

Master's Thesis Report

Formulation development, rheological characterization, and cosmetic evaluation of gel-creams

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Abstract

Oil in water (o/w) emulsions are thermodynamically unstable systems composed of small oil droplets dispersed in a continuous aqueous phase. This means that given enough time, the two phases will separate. Pharmaceutical emulsions are used as a method of administering the Active Pharmaceutical Ingredient (API) for topical applications. The API for this project will be dissolved in the aqueous phase. If the phases are to separate, the concentration of API will not be homogenous throughout product, which will alter the effect of the pharmaceutical. Therefore, the stability is crucial for the effect of a pharmaceutical emulsion.

The stability as well as cosmetic properties of an emulsion is affected by several different properties, referred to as Critical Quality Attributes (CQAs). These are attributes that directly influence the quality of a product. The CQAs are in turn affected by Critical Process Parameters (CPPs) which are specific parameters during the manufacturing process. To ensure a high-quality product with little variation between batches, the CPPs for manufacturing are to be identified, evaluated, and monitored.

In this project the temperature during manufacture, polymer quality, and homogenization speeds will be evaluated as CPPs and their effect on CQAs modelled using a Design of Experiments. From the models it was found that the homogenization speed is most critical to the formulation process, but further work is required to properly model how polymer concentration and temperature affect CQAs.

Populärvetenskaplig sammanfattning

Emulsioner är blandningar bestående av två olika vätskor (även kallade faser) som inte löser sig i varandra. Vanligtvis består den ena vätskan av någon typ av olja och den andra vätskan vanligtvis av vatten. Bägge faserna kan även bestå av andra vätskor eller fasta ämnen som är lösliga i antingen vattnet eller oljan. Om emulsionen är utformad så att oljan är fördelad i små droppar i vattnet, så kallas emulsionen för en olja-i-vatten emulsion. Vattnet är då vad som kallas den kontinuerliga fasen och oljan kallas den dispersa fasen.

Emulsioner är ofta vad som kallas semisolid material, så som krämer, lotioner och majonnäs. Detta innebär att materialet har både fasta och flytande egenskaper. Detta blir tydligt när man trycker ut en klick hudkräm från en tub på handen. Då kan krämen nästan rinna ut ur tuben, likt en vätska, men så snart den stannar tar den formen av en typisk klick hudkräm och kommer inte rinna mer efter det, likt ett fast material. När man sedan smetar ut hudkrämen på handen känns den flytande igen, och ger en enkel och len känsla på huden. Det är precis så man vill att en hudkräm ska bete sig, men detta innebär att det inte är helt enkelt att beskriva emulsionens egenskaper.

Emulsioner används som beredningsform för läkemedel då det gör det enkelt att ta rätt mängd och applicera det på rätt plats. Dessutom kan läkemedlet då ha goda kosmetiska egenskaper, vilket underlättar användandet. Dessvärre är emulsioner termodynamiskt instabila, vilket innebär att det är energimässigt gynnsamt för de två faserna att separera. Faserna vill inte vara blandade. Det finns fyra huvudsakliga mekanismer bakom separationen av de två faserna i en emulsion: gräddsättning, flockulering, koalescens och Ostwald-mognad (Ostwald ripening). Då emulsionerna i detta projekt är mest känsliga för gräddsättning ligger fokus främst på denna mekanism. Gräddsättning innebär att dropparna i oljefasen stiger mot ytan, vilket lämnar vattenfasen koncentrerad kvar i botten. Den drivande kraften för gräddsättning är densitetsskillnaderna mellan vatten och olja, där oljan är lättare än vattnet. För ett läkemedel som är upplöst i vattenfasen kommer detta innebära att koncentrationen av läkemedlet blir högre i botten av behållaren och lägre i toppen, vilket innebär risk för både över- och underdosering vid användning.

Stabiliteten för en emulsion kan däremot påverkas på olika sätt. Gräddsättning kan exempelvis påverkas genom att öka viskositeten, det interna motståndet mot flöde, för den kontinuerliga vattenfasen. Viskositeten kan ökas genom att tillsätta ett förtjockningsmedel i den kontinuerliga fasen. Inom matlagning är det vanligt att använda exempelvis majsstärkelse för att göra en tjockare

sås och inom läkemedel är det i stället vanligt att tillsätta en förtjockare som kallas karbomer. Karbomerer är utmärkta förtjockningsmedel och är därmed utbrett använda. Dessvärre är dessa klassificerade som mikroplaster enligt UNEP (United Nations Environment Programme) och kan vara skadliga för miljön. I detta projekt har i stället en biologiskt nedbrytbar förtjockare producerad från cellulosa använts och utvärderats som substitut till karbomerer.

Genom att öka viskositeten för den kontinuerliga vattenfasen blir det svårare för oljedropparna att stiga i lösningen. Flera andra attribut kan komma att påverka både stabiliteten av emulsionen och känslan av att använda produkten. Dessa attribut kallas för *kritiska kvalitetsattribut* och beskriver bland annat fysiska och kemiska attribut/egenskaper som är essentiella för produktens kvalitet. Vid tillverkningsprocessen för en emulsion finns flera tillverkningssteg som kan ha en drastisk påverkan på de kritiska kvalitetsattributen, så kallade *kritiska processparametrar*. Precis som när man bakar en kaka är det inte bara ingredienserna som bestämmer hur resultatet blir, utan hur hårt äggen är vispade, temperaturen i ugnen, tiden i ugnen, etcetera. På samma sätt som dessa steg är essentiella för både smak, saftighet och den totala upplevelsen för kakan, så finns det kritiska processparametrar som är essentiella för stabilitet och kosmetiska egenskaper för en läkemedelskräm. Detta arbete kommer att undersöka hur homogenisering, temperatur under tillverkning och förtjockarens kvalitet kan påverka den slutgiltiga produkten. Homogenisering är den process som fördelar oljefasen i den kontinuerliga vattenfasen och effekten beror på vid vilken hastighet som homogeniseringen utförs. Förtjockarens kvalitet kommer undersökas genom att variera mängden som tillsätts i ett försök att efterlikna minskad effekt. Temperaturen varieras då den exempelvis kan påverka förtjockarens effekt.

I detta arbete har en experimentell metod, faktorförsök, använts för att utvärdera hur de tre olika processparametrar påverkar emulsionen. De tre parametrarna är temperatur under tillverkning, intensiteten av homogeniseringen och variationer i förtjockarens kvalitet. Med denna metod kan man med få försök observera hur de enskilda faktorerna påverkar kvalitetsattributen, samt hur olika faktorer påverkar varandra, så kallade samverkanseffekter från faktorerna. Modeller har även skapats från resultaten för att kunna ge en komplett bild av hur dessa parametrar påverkar emulsionen i hela försöksområdet. Detta har gjorts för att försöka skapa en process som är robust, och identifiera de processteg som är viktiga att hålla god koll på. Man vill ju ha samma kaka varje gång.

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Abbreviations

O/W	Oil in water
W/O	Water in oil
API	Active Pharmaceutical Ingredient
CPP	Critical Process Parameter
CQA	Critical Quality Attribute
DoE	Design of Experiments
RH	Relative humidity
cP	Centipoise
RCF	Relative Centrifugal Force
RT	Room temperature

1. Introduction and Aims of the study

1.1 The basics of an emulsion

An emulsion is a mixture of two or more different liquids which on their own are insoluble in each other. These will typically be made up by an aqueous phase and an oil phase where one phase will be dispersed as small droplets in the other. An emulsion is a thermodynamically unstable system, meaning that given enough time, the two phases will separate [1]. To allow the two phases to remain dispersed for a longer period, an emulsifier is used. The emulsifier is surface active and will interact at the surface between the two phases, forming a thin surface layer around the droplets that hinder the droplets from merging with each other [2]. This study will be working with an oil-in-water (o/w) emulsion, meaning that the system will be composed of a continuous water phase with a dispersed phase of oil droplets throughout the continuous phase.

1.2 Emulsion stability mechanisms

Since an emulsion is a thermodynamically instable system it will separate into the two original phases given sufficient time. Phase separation occurs via four main mechanisms, creaming/sedimentation, flocculation, coalescence, and Ostwald ripening.

Creaming and sedimentation is the phase separation mechanism where gravity forces separation based on the density differences between the two phases. Creaming occurs when less dense droplets rise upwards in the emulsion, while sedimentation occur if the dispersed phase has a higher density than the continuous phase, causing droplets to settle towards the bottom. The effect gravity has on the droplets can be explained by Stokes law, $v = \frac{2a^2(\rho_0 - \rho)g}{9\eta_0}$. In this equation, the creaming speed (v) is dependent on the radius (a) of the droplets, the gravitational force (g) and the difference in density between the droplets (ρ) and the continuous phase (ρ_0). The rate of creaming is reduced by the viscosity (η_0) of the continuous phase [3]. Creaming will cause an uneven distribution of droplets and cause heterogeneity in the system, as well as it may increase the rate of flocculation.

Flocculation is caused by the affinity of particles for each other, causing them to aggregate in clusters rather than freely dispersed droplets. Flocculation will decrease the distance between droplets which in turn increases the probability of coalescence to occur.

Coalescence is the process of two or more droplets merging and forming one larger drop. This process will eventually lead to a complete collapse of the emulsion as the aqueous phase and oil phase eventually separates completely. Coalescence can be hindered by an emulsifier system.

Ostwald ripening describes a process where larger droplets grow on the expense of smaller droplets since larger droplets are more energetically favored. The smaller droplets will have a higher internal pressure, allowing their constituents to diffuse through the continuous phase into larger droplets that have a lower internal pressure. This process is driven by the solubility of the dispersed phase in the continuous phase and the Laplace pressure. However, this will not be further examined during this project.

1.3 Purpose of a thickener

This project will focus on incorporating a thickener in the form of an environmentally friendly polymer from a renewable source into an already formulated base formulation. The oil phase of the base formulation consists of biodegradable fat, an emulsifier system of both a hydrophobic and a hydrophilic emulsifier. The aqueous phase consists of pure water, the Active Pharmaceutical Ingredient (API), a buffering system to keep the pH at 5. Cetostearyl alcohol is used as a co-thickener to the polymer that will be incorporated into the dispersed oil phase of the formulation.

Cetostearyl alcohol have been used in the dispersed oil phase in other emulsion formulations at Galenica with a proven thickening effect. The exact mechanisms of how Cetostearyl alcohol acts as a thickener is yet to be determined, but since it has a thickening effect, the interactions are believed to affect the continuous phase despite being more soluble in the dispersed oil phase. This project will continue the development of a product with Cetostearyl alcohol as a thickener but will not further explore the mechanisms of how the thickening effect is achieved.

The base formulation has been extensively tested prior to this thesis, and it is presumed that the emulsifier system is robust enough to prevent coalescence from occurring. Instead, creaming of the oil phase is expected to be the main separation mechanism.

By incorporating a thickener into the continuous phase, the viscosity will increase, and thus the formulation's resistance to creaming. While an increased viscosity will aid in the formulation's stability, it is of great importance that the cosmetic properties remain pleasant, and that the formulation still spread easily.

1.4 Aim of the study

The aim of the study is to determine the influence of a polymer on a standard oil-in-water emulsion, as well as to determine how Critical Process Parameters (CPPs) will affect Critical Quality Attributes (CQAs) and cosmetic properties. The project will focus on incorporating environmentally friendly polymers as an alternative thickener to polyacrylates (also called carbomers), since polyacrylates are defined as microplastics [4].

A Design of Experiments (DoE) will be set up to model the process robustness as well as rheological and cosmetic properties of a formulation with the polymer incorporated. Experiments with the purpose of predicting emulsion stability will be performed as well as stability studies of the emulsion.

2. Theoretical Background

According to the European Medicines Agency's ICH guidelines, CQAs are defined as "A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.". This description is then further related to the definition of CPPs as follow "A process parameter whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure the process produces the desired quality.". [5]

During this project the CQAs under observation are viscosity, rheological properties, droplet size and textural properties. These will be monitored by altering two CPPs, the temperature and the homogenization process. One final factor that will be altered during this project is the polymer concentration of the chosen polymer, which also will influence CQAs, making a total of three different test factors.

The temperature during manufacture can affect the internal structures as the formulation is formed, as well as influence individual excipients, such as the viscosity, solubility and swelling of the polymer.

Homogenization is likely to have an effect of the droplet size of the formulation, where higher homogenization speeds will lead to a smaller droplet size and a lower rate of creaming. The decreased droplet size is also likely to affect the cosmetic properties of the formulation. Higher

homogenization rates could possibly affect the thickening effect of the polymer if the polymer is broken.

The polymer concentration is expected to be correlated to the viscosity and the perceived thickness of the formulation. It is also likely to affect the perceived stickiness of the formulation.

2.1 Selecting a suitable thickener

Four different thickeners were evaluated, Xanthan gum, Carrageenan, hydroxypropyl methylcellulose (referred to as Hypromellose) and Sodium alginate. The thickeners will be incorporated into the base formulation with the purpose of increasing viscosity, and as a result decrease the rate of creaming.

For the thickeners to be suitable to use in the formulation there are several requirements that must be met. Any thickener used in the formulation will have to be stable and non-reactive to the API as well as the other excipients in the formulation while remaining stable in the aqueous phase during long term storage. The thickener also needs to be stable during the production stages of the formulation, meaning a desired stability during both homogenization and heating of the product. To ensure homogeneity in the sample, the thickener also needs to be completely soluble in the aqueous phase, otherwise there is a risk of crystals or particles of thickener forming in the aqueous phase, as well as decreased thickening effect from the polymer.

2.2 Stability study of an emulsion

There are several methods and theories developed for predicting the stability of an emulsion, but there is only one way to know for sure, performing a stability study. During a stability study the sample will be kept under specific conditions such as a defined temperature and humidity during storage for the intended shelf-life of the product. At the beginning of the stability study, a series of tests will be performed to measure the physical properties of the formulation. Which tests are performed can vary, but any test that might indicate separation of the emulsion or reveal the separation mechanisms will be of great use during the stability study. The first measurements are referred to as the initial measurements and will serve as a reference for upcoming measurements. At different test times, a sample will be taken from storage to be tested once more. The results from this test will be compared to the initial to determine if any changes have occurred in the emulsion. Any major differences between the initial and later measurements are likely an

indication of instability, but if the results are uncertain or the difference is small, it is recommended to resume the controlled storage to observe the change over a longer time. By running tests at regular intervals, the long-term stability of an emulsion can be measured, and conclusions regarding the shelf-life and recommended storage conditions can be made.

Conducting a long-term stability study at 25°C/60% Relative Humidity (RH) from manufacturing until the desired shelf-life is met provides the most reliable assessment of the long-term stability. However, this process may go on over several months or even years. As an alternative approach, an accelerated stability study can be conducted simultaneously as the long-term stability study. This accelerated study stress tests the formulation, yielding results in a significantly shorter timeframe. While not as definitive as the standard study, it offers valuable insights into the emulsions long-term stability, however, some formulations that separate under accelerated conditions might still be stable at 25°C. Throughout this project, stability studies will be conducted at 25°C, 30°C, and 40°C.

