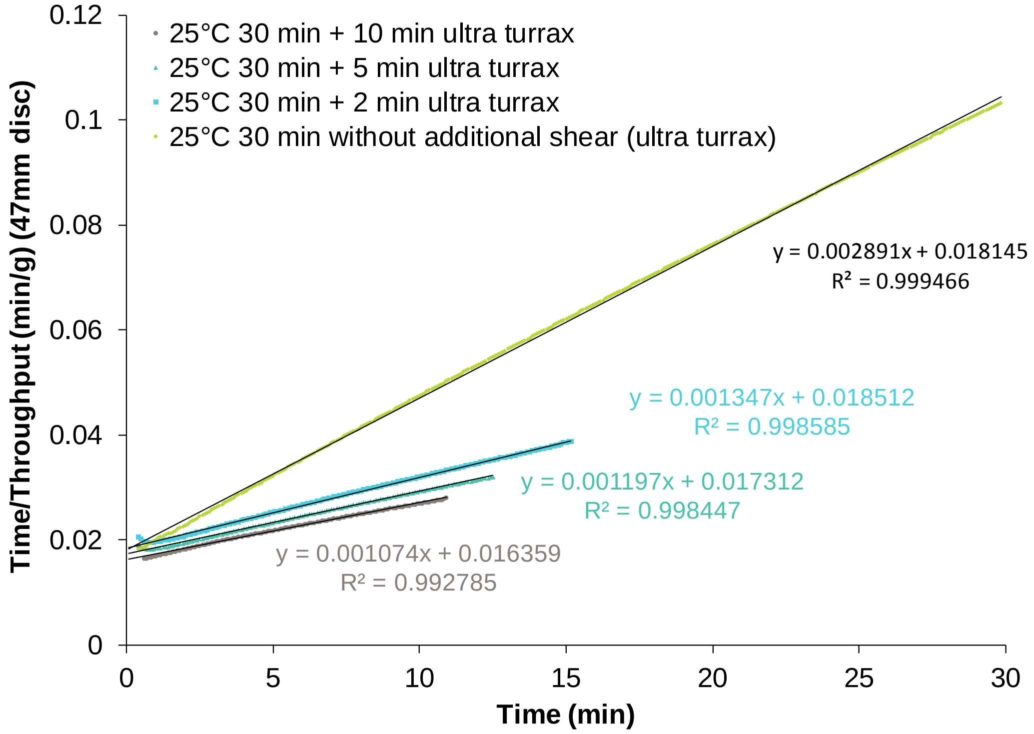

Figure 3: Time/throughput (min/g) versus time (min) of Blanose™ 7M31F to calculate *k* and therefore filterability.

Based on these results, the influence of the preparation method on sterile filtration was further analyzed with respect to preparation temperature, mixing time, and use of mechanical high shear of a 0.4% (w/w) solution of Blanose™ 7M31F. The influence of all factors on filtration performance was systematically evaluated by multiple linear regression at a significance level of $\alpha = 0.05$. A face-centered central composite design (CCF) was performed. The center point was conducted in triplicate to estimate the error of repetition (Table 3). The factor levels were based on previous results, e.g. the additional mechanical shear applied by ultra turrax disperser was set to 2 minutes, to avoid polymer degradation due to the high mechanical shear forces.

Table 3: Factors and levels of the DoE for filtration performance and viscosity analysis

Factor level	-1	0	1
Preparation temperature (Temp) (°C)	10	35	60
Additional mechanical shear (Shear) (min^{-1})	0	10,000	20,000
Mixing time (Time) (min)	60	135	210

An ANOVA analysis was performed considering the three factors as well as their interactions. After backward regression, several significant factors were found for the filtration performance of Na-CMC solutions, and the final model equation for filtration performance was simplified to:

$$y = b_0 + b_1 \cdot temp + b_2 \cdot shear + b_{11} \cdot temp^2 + b_{22} \cdot shear^2$$ **Equation 3**

The quality and regression parameters for the DoE are given in Table 4. The filterability of Na-CMC solutions was mainly affected by additional mechanical shear. With increasing shear forces (expressed as increasing min^{-1}) more mechanical stress was exerted on the Na-CMC particles in solution, enforcing better disaggregation. Thus, filtration was faster with less tendency of filter clogging. A quadratic influence of mechanical shear forces on filterability was detected. Better filtration performance was also achieved with lower preparation temperatures. The quality of the model was considered adequate based on the high values of the coefficients of determination, prediction and reproducibility.