2.3 Stress testing by centrifuge

Apart from storage under increased temperatures, the emulsion can be stress tested by centrifugation. Since creaming is affected by density differences between the two phases, applying a centrifugal force will accelerate the creaming and further accelerate phase separation of the emulsion. Therefore, by performing a centrifugal stress test on a sample, information regarding long-term emulsion stability can be achieved in only a few minutes. While centrifugation is a great way of quickly getting an indication regarding emulsion stability, it should be noted that some emulsions separate under intense centrifugation while they would be stable under regular conditions.

2.4 Measuring emulsion separation

As the emulsion separates, creaming will cause the oil phase to rise in the sample, resulting in the aqueous phase collecting at the bottom. During macroscopic phase separation this can be seen visually as an opaque fluid appearing at the bottom, but with the correct techniques, microscopic amounts of creaming can be detected by measuring how light is deflected throughout the sample.

During this project a Turbiscan LAB was used to observe physical changes in the emulsion over time. The Turbiscan works by emitting light with a wavelength of 880 nm at a sample and

measuring the amount of light backscattered (reflected) and transmitted (shines through) and can detect differences that are not apparent visually. While measuring, the light source will move from the bottom of the sample to the top. By doing this, the transmittance and backscattering will be measured throughout the sample. If there is no sign of phase separation (Mainly creaming, but flocculation and coalescence as well) the results should be uniform throughout the sample, but differences could appear from other factors like air bubbles, irregularities in the glass vials or other contaminants. Therefore, it is better to measure the samples over time, observing the difference from the initial measurements.

By measuring the difference between initial and later measurements, the Turbiscan can detect microscopic changes in the emulsion that will alter how light is refracted by the sample. These changes can then be related to phase separation mechanisms such as either creaming, flocculation, or coalescence of the sample. In this project, creaming is the most likely phase separation, and the focus will be on detecting creaming.

2.5 Predicting emulsion stability

During this project rheological testing will be used to characterize both cosmetical properties and make predictions/estimations about the stability of the emulsion. Tests using the rheometer is prepared by placing a sample on a temperature-controlled area and lowering the conical measuring bob onto the sample. Any excess sample is removed from the plates.

Once the sample is loaded, the measuring bob will be able to rotate, and the rheometer measures the torque and the deflection angle. The sample will provide a resistance to the applied torque and by comparing the deflection angle to the applied torque, or required torque for a certain deflection angle, the rheometer can determine the internal forces of the sample. [6]

In this project, the rheometer will be used to perform three different tests, one rotational test meaning that the cone will rotate in one direction with a controlled shear rate (CSR or CR viscosity test), and two different oscillating tests, amplitude sweep and frequency sweep, where the measuring bob rotates back and forth.

During the rotational test, the viscosity of the sample is measured at varying shear rates which will give a graph with the viscosity as a function of shear rate. This is desirable as many emulsions display shear thinning behavior, meaning that the viscosity will decrease with increased shear rate.

At lower shear rates, a region where the viscosity is linear with the shear rate will appear. This viscosity is called the zero-shear viscosity and is the viscosity of the sample at rest. This is a crucial property as it is the sample's resistance to internal movement such as droplets creaming or flocculating in the solution, thus aiding in the stability of the emulsion. The viscosity at higher shear rates is also important, as this will relate to the cosmetical properties when applying the product.

The two oscillating tests are performed to gain a better understanding of the inner forces and structures of the emulsions. Amplitude sweep is performed by applying a gradually increasing force (and as a result, shear stress) while oscillating back and forth at a constant frequency. This will result in a graph of both the elastic modulus (G') and the viscous modulus (G'') as a function of shear stress and will be used to determine the yield stress and the flow stress of the sample. The yield stress is the stress at the end of the linear viscoelastic (LVE) region, the range of which both G' and G'' remain constant during increased shear stress, though for evaluation, the curve of G' is often preferred by users [7]. The LVE region is the range of shear stress that can be applied on the sample without destroying any structure in the sample, and the yield stress is the maximum stress that can be applied to the sample before the structure breaks. The flow point is the point in the graph where G' and G'' are equal. At this shear rate, G'' will overcome G' , and the sample will transition from acting as a solid to acting like a liquid. [8]

Frequency sweeps are instead performed at a constant controlled stress, while the oscillation frequency increases gradually. By performing the frequency sweep in the non-destructive deformation range (i.e., the LVE region), the test can give information about time-dependent behavior of a sample. The high frequency simulates how fast motion affects the sample on short timescales, while low frequencies simulate low motion or sample at rest under long timescales. Therefore, by observing the difference between G' and G'' at low frequencies, some information regarding the formulation's stability from internal forces at rest can be collected. If G'' exceeds G' at low frequencies, the sample act as a liquid at rest, indicating that it is susceptible to segregation by creaming or sedimentation. [9]

2.6 Cosmetic properties

The textural properties will determine the sensory effects of an emulsion. In this project there are four textural properties that will be measured by texture analysis, firmness, consistency,

cohesiveness, and adhesiveness. These properties can be analyzed using a Texture Analyser, which is an instrument that moves a probe vertically up and down while measuring the forces applied to the probe together with time and position of the probe. By doing so, the instrument can measure the forces applied by the sample on the probe, which can be related to the textural properties of the sample. For full equipment description, see heading 3.2 Equipment.

Firmness is calculated as the peak of the positive force curve when the probe is moving into the sample. It will yield a positive value and a higher value will mean a firmer sample [10,11]. Consistency is calculated by the area under the positive curve. Consistency relates to the total amount of work needed to deform the sample. A higher consistency value will relate to thicker sample. The cohesiveness is measured by the maximum negative force when withdrawing the probe from the sample. Since cohesiveness is a negative force, a lower value of cohesiveness will mean a more cohesive sample [11]. Adhesiveness or stickiness is measured as the negative area acquired when the probe is withdrawn. It can be related to the negative (upwards) work needed to separate from the sample. [12,13]

2.7 Theory behind pre-experiments

2.7.1 Melting and solidification point of oil phase

When constructing an emulsion, each excipient is maintained above its melting point or dissolved in the aqueous or oil phase. This ensures that each phase has a homogenous distribution of components. Mixing the two phases at similar temperatures is suggested to prevent separation due to solidification. While the phases remain in a liquid state, homogenization is performed to disperse the oil phase into small droplets throughout the aqueous phase, forming the emulsion. Temperatures are maintained to ensure that both phases remain in a liquid state, preventing any phase transitions in the emulsion. Following the homogenization step, the temperature is allowed to gradually decrease during stirring. As the emulsion continues to cool, structures may be formed due to solidification of excipients that impact the emulsion's textural and stability properties.

If homogenization was performed at a temperature that allows for the oil phase to solidify, it would likely lead to an uneven distribution of droplet size, and uneven aggregation of oil phase across the emulsion. It is also possible that structures built by emulsifiers are broken, resulting in vastly different properties of the finalized product. Thus, when altering the production temperature, it is important to be aware of the temperature at which the oil phase solidifies.

The temperature of solidification will set a lowest possible handling temperature when producing the emulsion. For this study, the assumption will be made that the emulsifiers start building structures in the emulsion at the same temperature as the oil phase will solidify. Thus, the thickening temperature can be measured by measuring the temperature when the oil phase transition from liquid to solid. This temperature is the lowest possible temperature for the design space in the DoE, but to ensure that the temperature is not crossed it is suggested to maintain temperatures significantly above the solidification point of the oil phase.

2.7.2 Effect of homogenization and heating

During the manufacturing process of the emulsion, the products will undergo both heating and homogenization, two processes that may alter excipients incorporated in the formulation.

The polymer may be degraded during homogenization or heating. Heating can cause changed properties of the polymer. If the changes are not reversible it can cause altered rheological properties of the formulation and in turn lead to decreased stability or unfavorable cosmetic properties of the emulsion. During both heating and homogenization, it is possible that the polymer breaks, causing a decrease in the polymer length, resulting in a decrease in the viscosity of the solution. Since viscosity is a potential QCA of the formulation, the effect of heating and homogenization will be examined prior to the execution of the DoE.

2.7.3 Accounting for variations in the thickener

According to the European Pharmacopoeia (Ph. Eur.), the variation of viscosity for monograph for Hypromellose is between 75 to 140% of the nominal value. This results in a viscosity between 3000 and 5600 centipoise (cP) for a sample with the nominal viscosity of 4000 cP [14]. Though Certificates of Analysis (CoA) from supplier display much less variation, the allowed variation in viscosity must be accounted for. This batch-to-batch variation of viscosity of the thickener is likely to affect the complete formulation. To evaluate how the formulation will be altered by changes in the thickener, it is valuable to perform experiments with samples with viscosities of 3000 and 5600 cP respectively. However, there are no samples at hand with these specific nominal viscosities. Instead, the polymer concentration (of polymer from the same batch) will be altered to mimic the limits of viscosities in the Ph. Eur monograph. This is believed to yield similar differences in viscosity, but there is a possibility that a higher polymer concentration of lower quality will give a stickier sensation, however, this is not further evaluated in this thesis.

To determine how much the thickener concentration should be altered to give a viscosity at 75% and 140% of the nominal value, a series of experiments were set up to examine how the viscosity changes with polymer concentration. Once determined, the polymer concentration for the upper and lower limits of viscosity is used in the DoE to observe if low/high viscosity batches of Hypromellose will disturb the process robustness or how it will affect CQAs.

2.8 Theory behind Design of Experiments

A Design of Experiments (DoE) is an experimental method of modelling if and how a specific variable is affected by multiple different factors. Each factor will be placed along their own separate axis, meaning that by having two factors, a two-dimensional design space will be made, and by having three factors, a three-dimensional design space and so on. The result will be a model where each point in the design space will correlate to a value of the examined variable. Multiple different variables can be examined from the same series of experiments, but each observed variable will return their own separate model.

During the following project, a screening, full factorial screening DoE will be performed, meaning that each corner of the design space will be experimentally determined, together with a series of replicates at the center of the design space. From this, a model can be made to predict how any changes of the factors will alter the variables under observation.

2.8.1 Test factors and design space

When designing a DoE, the test factors are supposed to be selected as parameters that are controllable and is suspected to have an influence on the observed parameters. If the factors do not affect the observed parameters, no models will be found, and thus no predictions can be made. However, as long as one or more test factors does influence the measured variables, it is possible to create a model. A DoE is usually designed around a previously performed experiments, with test factors being increased and decreased to observe the response. The experiment that the DoE is centered around is referred to as center-point. [15]

The center point of the DoE will be defined as the middle of each test factor, and as each test factor is increased and decreased the same amount, a cubic design space with the center-points placed in the middle appear. The center points serve both as a way of determining the standard deviations

of the experiments, as well as to determine if the response from the factors have a linear effect on the test parameters or not. [15]

Once the center point of the DoE is determined, the size of the design space is to be determined. The size of the design space refers to the range of how much the chosen test factors will alter from their high to low values. When selecting the range of test factors, three criteria are to be met: the experimental feasibility, experimental objective, and the experimental noise. Experimental feasibility refers to the relevance of each test factor scale, ensuring that the design space is still within a region that is relevant for the DoE. Experimental objectives specify that the range of the test factors is big enough to see the effect of the specified test factors. Experimental noise attributes to ensure that the effect of the test factors is large enough to overcome any noise or error of margin from the experiments. [15]

2.8.2 DoE model terms

Once a DoE is performed and a model is acquired, the model will be evaluated using four important diagnostic tools: goodness of fit (R^2), goodness of prediction (Q^2), model validity, and reproducibility. Goodness of fit describes how well the experimentally measured data fit the regression model. R^2 will be given as a number between 1 and 0 where 1 indicates a perfect model and 0 means no model at all. While R^2 is a good metric to determine how well a model is fitted to experimental data, it does not describe the usefulness of the model. However, Goodness of prediction does give an indication of the model's predictive power and can therefore be used as a metric to determine how useful the model is. Just like R^2 , the upper limit of Q^2 is 1, and would indicate a perfect model, but unlike R^2 , Q^2 have no lower limit. [16]

When analyzing a model both R^2 and Q^2 should be compared. A difference higher than 0.2 or 0.3 between R^2 and Q^2 should be seen as a warning of an inappropriate model. The limits of what can be seen as a good model varies depending on application, but a value of Q^2 higher than 0.5 is typically regarded as good, and a value of Q^2 higher than 0.9 regarded as excellent. [16]

Model validity is a numeric value describing if the model is appropriately fitted to the experimental data where a higher value suggests a more valid model. Here, a value higher than 0.25 indicates a valid model. [16]

The final diagnostic tool for evaluation of the model is the reproducibility. The reproducibility is a metric to determine the size of replicate errors compared to the variability of the entire design. A reproducibility above 0.5 is desired, and if found to be below 0.5 indicates large pure errors and a lack of control during the experimental procedure. [16]

3. Material and Methods

3.1 Material

The material used during this thesis is the excipients and API needed for the base formulation provided by Galenica, as well as four different thickeners, Xanthan gum, Carrageenan, Hypromellose and Sodium alginate that are to be incorporated into the base formulation for evaluation.

When performing the DoE, the selected thickener will be hypromellose, and the same lot will be used for each batch produced for the DoE to avoid any lot-to-lot differences.

3.2 Equipment

The following analytical equipment was used during the project. For all rheological measurements a rheometer CVO Int 50 from Bohlin equipped with a water bath as temperature control. A viscometer DVNext RV from Brookfield was used and was equipped with a Helipath drive motor, also from Brookfield. The turbiscan measurements was taken using a Turbiscan LAB from Formulation. The centrifugations were performed by using a Labofuge 400 from Heraeus. The texture analyzer used in the project is a TA50/650E Texture Analyzer TA.XTPlusC from Stable Micro Systems, equipped with a ½ inch hemispherical stainless steel probe and a 500g loadcell. Data from the texture analyzer was processed in the software Exponent Connect.

During manufacturing of smaller scales, the homogenizer in use was an Ultra Turrax T25 Basic from IKA. Each ingredient used was weighed using a 6200C SCS scale from Precia if the contents was above 6 grams, otherwise a XS204 scale from Mettler Toledo was used. The heating plate stirrer used was a RCT basic from IKA. The thermometer used was a model 620-2079 from Control Company (VWR). Water used in the formulation was purified using a Milli-Q Water Purification System model Advantage A10 from Millipore. During emulsion production of larger scales, up to 3 liters, an Unimix LM3 from Ekato Systems was used for homogenization, heating, cooling, and stirring of the product.

The software MODDE was used to create the DoE experiments and run order, as well as used for creating, evaluating, and displaying the models created.

3.3 Methods

For the DoE, 2.5 kg batches of emulsions were manufactured in the Unimixer with a prewritten batch protocol. The exact batch protocol for manufacturing is not shared in this report, but only a short summary. The aqueous phase was prepared in the Unimixer under low homogenization to ensure that each excipient was dissolved and evenly dispersed. Hypromellose was added last to the aqueous phase during homogenization at a low rate to reduce the risk of forming lumps of Hypromellose. The aqueous phase and oil phase were heated individually before the oil phase was added to the aqueous phase and mixed in the Unimixer vessel. Homogenization was performed in the Unimixer while maintaining temperatures. The formulation was then cooled to the same temperature at a controlled shear rate, with the same rate of cooling for each batch, resulting in different cooling times between batches of different manufacturing temperatures.

When preparing 2% polymer solutions for analysing viscosity of the raw material according to the monograph for Hypromellose in the European Pharmacopeia, 10.00 g of dry Hypromellose was weighed in a 1 L beaker. Hot water (90-99 °C) was added until a total volume of 500 g was achieved. A lid was placed on the beaker and the substance was stirred at a rate of 400 ± 50 rpm for 10-20 minutes until the particles were thoroughly dispersed and wetted. Any material stuck on the side of the beaker was scraped down with a spatula. The beaker was then placed in a water-bath maintained below 10 °C and stirring continued for another 20-40 minutes. The mass of the solution was adjusted to a total of 500 g if needed. The viscosity of the solution was determined at a temperature of 20 °C.

During rheological testing, three different tests were performed on the rheometer, while one viscosity test was performed on the viscometer and the textural properties were measured using the texture analyzer.

The viscosity CR tests performed on the rheometer used a linear increase in shear rate from 0.0006 s^{-1} to 300 s^{-1} . The sweep time was 480 seconds and 50 sample points were placed with a logarithmic distribution. The gap size was set to 150 μm .

The amplitude sweeps were performed at a frequency of 1 Hz with a controlled stress ranging from 0.001 Pa to 50 Pa. 30 sample points were taken with a logarithmic distribution and the delay time was set to 2 seconds. The gap size was set to 150 μm .

The frequency sweep was performed at a controlled stress within the LVE region acquired from the amplitude sweep, but for each emulsion in the DoE this was set to 1 Pa. The frequency ranged from 0.001 Hz up to 20 Hz and 60 samples were taken over the test with a logarithmic distribution. The gap size was set to 150 μm .

When measuring the viscosity using the viscometer on emulsions manufactured for the DoE, a 60 ml glass vial was filled with sample. The viscometer was equipped with a T-C spindle as well as a helipath to ensure the spindle does not rotate at the same place in the sample during the test. The spindle was set to rotate at 12 rpm for 30 seconds roughly 0.5 cm under the sample surface before the helipath was started. Once the helipath was turned on, the spindle was kept rotating at 12 rpm while 5 measuring points were taken with 10 seconds in between each measurement.

During the texture analyzer tests the test mode was set to compression. Pre-test speed, test speed and post-test speed were all set to 1.00 mm/sec. The trigger force to start measurements was set to 0.1 g. The target mode was set to distance at 5 mm, after which the probe returned to its initial position before ending the test. Each test was performed in triplicates and the software was set to calculate absolute positive force, positive area, absolute negative force, negative area, and negative distance for each test as well as the average from the triplicates.

The centrifugation stress testing was performed by filling Turbiscan vials with emulsion and centrifuging for 2 minutes at a Relative Centrifugal Force (RCF) of 1000 to remove any trapped air from filling the samples. An initial scan was taken in the Turbiscan and kept as reference. The sample was then centrifuged three times at 3500 RCF for 10 minutes each time. A Turbiscan measurement was performed after each centrifugation, resulting in 4 total Turbiscan measurements, and 30 minutes of centrifugation at 3500 RCF.

3.4 Preparation of pre-experiments

Prior to performing the Design of Experiments, a series of pre-experiments have been performed to gain a better understanding of the design space and finalizing the formulation.

3.4.1 Stress testing and selecting thickeners

Creaming is the most likely mechanism of separation, and sufficient creaming will lead to macroscopic phase separation of the continuous aqueous phase at the bottom of the container. The emulsifier system is known to be robust, and coalescence has not been observed in this emulsifier system before. Therefore, rheological testing, centrifugation in combination with Turbiscan measurements and storage under both 25°C as well as under increased temperatures, also assessed using Turbiscan, was performed, as these tests can give indications of the rate of creaming. Each thickener (xanthan gum, carrageenan, hypromellose and sodium alginate) was incorporated into the same base formulation provided by Galenica for stress testing.

A Turbiscan vial with each formulation was prepared and to remove any air bubbles and other possible irregularities, the samples were first centrifuged at a relatively low force of 1000 RCF for 2 minutes before making an initial turbiscan measurement that will serve as a reference for the following scans. Stress testing of the different formulations was then performed by using a higher speed and longer centrifugation time. The samples were centrifuged three times at 3500 RCF for 10 minutes and analyzed in between each centrifugation by turbiscan measurements. After centrifugation the samples were also investigated visually for phase separation.

3.4.2 Melting and solidification point of oil phase

The melting point and the solidification point of the oil phase was determined by placing a prepared sample of the oil phase in a water bath on top of a heating plate. A magnet stirrer was placed in both the water bath and the oil phase to ensure an even temperature distribution in both beakers. A thermometer was placed in both the water bath and the oil phase to control the rate of heating. During heating, some excipients will melt before others, which is to be expected. However, this test aims to define the temperature where all excipients are dissolved.

When the oil phase is completely dissolved, the temperature was noted as the melting point and the heating plate was turned off. The water bath was removed, and the oil phase was cooled to ambient temperature during stirring. The temperature where solid particles are first formed in the oil phase has been considered the solidification temperature of the oil phase.

3.4.3 The effect of homogenization and heating on the chosen polymer

Polymers are colloidal large chemically linked molecules that may degrade during homogenization and heating. It is important to have a manufacturing process that does not significantly decrease

the viscosity of the aqueous phase. Therefore, both the influence of homogenization and heating have been investigated.

According to the manufacturer, hypromellose of the quality that was used has gelling temperatures at 70 and 85°C in water. This is claimed to be a reversible gelation upon cooling but could be disadvantageous when forming an emulsion. However, the gelling temperature will be affected by other excipients in the aqueous phase, and this will need to be kept under consideration when heating the polymer.

Heating

To examine how heating will affect the thickener, an aqueous phase was prepared and then heated to 90°C during stirring. Samples were taken at temperatures of 70, 80 and 90°C and were allowed to cool to room temperature under ambient conditions. To decrease the amount of water evaporated during heating, aluminum foil was used to cover the sample. Rheological testing was performed at room temperature and used to observe changes in zero-shear viscosity from the applied heat.

Rheological testing was performed at room temperature and used to observe changes in rheological properties of the zero-shear viscosity and viscosity under higher shear.

Homogenization

To investigate if homogenization may degrade the thickener, the aqueous phase was homogenized at 10 000 rpm using the Ultra Turrax homogenizer during a total time of 60 minutes. Samples were removed before homogenization, and then after 10, 20, 30, 40 and 60 minutes of homogenization. In case of degradation, the viscosity is reduced by homogenization which will be examined by performing tests with rheometer. Zero-shear viscosity is suggested to be important for the emulsion stability and it has therefore been examined how it is altered by homogenization time.

3.4.4 Examination of viscosity variation when altering polymer concentrations

The monograph for hypromellose in Ph. Eur. Presents a method for preparing and measuring the viscosity of a 2 wt.% hypromellose dissolved in water. For a solution with a nominal viscosity of 4000 cP a 75-140% variation of the nominal viscosity is allowed, meaning that a variation in viscosity of 3000-5600 cP is allowed [14]. An experiment was designed to determine how the viscosity changes with the polymer concentration. The experiment was set up by preparing five batches of hypromellose dissolved in water with hypromellose concentrations of 1.5, 1.8, 2.0, 2.2

and 2.5% by weight. Each batch was prepared and analyzed according to the test for viscosity in the Ph. Eur. monograph for hypromellose with an exception for spindle setup and the viscometer. The viscosity of each sample was measured using the viscometer but with a different spindle setup, as the spindles used in Ph. Eur. were not available at Galenica. The spindle used was a T-B spindle, set to rotate at 60 rpm for two minutes before taking 5 measurements with 10 seconds in between each. After measurement the spindle waited 2 minutes before repeating the analysis twice more. The viscosity is reported as the average of measurements.

Since the setup of the performed experiments differ from the European Pharmacopoeia, the obtained viscosity will differ as well, but the relative differences in % is assumed to be proportional and it is used to compare how the viscosity changes with the polymer concentration and will be used to model viscosity variation that is acceptable in Ph. Eur. [14]

3.5 Method for Design of Experiments

3.5.1 Defining the design space

For the DoE study, three process parameters (referred to as test factors) were varied, polymer concentration, homogenization speed and the temperature during manufacture of the emulsion. This results in a three-dimensional, cubic design space with one parameter on each axis. One experiment for each corner of the design space was performed, giving eight batches with different variation of either high or low values of the examined test factors. In addition to the eight experiments, three batches at the center of the design space were manufactured as replicates to determine errors of margin as well as information at the center of the design space. In total, eleven batches of 2.5 kg were produced for the DoE using the unimixer semi solids vessel.

The main purpose of the altered polymer concentration is not to see what effect polymer concentration will have on the mixture, but rather to identify if viscosity differences between batches of hypromellose may result in changes of formulation properties and stability.

The design space of the DoE is based on information gathered from previous batches and pre-experiments. Since a formulation tested with 0.7% Hypromellose proved promising results during stress testing, the center point of polymer concentration was selected to 0.7%. The upper and lower ends of polymer concentration were based of the viscosity monograph from Ph. Eur. of hypromellose. It was decided to have a variation in polymer concentration of $\pm 10\%$, setting the

lower bound at 0.63% and the upper at 0.77%. The explanation as of how the polymer concentration variation was selected is described under heading “4.1 Pre experiments”.

The design space for the homogenization speed was set to 5000 to 12 000 rpm and was based on previous experiences of two different commercial manufacturers. The lower homogenization speed of 5000 rpm has in a previous internal investigation at Galenica been determined to result in a similar droplet size as a commercially manufactured emulsion. The upper homogenization rate of 12 000 rpm is the highest possible speed of the unimix semisolids vessel.

The limits of temperature were based of the pre-experiments observing the melting and solidification point of the oil phase for the formulation, as well as the experiment observing the behavior of the thickener (hypromellose) under and after heating. The limits were set to 60 and 75°C respectively giving a center point at 67.5°C. A summary of the design space and the eleven experiments can be found in table 1.

Table 1: The batches set up for the DoE with their respective test factors.

Exp No	Exp Name	Run Order	Incl/Excl	Temperature [°C]	Homogenisation speed [rpm]	Polymer concentration [wt.%]	Batch no
1	N1	10	Incl	60	5000	0,63%	529-2307-11
2	N2	9	Incl	75	5000	0,63%	529-2307-10
3	N3	1	Incl	60	12 000	0,63%	529-2307-02
4	N4	6	Incl	75	12 000	0,63%	529-2307-07
5	N5	11	Incl	60	5000	0,77%	529-2307-12
6	N6	3	Incl	75	5000	0,77%	529-2307-04
7	N7	2	Incl	60	12 000	0,77%	529-2307-03
8	N8	5	Incl	75	12 000	0,77%	529-2307-06
9	N9	7	Incl	67,5	8500	0,70%	529-2307-08
10	N10	4	Incl	67,5	8500	0,70%	529-2307-05
11	N11	8	Incl	67,5	8500	0,70%	529-2307-09

3.5.2 Experimental methods of DoE

Each batch produced for the DoE will be analyzed by viscometer, rheometer, texture analyzer and under microscope to measure their physical properties. These results will be used to model how the three process parameters will alter the physical properties of the formulation.

Viscometer

The samples are analyzed using a viscometer RV equipped with a T-C spindle as well as a helipath, moving the spindle down in the sample while measuring. The analysis was performed by rotating the spindle at 12 rpm at the surface of the sample for 30 seconds, before starting the helipath. Once started, the viscometer took 5 measurements with 10 seconds intervals. The average viscosity was calculated and reported for each sample.

Rheometer

The rheometer was equipped with a 4° cone and the gap size was set to 150 µm. The rheometer was used to perform three different tests, controlled rate viscosity test, amplitude sweep and frequency sweep. From the viscosity-shear test the zero-shear viscosity and the viscosity at high shear is reported. From the amplitude sweep the yield point and flow point is reported, and from the frequency sweep the crossover point between G' and G'' is reported if identified, otherwise reported as no crossover point.

Texture analyzer

The texture analyzer was used to measure each sample of the DoE, and the reported properties are the firmness, consistency, cohesiveness, and adhesiveness of each batch. The tests are performed in triplicates and the average of each property is reported.

Microscope

The microscope is used to gain an estimation of the droplet size of the emulsion. The droplet size is measured in the image processing software of the microscope.

4. Results and Discussion

4.1 Pre experiments

The results of the pre-experiments determined the boundaries for the DoE design space.

4.1.1 Stress testing and evaluation of thickeners

The four different thickeners incorporated into the formulation was stress tested under centrifugation of 3500 RCF for a total of 30 minutes with turbiscan measurements after each 10 minutes of centrifugation. The result from xanthan, hypromellose, sodium alginate, and carrageenan can be seen in Figure 1, Figure 2, Figure 3, and Figure 4 in that order. None of these

tests resulted in macroscopic phase separation detected visually, but as can be seen in the measurements, there is a minor indication of phase separation for each thickener apart from Hypromellose. The difference can be observed by the difference in height between measurements of the samples. While these indications are not definitive, it was reason enough to continue development with hypromellose as a thickener.