Table 4: Results from the DoE (filtration performance and solution viscosity): power of the model (coefficient of determination, R^2; coefficient of prediction, Q^2; lack of fit, P, and reproducibility) and coefficients for factors (preparation temperature, Temp; and additional shear applied, Shear) to the response variables (coefficient ± confidence interval (α=0.05)).

Parameter	Filtration performance V_m
R^2	0.985
R^2 (adj.)	0.980
Q^2 (>0.5)	0.968
R^2 - Q^2 (<0.2)	0.017
Reproducibility	0.975
Constant	426.009 ± 22.063 (g)
Preparation temperature (Temp)	-42.767 ± 16.931 (g)
Additional mechanical shear (Shear)	200.811 ± 16.931 (g)
Temp*Temp	50.216 ± 30.765 (g)
Shear*Shear	67.192 ± 30.765 (g)

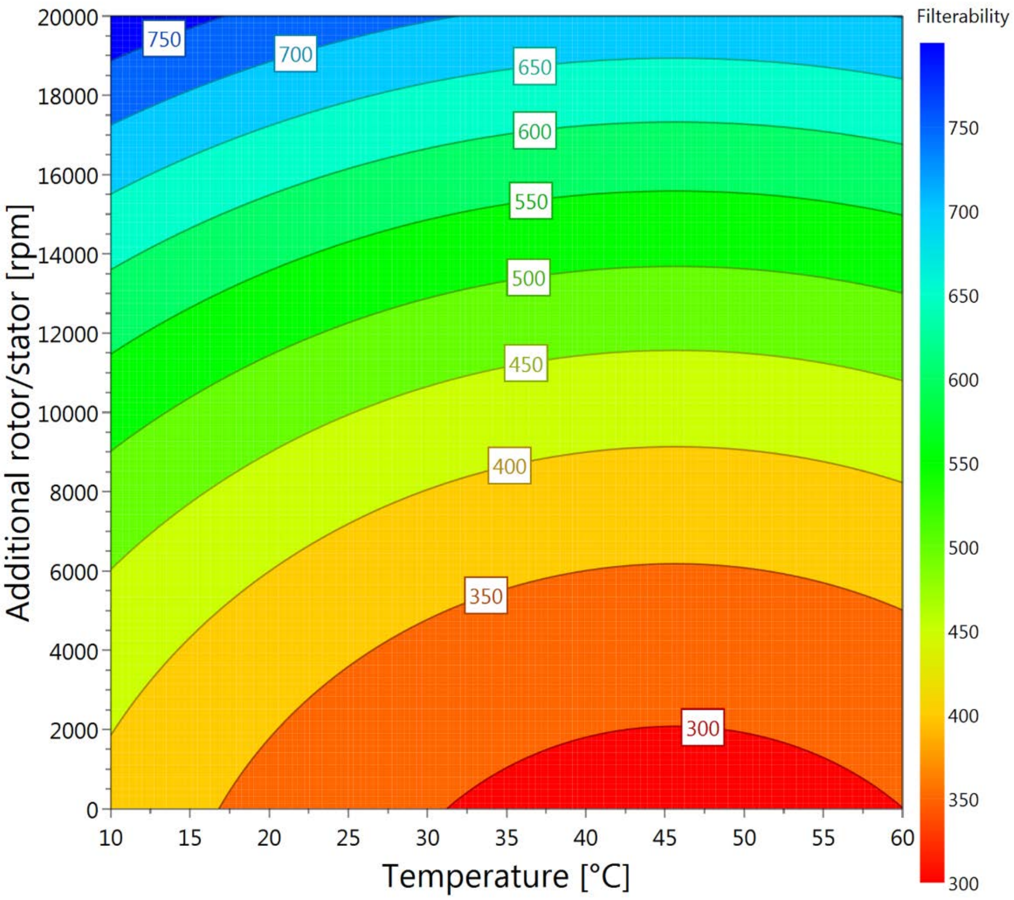

Figure 4: Contour plot of DoE results with temperature and additional high shear as significant factors.

Figure 4 shows a contour plot generated with two significant factors from the DoE. Higher shear forces and lower temperatures result in better filterability. The main influence on filterability is additional mechanical shear, which will be incorporated in all future preparation processes leading to sterile filtration.

Influence of various molecular weight grades of Na-CMC on filtration rate

As a sampling of available molecular weight grades, four grades of Na-CMC at common viscosities relevant to customers were tested for filterability. All grades were prepared using both the traditional method without additional high shear mixing as well as the improved method including 2 minutes of high shear mixing at the end of the process. The concentrations were adjusted to achieve a viscosity of approximately 26 mPa·s prior to additional high shear mixing. Table 5 lists the various use levels of Na-CMC in this study.