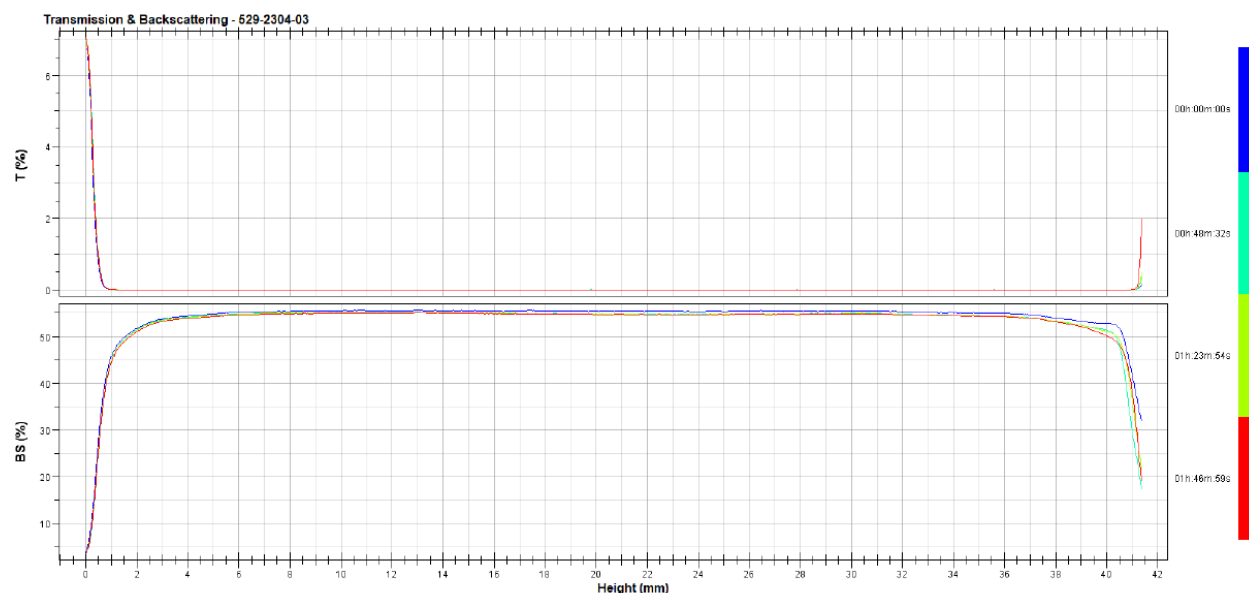


Figure 1: Transmittance and Backscattering measurement of the centrifugal stress test of xanthan gum incorporated to the base formulation.

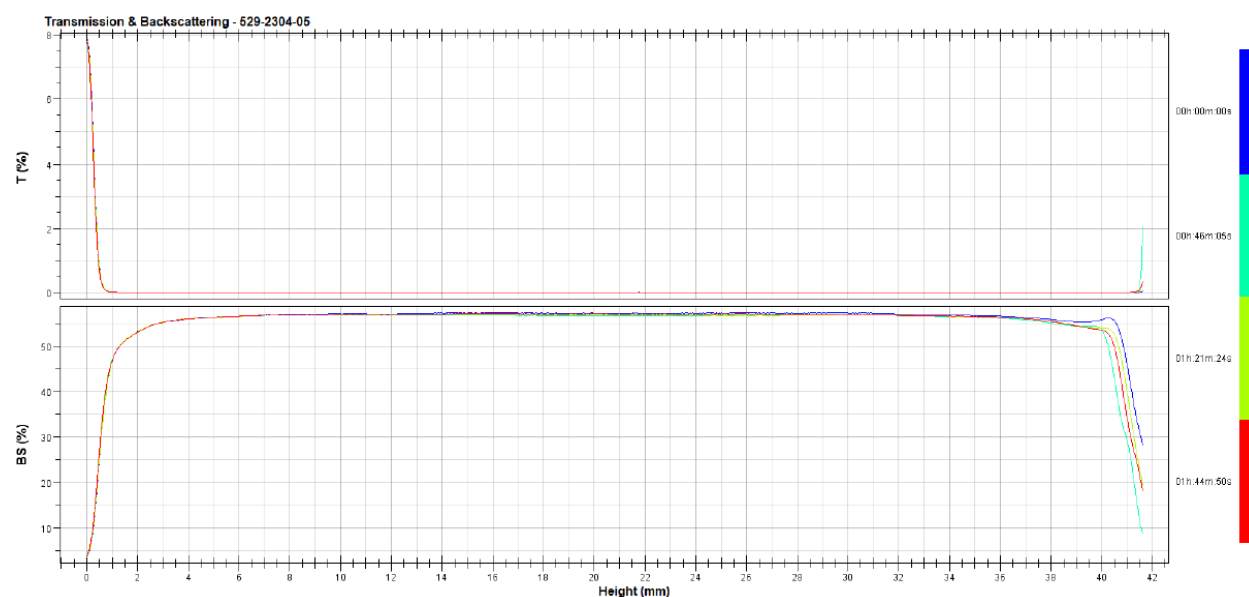


Figure 2: Transmittance and Backscattering measurement of the centrifugal stress test of hypromellose incorporated to the base formulation.

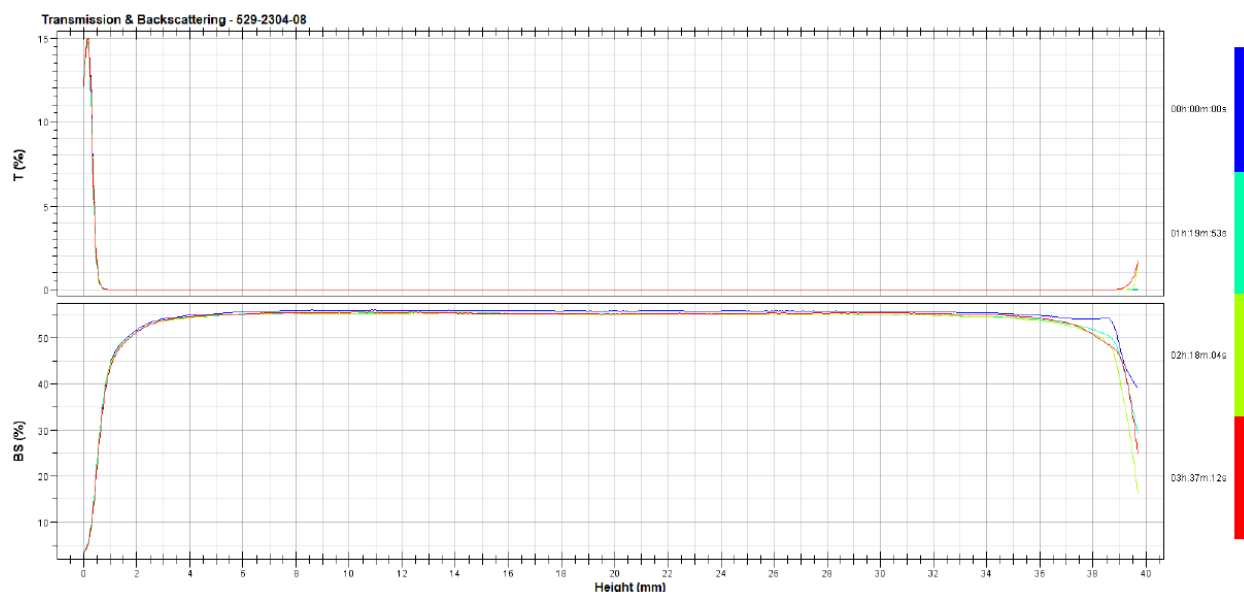


Figure 3: Transmittance and Backscattering measurement of the centrifugal stress test of sodium alginate incorporated to the base formulation.

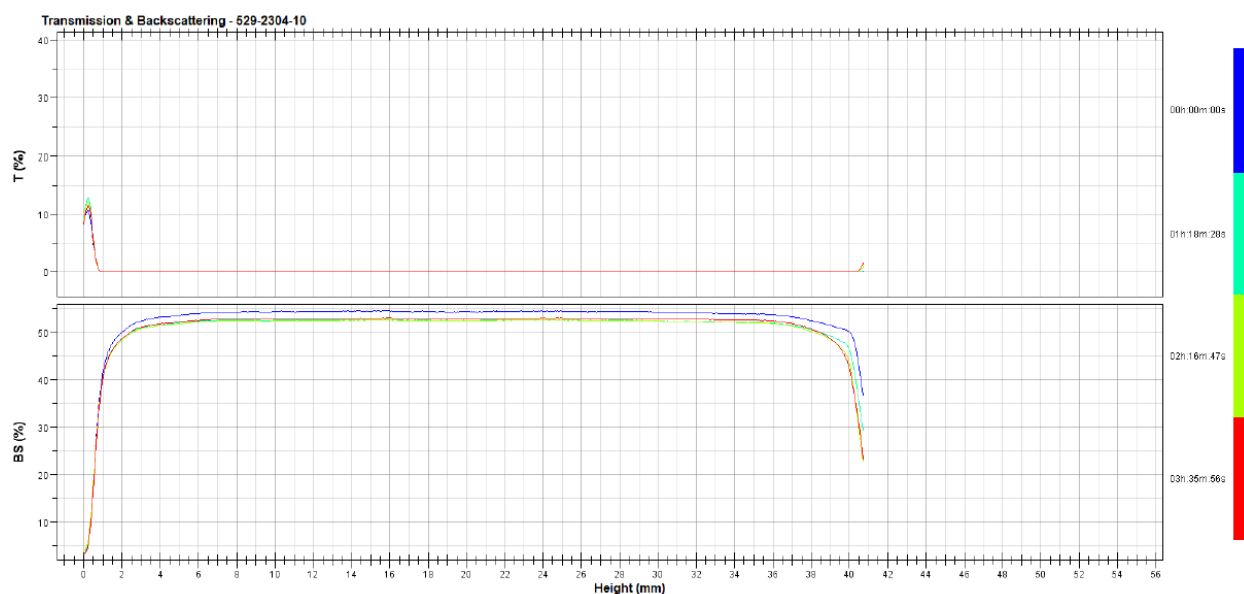


Figure 4: Transmittance and Backscattering measurement of the centrifugal stress test of Carrageenan incorporated to the base formulation.

4.1.2 Determination of melting and solidification of oil phase

The oil phase was prepared and placed on heating. At 33°C the solution was partially liquid with a lot of particles and solid material, and a completely homogenous solution was first achieved at 57.2°C. To ensure complete dissolution the sample was kept on heating up to 90°C. Once the oil phase reached 86.7°C the cloudy but homogenous solution turned completely clear. This is

believed to be a result of lot-to-lot differences from the emulsifier system, as the oil phase has appeared completely clear at different temperatures prior to this experiment. The transition was not further studied, and the melting point was denoted as 57.2°C.

The heating plate was then turned off and the oil phase was removed from the water bath. As stirring continued the solution was observed for any changes. At 48.9°C, white solid particles were forming on the edge of the beaker, indicating that around this temperature, the oil phase will solidify, and thickening will begin in the emulsion.

4.1.3 The effect of homogenization and heating on the chosen thickener

To determine how the thickener will be affected by the formulation process, a series of tests were performed on the polymer hypromellose dissolved in the aqueous phase. The primarily suspected factors that might have effect on the thickener is homogenization and heating. Two experiments were conducted where the aqueous phase was either homogenized or heated at varying times or temperature.

Homogenizing of thickener

Each homogenized sample was tested using a rheometer, giving a specific viscosity depending on the shear rate as can be seen in Figure 5. No major differences were observed between the samples homogenized from 0 to 30 minutes, and for the batches homogenized 40 and 60 minutes, no definite trend of decreased viscosity was detected.

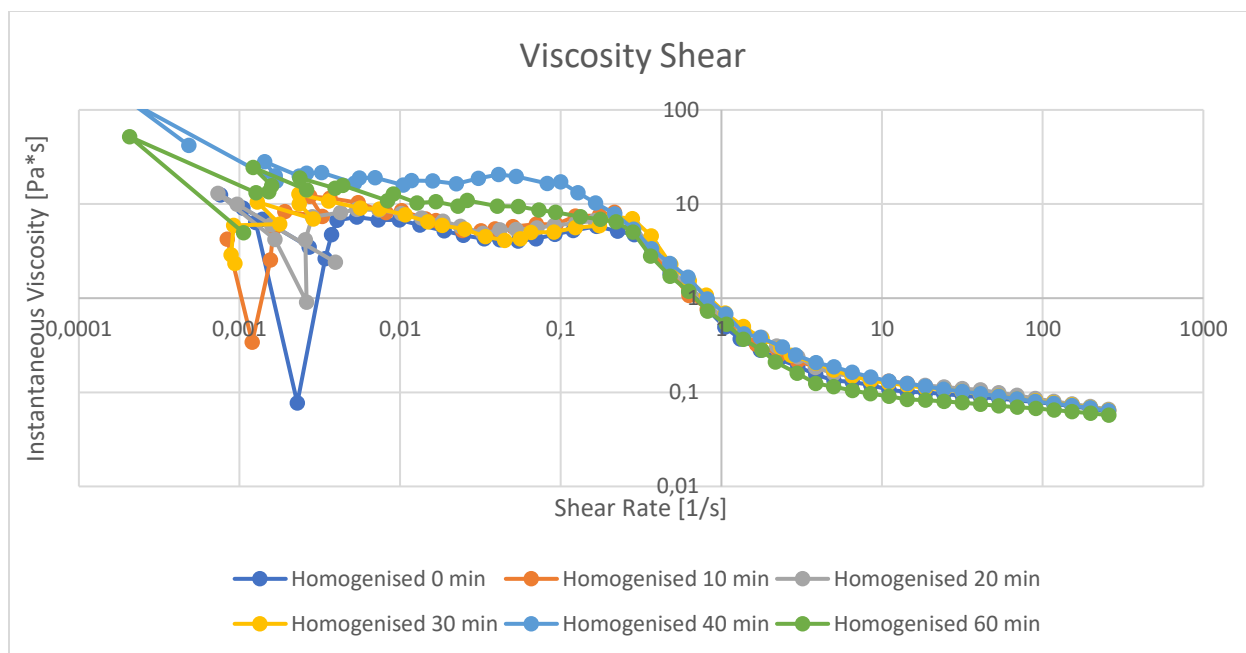


Figure 5: Viscosity as a function of shear rate of aqueous phases homogenized different times.

The two batches homogenized 40 and 60 minutes differ from the other four measurements at lower shear rates. The expected result would be a linear region during low shear followed by decreased viscosity as the shear rate increases, a so-called shear thinning behavior. The samples homogenized 0 to 30 minutes do, however, seem to have an increase in viscosity between the angular frequency of 0.06 s^{-1} and 0.25 s^{-1} before displaying the expected shear thinning behavior. This increase in viscosity was not observed in the batches homogenized 40 and 60 minutes, which instead display the expected linear viscosity at low shear rates.

When the test was repeated with a longer equilibration time for each measurement point over the same shear rates, there were no significant differences between the samples, and most importantly, no significant decrease in zero shear viscosity. Therefore, it is concluded that the thickener hypromellose is not unstable during homogenization. The odd behavior of a viscosity increase between shear rates of 0.06 s^{-1} and 0.25 s^{-1} remains, as can be seen in Figure 6. An explanation/theory could be that the polymer builds some crosslinked structure that causes some resistance to shear, and is broken, allowing the regular shear thinning behavior. However, this is not further investigated in this diploma work as it does not appear in the final formulation.

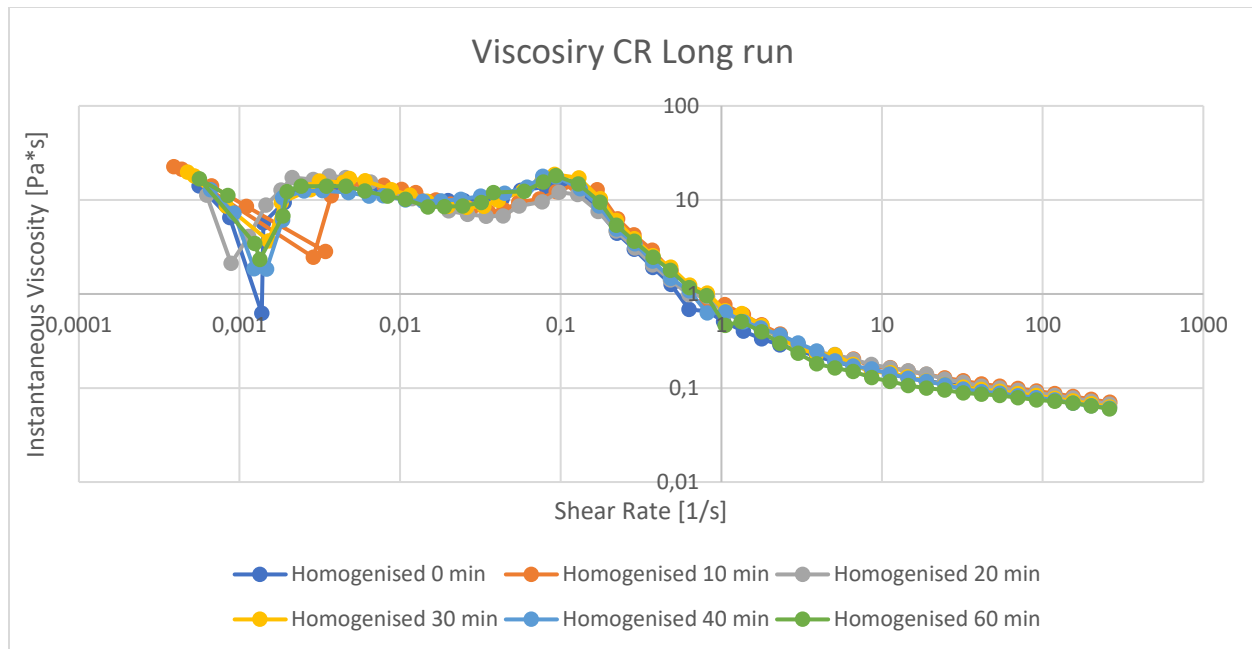


Figure 6: Viscosity as a function of shear rate performed over a longer test time.

Heating of polymer

Rheological viscosity measurements were performed at room temperature and analyzed of three samples heated to 70, 80 and 90°C as well as the reference kept at room temperature (RT). The results for each measurement can be seen in Figure 7. Looking at the graph, it is apparent that the sample at room temperature and the sample heated to 80°C appear very similar. The sample heated to 70°C does have a slightly higher overall viscosity, and the sample heated to 90°C have a slightly lower viscosity curve.

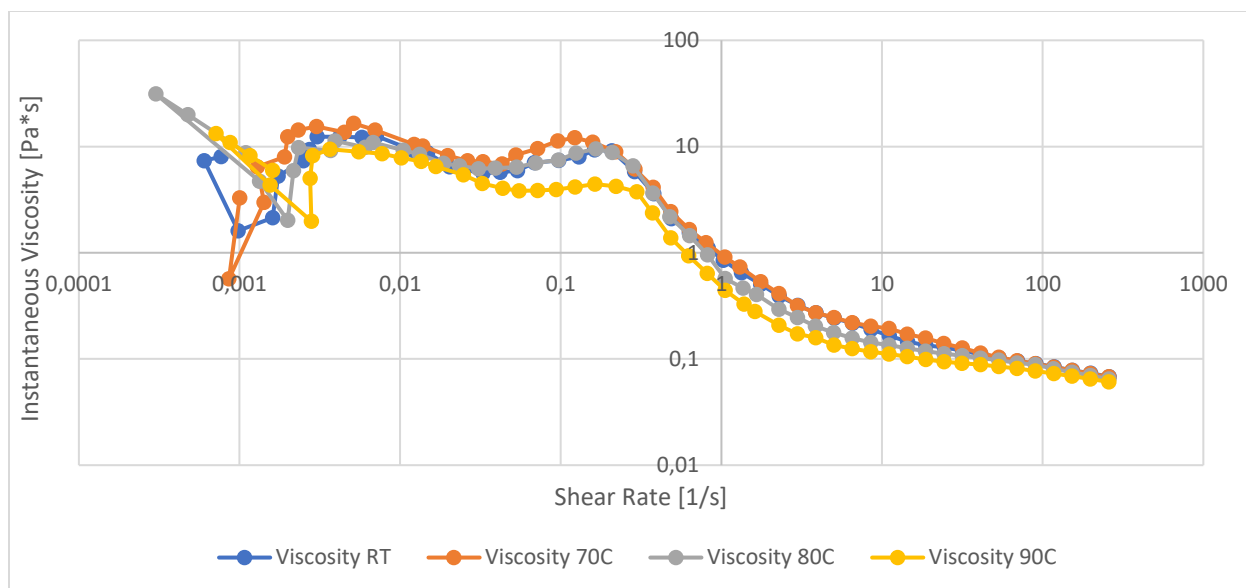


Figure 7: Viscosity as a function of shear rate for aqueous phases heated to different temperatures.

The sample heated to 70°C has a slightly higher viscosity than the other samples and could possibly be an effect of water evaporating during heating, effectively increasing the polymer concentration. If this is the case, the other samples heated to 80 and 90°C could be lowered due polymer instability, decreasing the thickening effect even if some further evaporation of water occur. This would indicate that the polymer is instable in the aqueous phase at temperatures around 80°C and above, and it is suggested that any formulation process try to avoid temperatures that high.

It is also quite likely that the variations between the samples are a result of errors of margin and variation from the rheometer measurements. To confirm whether differences are due to systematic differences or error of margin, several replicates would have to be performed and compared. This was not performed as the variation in viscosity was not great enough to cause concern regarding loss of polymer function. The differences between RT, 70°C and 80°C were assumed to be a result of errors of margin or negligible degradation of the polymer. The decreased viscosity at 90°C was regarded as a possible degradation of the polymer.

Since polymer stability at 90°C is not guaranteed, but the viscosity curve of the 80°C sample was nearly identical to the sample at RT, the polymer in the aqueous phase is assumed to be stable at temperatures up to 80°C.

There was no indication of gelling of the polymer during this experiment.

4.1.4 Examination of viscosity variation when altering polymer concentrations

The three batches with hypromellose concentrations of 1.5, 1.8, 2.0, 2.2 and 2.5 weight% were prepared in hot water according to the Ph. Eur. and measured using the viscometer [14]. The result of the viscosity measurements can be seen in Appendix I, and the average values can be seen in Figure 8, together with a trendline. For the trendline, a polynomial fit was applied as it appeared to make a good fit, and the viscosity is not believed to follow a linear trend. The equation achieved is $y = 1472.8x^2 - 2170.9x + 974.52$ and it describes the viscosity (y) as a function of hypromellose concentration (x). Using the equation, a variation in viscosity can be related to a change in polymer concentration. The sample prepared with 2% Hypromellose had a measured viscosity of 2607 cP

And according to the CoA for the batch in use, the nominal viscosity was 4350 cP, or 108.75% of the nominal value. Assuming that the value of our measurements is also elevated by 108.75%, a viscosity of approximately 2397.24 cP would have been measured if the nominal viscosity was 4000 cP. By applying the allowed variation of 75-140% on the theoretical viscosity of 2397.24 cP, a variation between 1797.93 and 3356.14 cP is acquired.

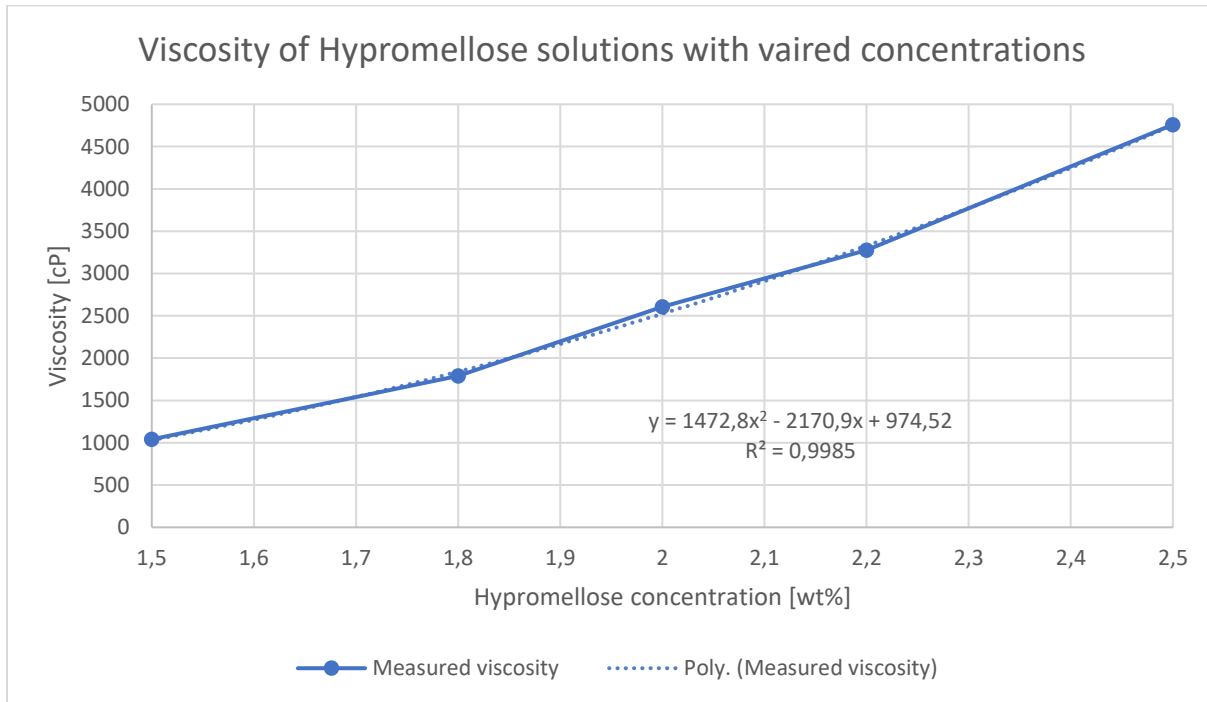


Figure 8: Average measured viscosity as a function of Hypromellose concentration.

By inserting the values of 1797.93 cP and 3356.14 cP as the y-values in the equation above, a polymer concentration (x-value) of approximately 1.79 and 2.21% is achieved.

It is found that the allowed viscosity variation between different batches of hypromellose corresponds to a difference of polymer concentration of 89.5% to 110.5%, or a difference of -10.5% or +10.5%. This relative difference in polymer concentration will be used as the basis for the design space of the DoE. The upper and lower limits for the DoE were set to $\pm 10\%$ of the center value.

4.2 Centrifugation stress testing and Stability study

Each batch from the DoE was put through stress testing by centrifuge to gain some immediate information regarding stability, as well as an initiated stability studies at 25, 30 and 40°C. The samples were analyzed by running Turbiscan measurements.

After a total of 30 minutes of centrifugation at 3500 RCF with a turbiscan measurement every 10 minutes, no distinct creaming was observed when observing the formulation visually or when using turbiscan. There are changes occurring in the formulation, but for this specific test it is creaming that is of interest. Figure 9 and Figure 10 display an example of a turbiscan measurements of another formulation that have undergone macroscopic phase separation by creaming. This formulation is of a fundamentally different sample and the measurement is only used as a reference. As can be seen, there is a clear dip in backscattering compared to the baseline in Figure 9, and in Figure 10 the backscattering and transmittance have clear opposing peaks. This dip in backscattering appear as the aqueous phase does not scatter light and thus, the backscattering is reduced.

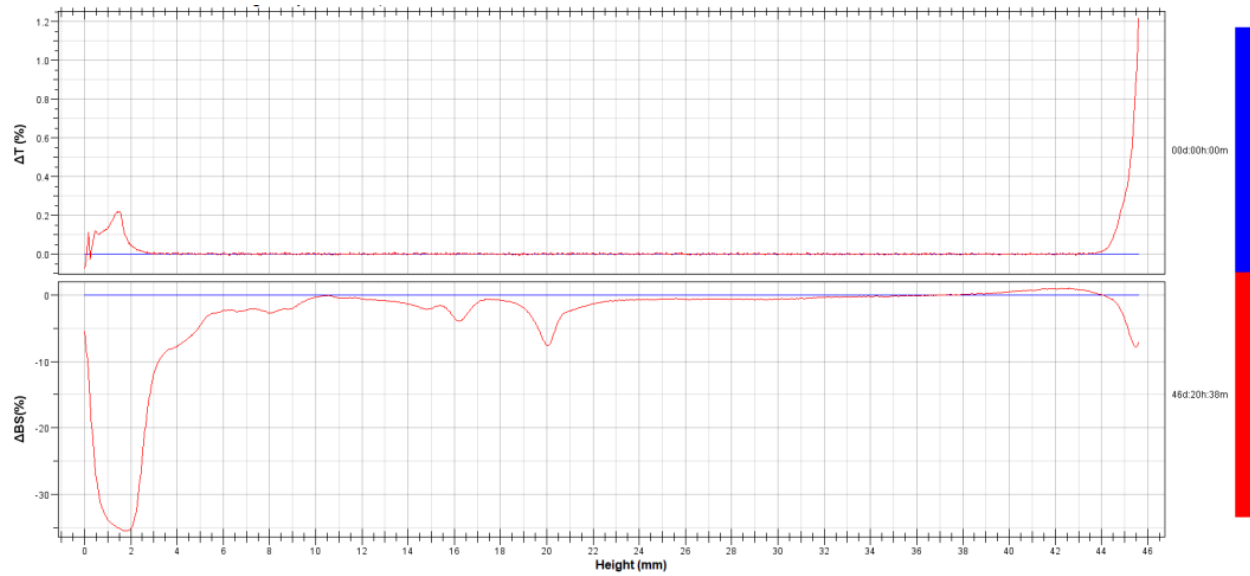


Figure 9: Δ Transmittance and Δ Backscattering measurement of a formulation that has undergone macroscopic creaming.