Table 5: Selected concentrations of Na-CMC grades for comparative experiments

Polymer Grade	Concentration (%)	Viscosity (mPa·s)
Aqualon™ L2P	2.3	26.2
Blanose™ 7LP EP	1.65	26.6
Blanose 7LF	2.0	26.4
Blanose 7MF	0.65	24.9

Figure 5 shows the results of the sterile filtration runs using various Na-CMC grades adjusted to a viscosity of ~26 mPa·s. Blanose™ 7MF shows the best filtration rate compared with all other grades. Even without the use of additional high shear mixing, Blanose™ 7MF has better filterability than all other grades after being subjected to high mechanical shear forces. All grades show filter blocking after a certain amount of time. For lower molecular weight grades the clogging is almost immediate, resulting in very low throughput even after 30 minutes. The data from this investigation suggest that increasing the molecular weight of Na-CMC for sterile filtration will in turn increase the filtration rate. There is a clear ranking with the highest molecular weight grade on top, followed by both (relative) intermediate grades and the lowest molecular weight grade showing the worst filtration rate. However, only a few different molecular weight grades (especially low molecular weight grades) were analyzed and this conclusion might not hold true for higher molecular weight grades, which were not investigated in this study and could show lower filterability.

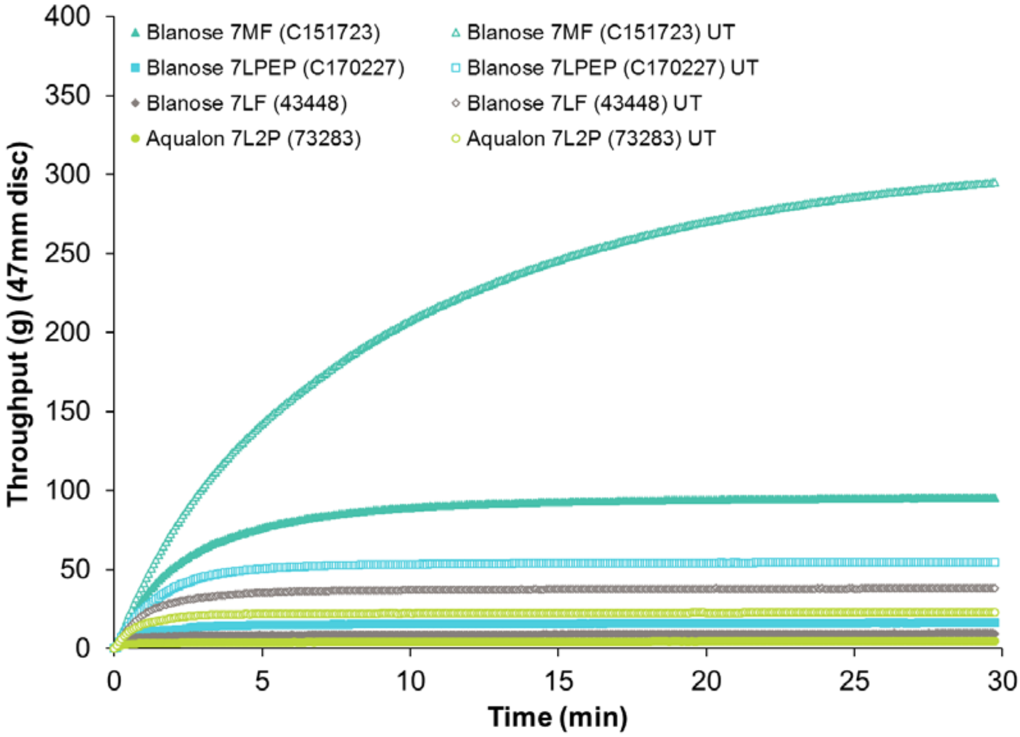

Figure 5: Sterile filtration of various Na-CMC grades prepared with and without additional high shear forces.

Formulation of Aqualon™ 7L2P and Blanose™ 7LF

To investigate the issues with sterile filtration of the customer using their own formulation of Aqualon 7L2P and Blanose 7LF, a solution with both polymer grades and a viscosity of 42 mPa·s was prepared and compared with solutions of the individual polymers at the same viscosity. Table 6 lists the concentrations of Na-CMC in each solution.

Table 6: Use levels of various Na-CMC grades for customer relevant formulations

Polymer Grade	Concentration (%)	Viscosity (mPa·s)
Aqualon™ L2P	1.35	42.3
Blanose™ 7LF	1.175	
Blanose 7LF	2.35	42.9
Blanose 7L2P	2.7	42.0

Figure 6 shows the results of sterile filtration runs for the investigated solution containing both Aqualon™ 7L2P and Blanose™ 7LF as well as the respective solutions containing a single grade. All filtration runs resulted in early clogging of the filters after a short amount of time. This clogging prevents further solution from being filtered, resulting in a throughput of only 10–20 g after 30 minutes of filtration. All of these used the optimized preparation process with 2 minutes of additional high shear after stirring for 60 minutes using a laboratory propeller stirrer.

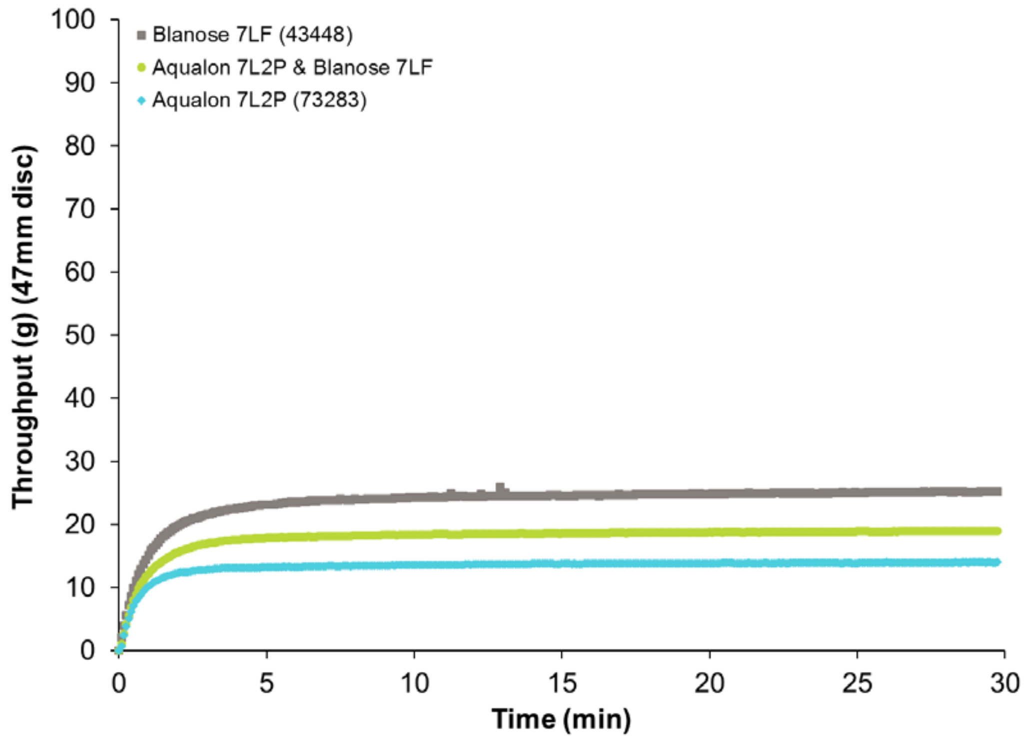

Figure 6: Filtration of a combination of Aqualon™ 7L2P and Blanose™ 7LF compared with individual polymer solutions at 42 mPa·s.

Conclusions and recommendations

Filtration rate of Na-CMC solutions is affected by various preparation steps. An additional period of high shear forces (2 minutes) more than doubled the filtration rate. On the other hand, the 2 min interval did not significantly lower the viscosity compared with no additional shear. A DoE was conducted to investigate the influence of solution preparation on filtration rate. The filterability of Na-CMC solutions was mainly affected by additional mechanical shearing. With increasing shear forces, more mechanical stress was exerted, enforcing better disaggregation of Na-CMC aggregates. Thus, filtration was faster with less tendency of clogging. A quadratic influence of mechanical shear forces on filterability was observed. Better filtration performance was also achieved with lower preparation temperatures. Mixing time, however, did not significantly influence the filtration rate.

The investigation of several molecular weight grades of Na-CMC suggested better filtration performances for grades with higher molecular weight compared with those of lower molecular weight. However, only a few different molecular weight grades (especially low molecular weight grades) were analyzed and this conclusion might not hold true for higher molecular weight grades. Higher molecular weight grades were not investigated in this study and might show lower filterability

A formulation suggested by a customer using Aqualon™ 7L2P and Blanose™ 7LF to create a solution with a viscosity of ~40 mPa·s was investigated for filtration rates. The formulation was tested against single polymer solutions. All solutions showed nonsatisfactory throughputs after 30 minutes.

We recommend that all customers use the optimized preparation procedure for future formulations needing sterile filtration.