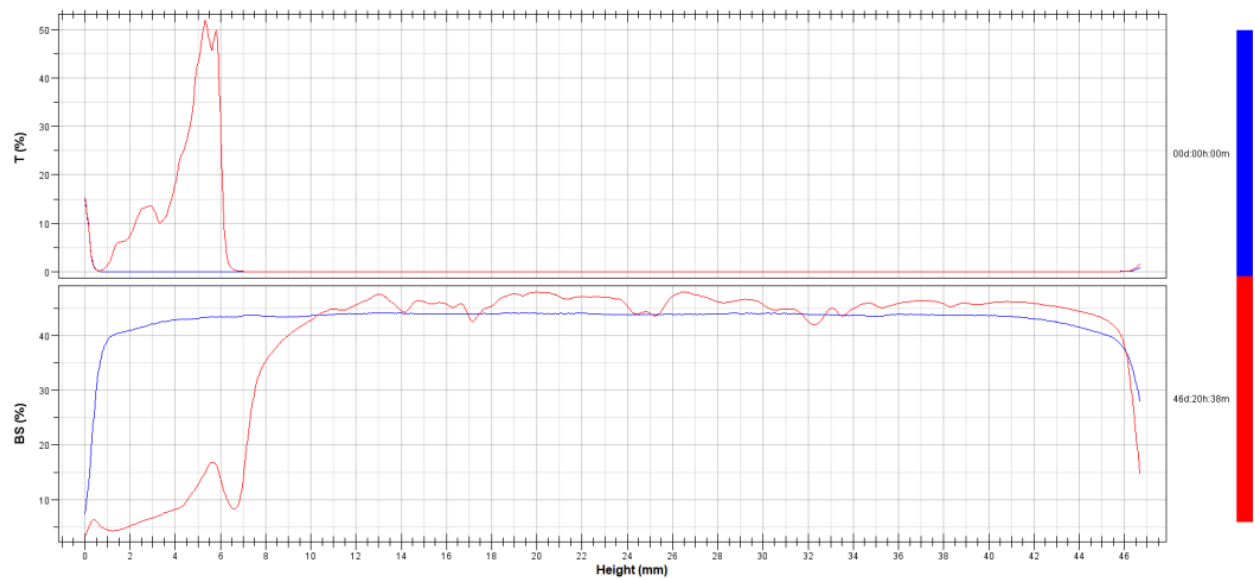


Figure 10: Transmittance and Backscattering measurement of a formulation that have undergone macroscopic creaming.

For each experiment from the DoE no macroscopic phase separation was observed, and very limited indications of creaming could be seen by comparing turbiscan measurements. Figure 11 is an image of experiment number 7, manufactured using low temperature, but high polymer concentration and homogenization speeds. As can be seen, each series of centrifugation does make the backscattering differ incrementally. However, there is little to no difference in the beginning of each measurement, and the transmittance does not show any significant differences. This is no

clear indication of creaming, as under creaming backscattering decreases in the lower end of the sample.

This is not a result of creaming but could be caused various other factors such as differences in temperature between measurements, changes in droplet size due to Oswald ripening or coalescence or come from changes of the internal structure in the formulation. This is not further studied in this project since it is not an indication of creaming, but remains a subject for future studies.

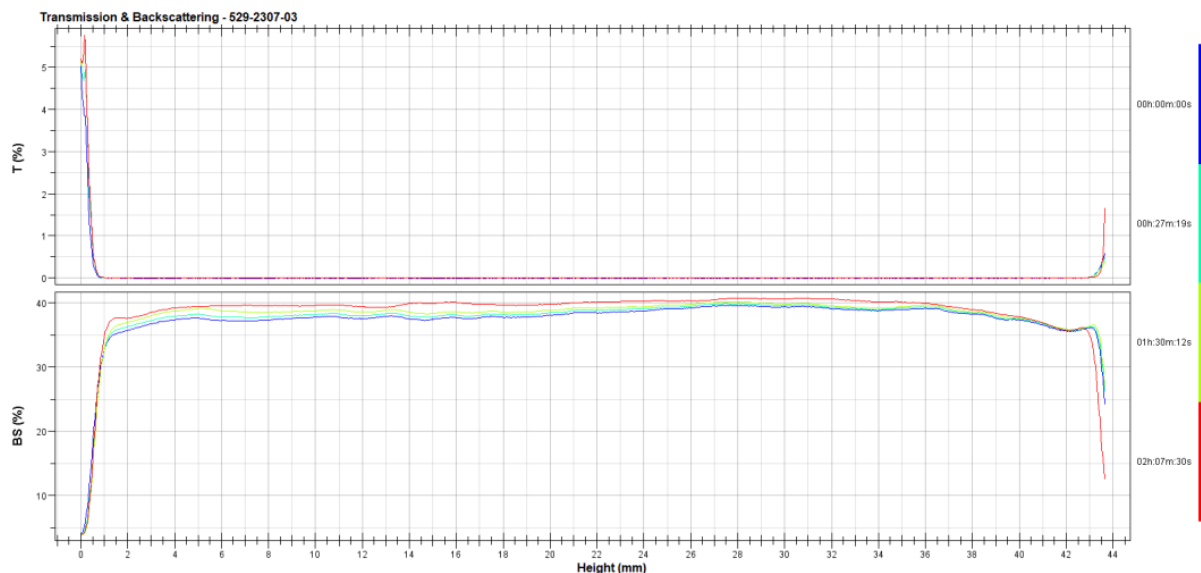


Figure 11: Transmittance and Backscattering measurement of experiment number 7, batch number 529-2307-03, centrifuged for 30 minutes.

Measurements from the stability study performed at 25, 30 and 40°C was taken at the beginning of the study, after 14 days and after 22 days. Just like with the centrifugation stress test, no significant creaming was recorded in the formulation. In Figure 12 a slight difference can be seen throughout the sample, but with little to no difference at the start of the measurement. Figure 13 displays the difference between the measurements with the initial time point as reference. Here it can more clearly be seen that the backscattering does slightly decrease with time, but the difference is so small that no definitive conclusion can be made.

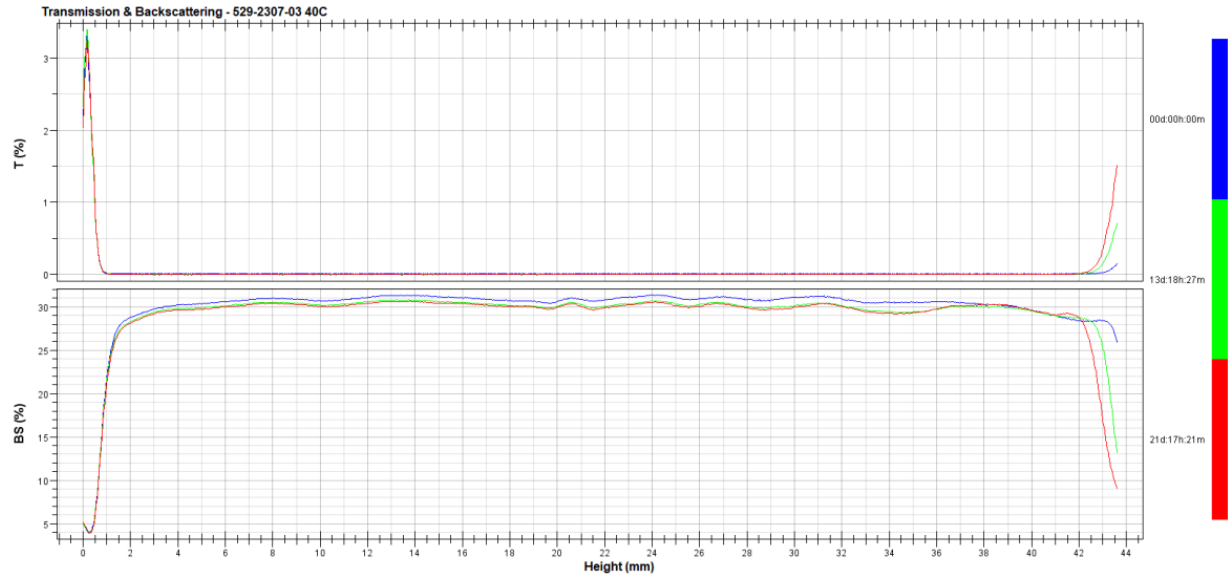


Figure 12: Transmittance and Backscattering measurement of experiment number 7, batch number 529-2307-03 stored in 40°C.

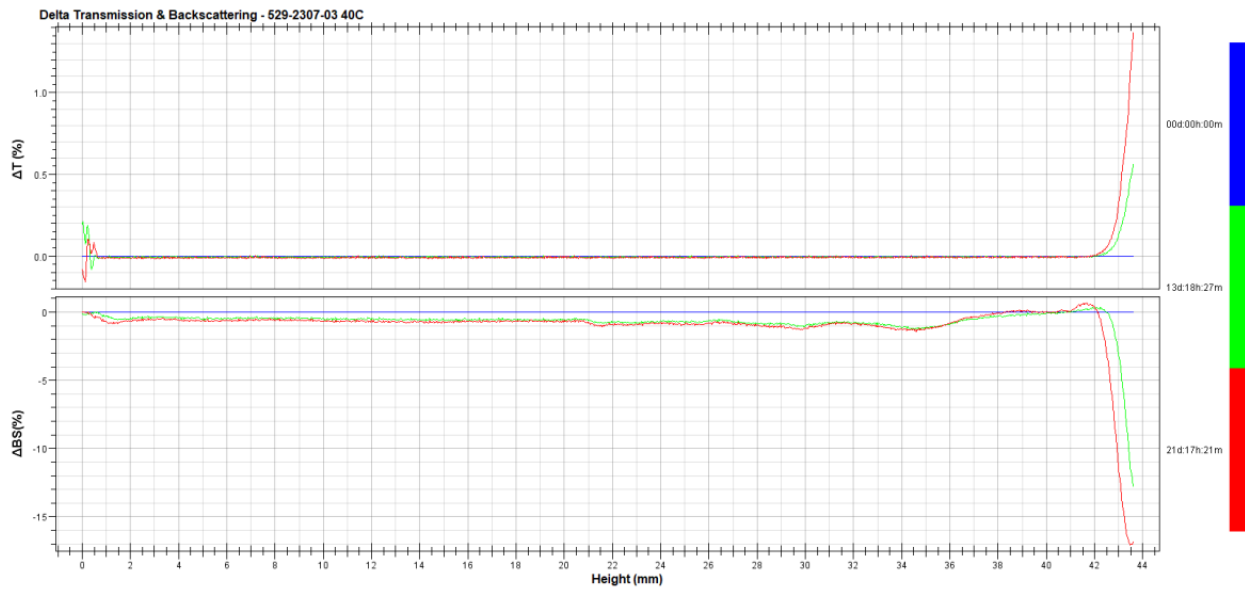


Figure 13: Δ Transmittance and Δ Backscattering measurement of experiment number 7, batch number 529-2307-03 stored in 40°C.

The full results from the turbiscan measurements from both centrifugation stress test and stress tests under controlled conditions can be seen in appendix II and appendix III.

4.3 Experimental result from Design of Experiments

4.3.1 Experimental data from DoE

The batches formulated for the DoE were each tested by viscometer, rheometer, and texture analyzer. The results are displayed in Table 2 and were imported into MODDE as the response variables when creating models from the DoE. The viscosity acquired from the viscometer is only labeled as “Viscosity”, while any viscosity acquired from the rheometer is labeled with the specific shear. Models that are created from the measurements: viscosity, viscosity zero-shear, viscosity high shear, and flow stress will be referred to as rheological models, while models based on firmness, consistency, cohesiveness, and adhesiveness will be referred to as textural models.

Table 2: Each batch in the DoE with the raw data of each reported response variables

Exp No	Run Order	Temp [°C]	Homogenization [rpm]	Polymer [wt%]	Viscosity [cP]	Viscosity Zero-shear [cP]	Viscosity high-shear [cP]	Flow stress [Pa]	Firmness [g]	Consistency [g*s]	Cohesiveness [g]	Adhesiveness [g*s]
1	10	60	5000	0,63	32 782	1381	0,946	40,4	5,512	17,206	-2,534	-14,816
2	9	75	5000	0,63	37 032	1106	1,066	43,2	5,2	16,253	-2,519	-17,315
3	1	60	12 000	0,63	18 816	306,4	0,4957	21,8	2,419	10,247	-1,291	-8,237
4	6	75	12 000	0,63	19 050	345,2	0,5512	22,4	2,478	10,033	-1,402	-8,705
5	11	60	5000	0,77	35 650	993,9	1,036	32,6	4,886	15,276	-2,394	-16,188
6	3	75	5000	0,77	35 502	1126	1,116	51,1	4,693	14,807	-2,382	-18,422
7	2	60	12 000	0,77	24 966	663,1	0,693	34,9	2,78	11,037	-1,664	-11,102
8	5	75	12 000	0,77	27 684	459,5	0,7306	41,2	3,003	11,613	-1,751	-11,26
9	7	67,5	8500	0,7	23 750	350	0,6254	26,3	2,589	10,251	-1,57	-10,686
10	4	67,5	8500	0,7	24 768	527,2	0,6541	30,7	2,767	10,721	-1,582	-10,767
11	8	67,5	8500	0,7	23 868	472,9	0,6412	27	2,706	10,608	-1,578	-10,113

When performing the frequency sweep of each formulation, it was found that none of the experiments had a crossover point in G' and G'' , and thus, no further analysis of the frequency sweep was performed or reported DoE models.

The microscope was used to measure the droplet size of each formulation manufactured for the DoE. Each formulation measured a droplet size of approximately 0.8 μm . However, this is below the range of which accurate measurements can be performed, and thus, the droplet size was not further investigated.

4.3.2 Experimental models using linear terms

Rheological models using linear terms

Due to previous experiments performed at Galenica, there is already a suspicion that the homogenization speed might not have a linear effect on the CQAs, but rather an exponential effect. This assumption was further verified as the data was inserted into MODDE and models were evaluated using the summary of fit bar graph seen in Figure 14. The model for both Viscosity from the viscometer and Viscosity at high shear from the rheometer returned with a model validity below 0.25, indicating that there are systematic problems with those models. The model for flow stress had a R^2 value of 0.7 indicating a good fit to the data, but a Q^2 value is in the negatives, indicating that the predictability of other points in the model will be poor, and the big difference between R^2 and Q^2 indicates an inappropriate model. What should be noted is that the reproducibility for each rheological model is high, indicating that errors in the models are unlikely to stem from errors in the experimental procedure.

The only experiment that does result in a statistically significant model was the model for zero-shear viscosity. The model is acceptable with a R^2 value of 0.696 and a Q^2 value of 0.566, but it is far from perfect. The model also requires that no other factor than homogenization speed is accounted for, as can be seen in Figure 15.

Figure 15 displays the coefficient plot for each rheological model. As can be seen, many coefficients have been removed to optimize each model, and still, the only model that is statistically valid is the model for zero-shear viscosity that only accounts for the homogenization speed.

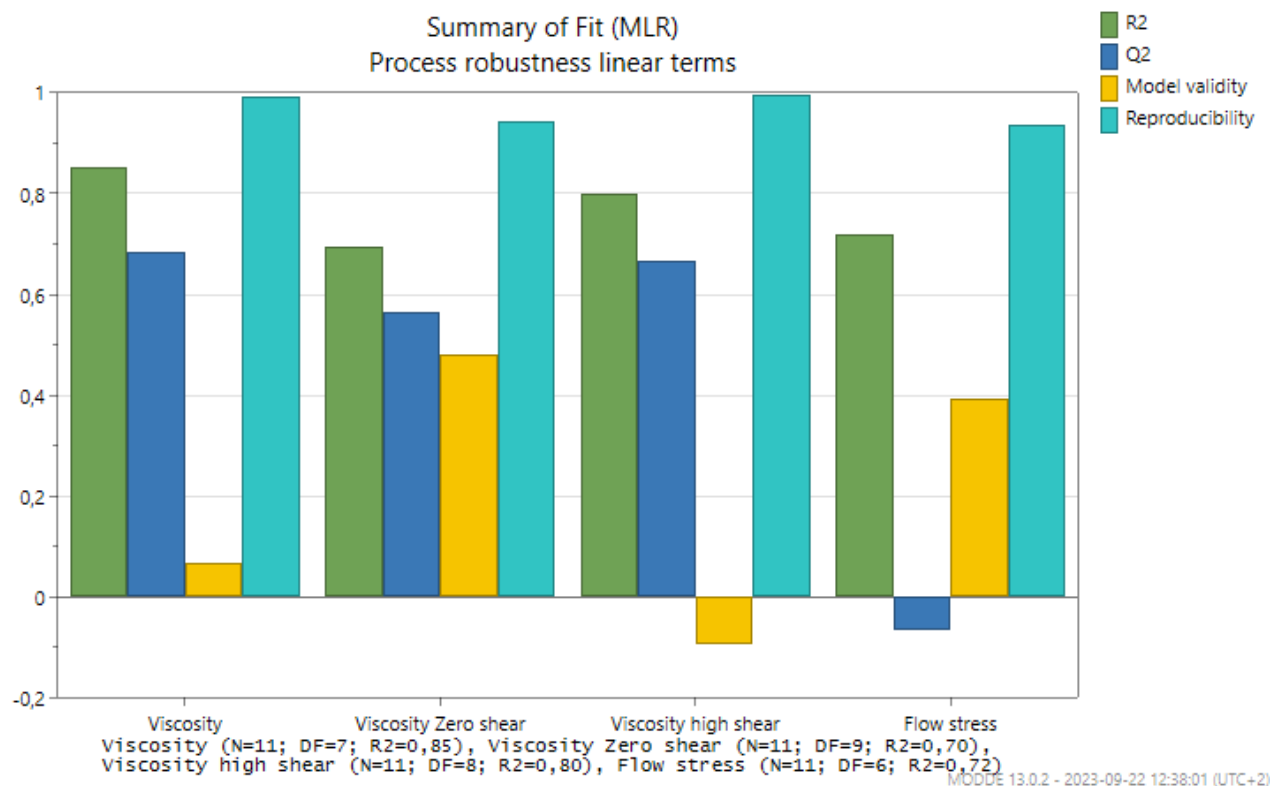


Figure 14: Summary of fit for rheological models using linear terms.

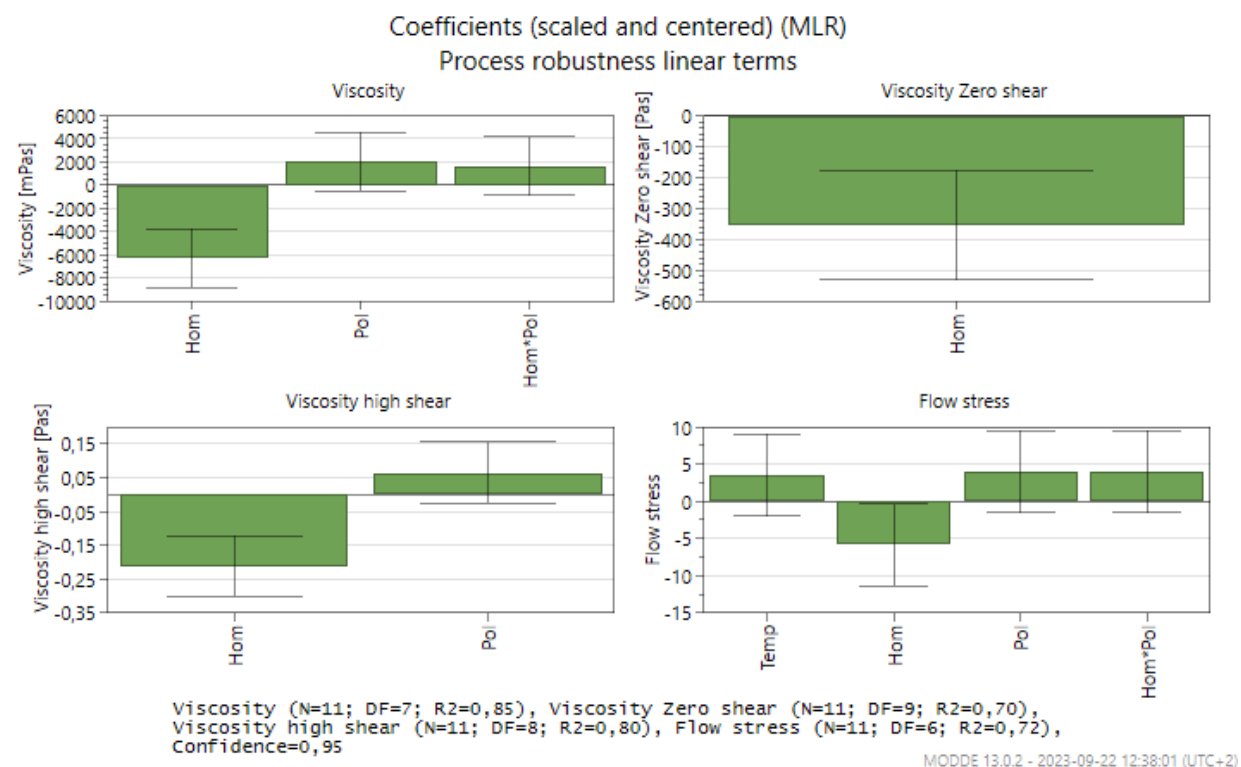


Figure 15: Coefficients of rheological models created using linear terms.

The predicted responses for each rheological model can be seen in Figure 16, with the homogenization speed on the y-axis, polymer concentration on the x-axis, with the temperature set constant at 67.5°C. The only relevant model of the four depicted in Figure 16 is the top right model describing zero-shear viscosity, and the model does not account for changes in any factor but homogenization speed, resulting in straight lines unaffected by polymer concentration or temperature. While the model does very little for describing the entirety of the design space, it does further confirm the suspicion of the homogenization speed as a CPP, and homogenization is to be observed closely under future experiments.

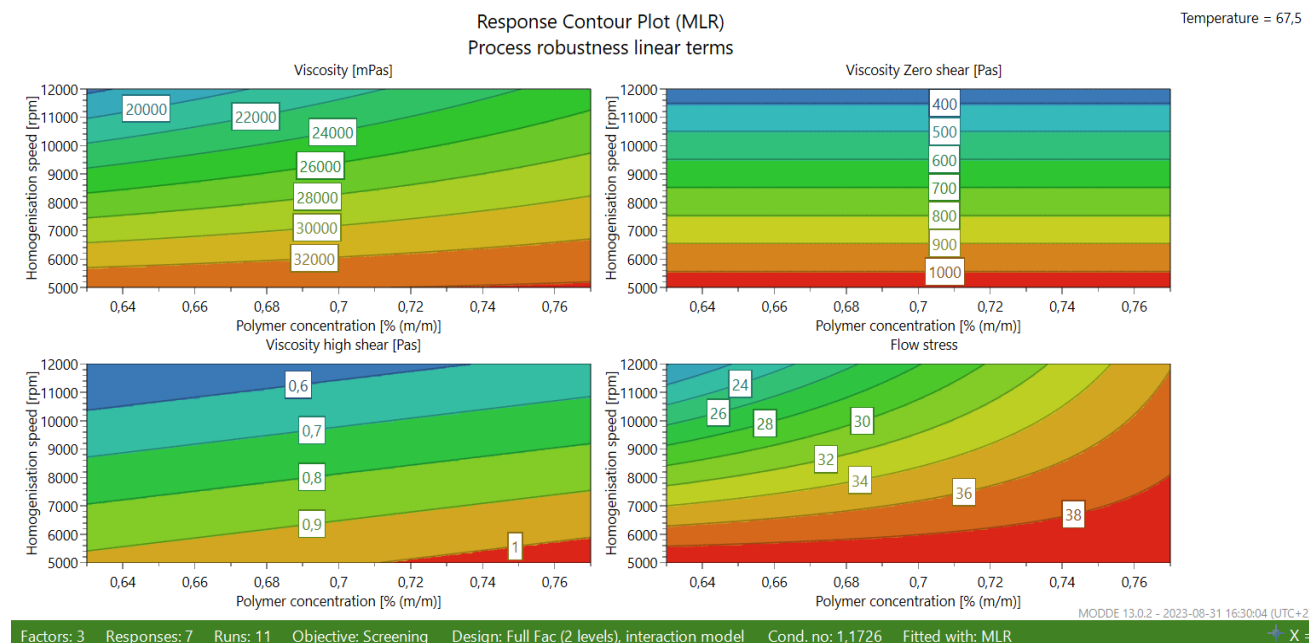


Figure 16: Response contour plot at 67.5°C for rheological models created using linear terms.

The full report from the DoE models created for rheological properties using linear terms can be found in appendix IV.

Textural models using linear terms

The summary of fit for the models of the textural properties are displayed in Figure 17. Here it can be seen that while R^2 and Q^2 are both above acceptable values, and the difference is not greater than 0.2. However, the model validity of each model is poor, indicating that the linear model fit is unlikely to be an accurate description of the behavior in the design space. The four models are also only acquired by eliminating several test factors as can be seen in Figure 18. Here it can be seen

that none of the model account for temperature, and the homogenization is the only statistically significant term.

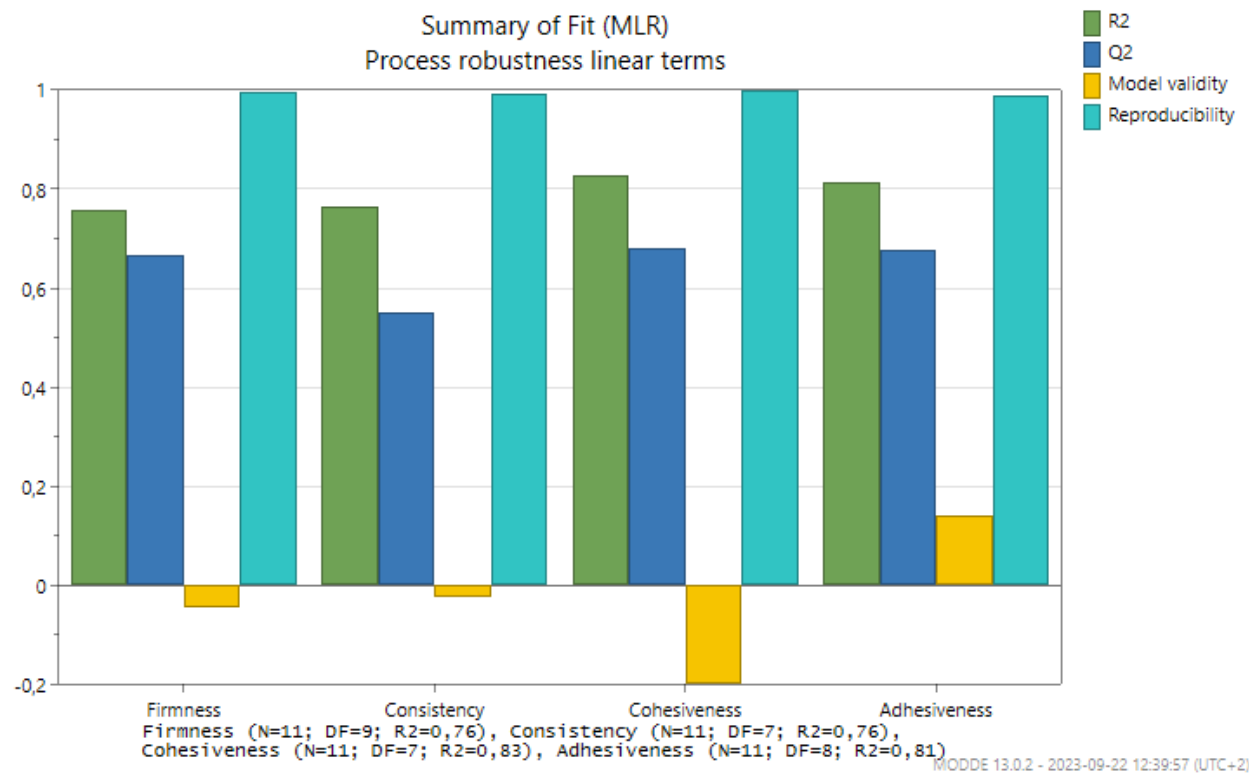


Figure 17: Summary of fit for textural models using linear terms.

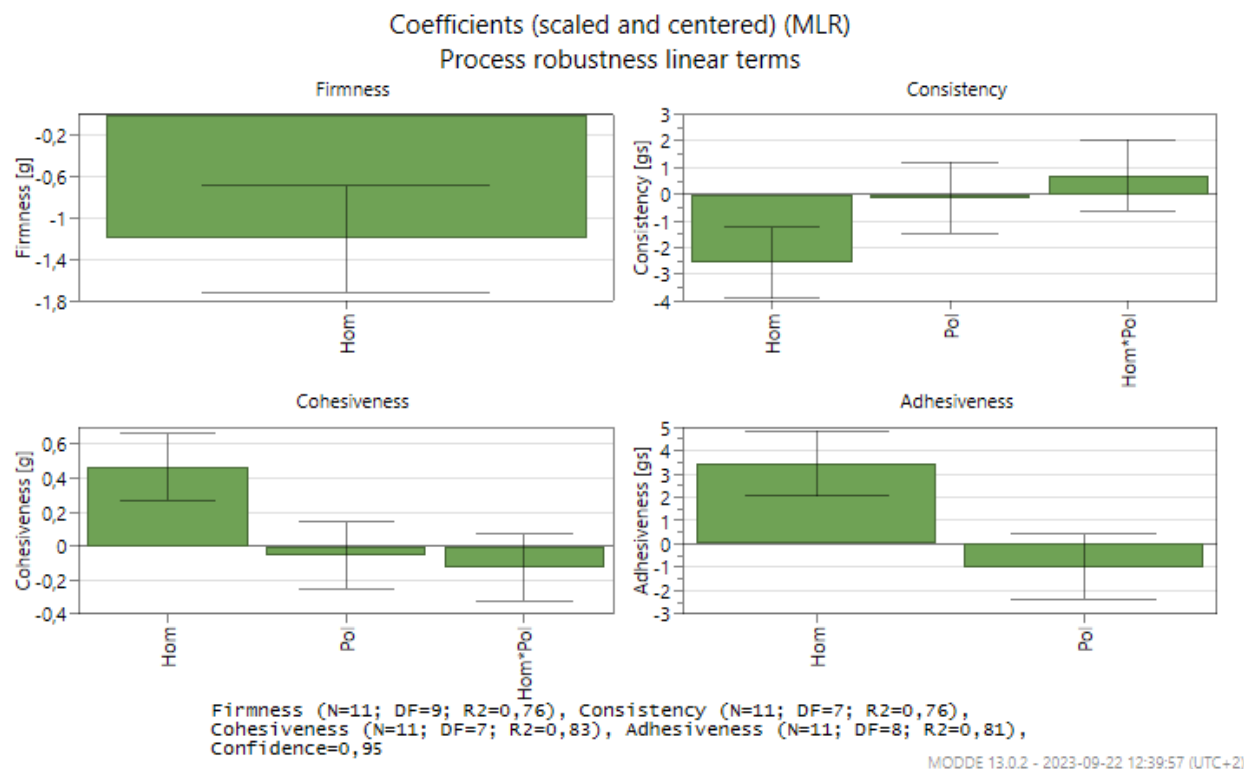


Figure 18: Coefficients of textural models created using linear terms.

While the model validity is low for the textural models, the indication that homogenization speed is a CPP still persists as it is observed to have a high effect. The term for polymer concentration is also included in most models, but its effect is not as significant, and its effect is not greater than the noise of the experiment. This gives an indication that while polymer concentration might influence the formulation properties, it is unlikely to be critical to the process within the region of the DoE.

The full report from the DoE models created for textural properties using linear terms can be found in appendix V.

4.3.3 Experimental model using a square term

While a screening full factorial design does not support the use of a square term, models with square terms are still possible to be made. When doing this, it is important to note that 6 further experiments are needed to verify the application of a square term to the model. However, since there is a suspicion of non-linear responses from the test factors, a square term was applied to observe the effect of the models.

Rheological models using a square term

By applying a square term to the rheological models, the R^2 values, Q^2 values and model validity improved for each model. The summary of fit plot for each model using the square term can be seen in Figure 19, and when compared to models using linear terms in Figure 14, vast improvements can be seen.

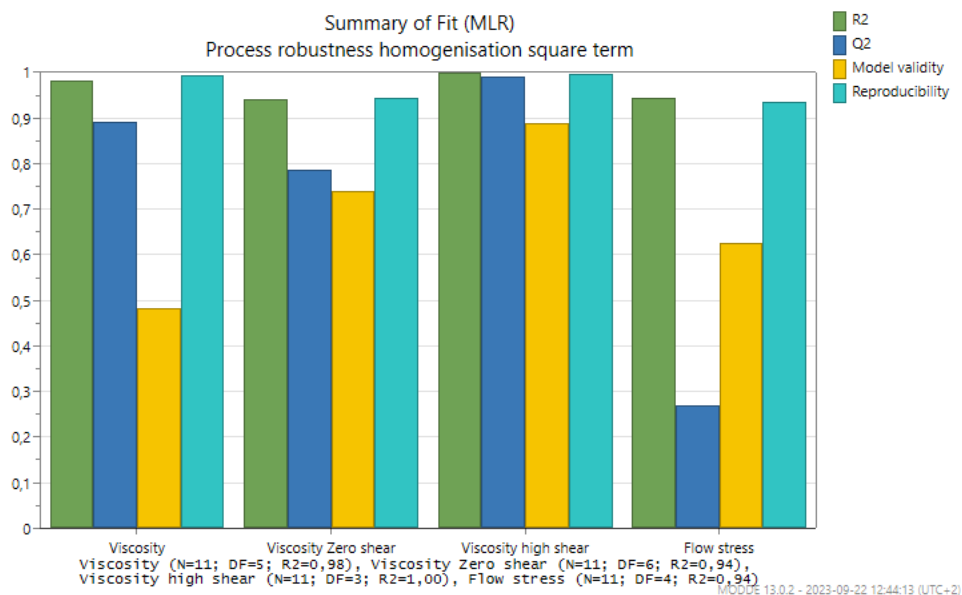


Figure 19: Summary of fit for rheological models using a square term.

Each model displayed improvements in R^2 , Q^2 and model validity when introducing the square term. With both an R^2 and Q^2 value above 0.5, a difference between R^2 and Q^2 less than 0.2 and a model validity above 0.25, a model to describe the behavior of viscosity, zero-shear viscosity and high-shear viscosity was achieved. However, while the model to determine how flow stress behaved displayed an R^2 value of 0.943, the Q^2 value was only 0.268. The high R^2 value and low value of Q^2 indicates that even though the model is well fitted to experimental data, there is very little predictive power of the model. The difference between R^2 and Q^2 is 0.675 which is too large for a significant model. This is an indication of systematic errors, such as another factor that is affecting the response variable. No proper model for flow stress is acquired, and this is an indication that other factors not accounted for in this experiment does influence the flow stress. One such factor might be the time between manufacturing and testing, as this varied a fair amount between batches. A theory is that the emulsifier system keeps building internal structures in the

formulation that increase the flow stress over time. This was not further examined in this project, but it is suggested to further investigate if a model for flow stress is to be developed.

The biggest improvement was seen for viscosity at high shear. By applying a square term to homogenization speed, the model validity went from -0.1 to 0.8, indicating that the square homogenization term is very likely an appropriate description of the effect of homogenization. The values of both R^2 and Q^2 were increased to 0.999 and 0.990 meaning that both the model fit, and any predictions made from using the model are highly reliable. This further confirm the assumption that homogenization speed has an exponential effect on the CQAs, however, further experiments are still needed to confirm the models using square terms which could alter the appearance of the summary of fit plot once applied.

The coefficients plot for the rheological models are displayed in Figure 20, and display that for each rheological property apart from zero-shear viscosity, the polymer concentration has a statistically significant effect, as well as synergistic effects with the homogenization speed. The fact that polymer concentration (and thus in turn, polymer quality) does not seem to affect the zero-shear viscosity of the formulation is a positive result, as zero-shear viscosity is considered critical to the formulation stability. This indicates that the process will remain robust in cases of polymer variation and the stability is likely to be unaffected by lot-to-lot variations from the polymer.

From the coefficients plot in Figure 20 it is also seen that the temperature during manufacture does not have a critical effect on the formulation properties, however, the model for zero shear viscosity does not account for the temperature factor. Here further studies must be performed, but it is an indication that the process will remain robust during temperature fluctuations.

The significant effect that they homogenization speed has on the CQAs of the formulation is believed to be attributed to the droplet size of the finalized formulation. It is also possible that a more intense homogenization breaks internal structures of the formulation, which would explain the decrease in viscosity with increased homogenization, however, further studies would need to be conducted to confirm or reject this theory.

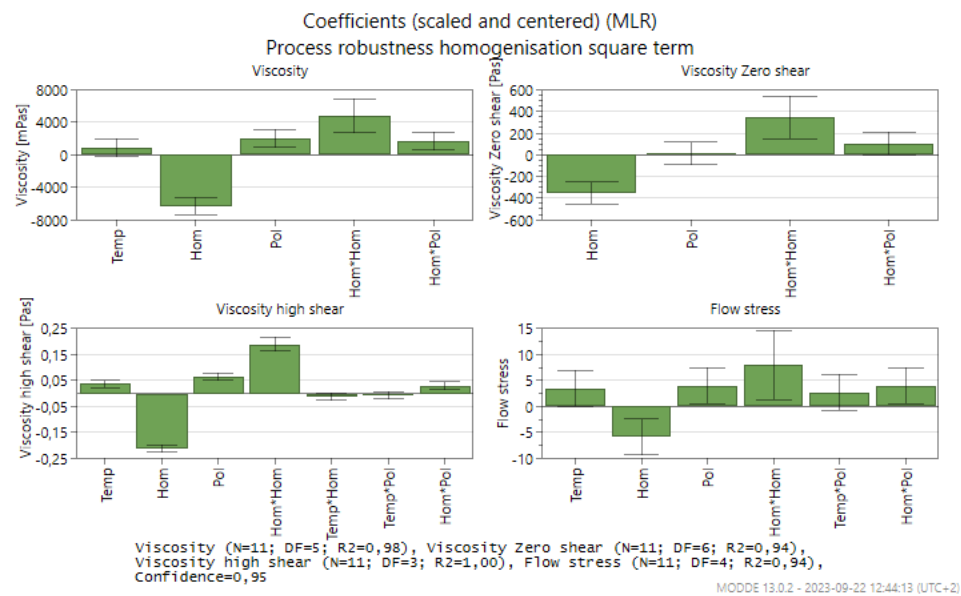


Figure 20: Coefficients of rheological models created using linear terms.

The models created for rheological properties using a square term are displayed in Figure 21, Figure 22, Figure 23 below. Here it can be seen how homogenization has a large effect on each parameter in the lower ranges around 5000 to 8000 rpm, while the process is more robust in the higher ranges above 8000 rpm. Polymer concentration does also influence the attributes, but not to the same degree as the homogenization speed. By comparing the three images it is possible to see the effect of temperature as well, but it can be more clearly seen in Figure 24 below, with the temperature instead of homogenization speed on the y-axis, polymer concentration on the x-axis and the homogenization speed locked at 8500 rpm.

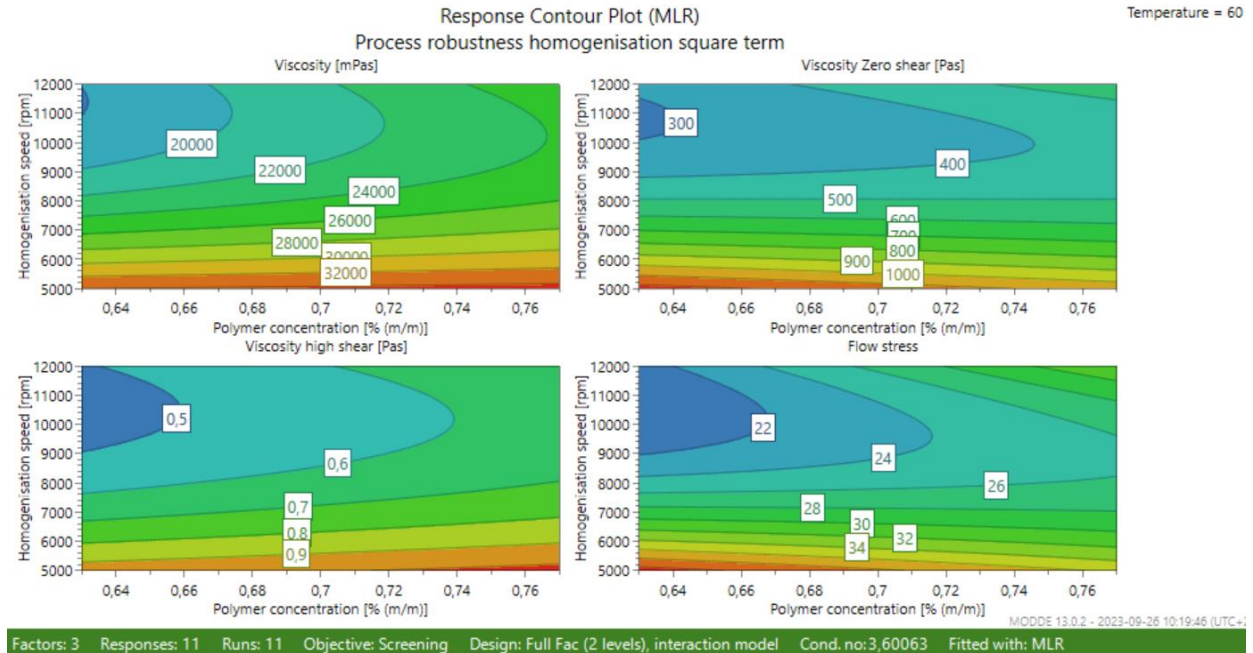


Figure 21: Response contour plot at 60°C for rheological models using a square term.

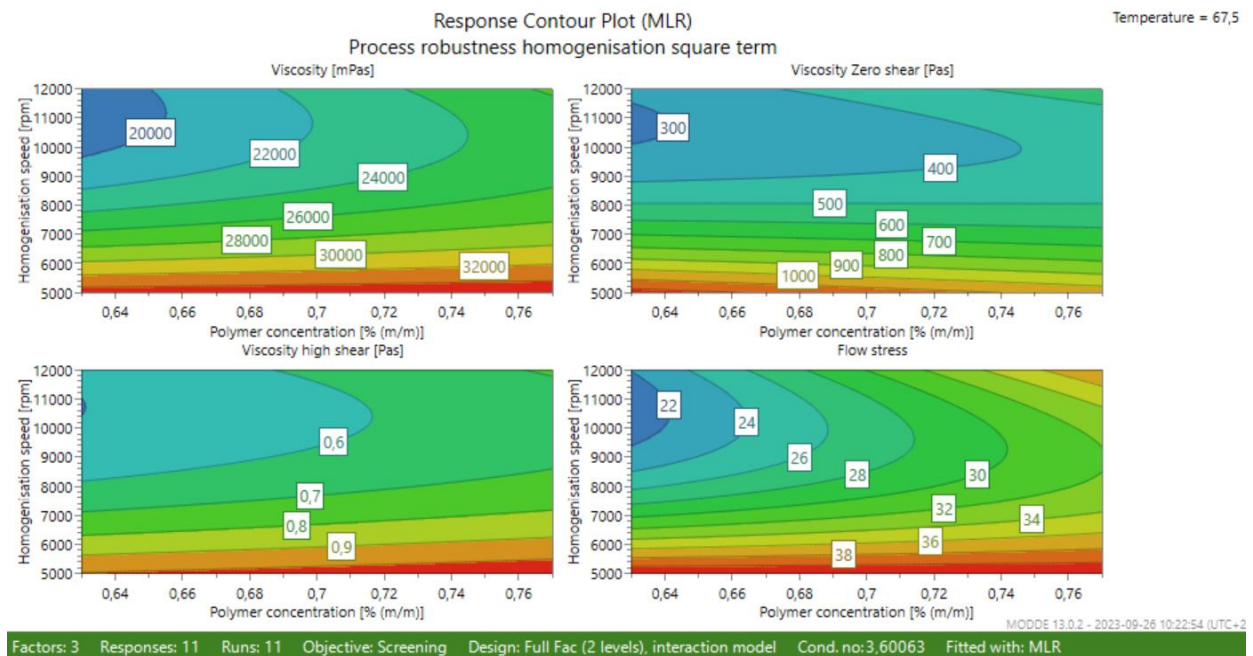


Figure 22: Response contour plot at 67.5°C for rheological models using a square term.

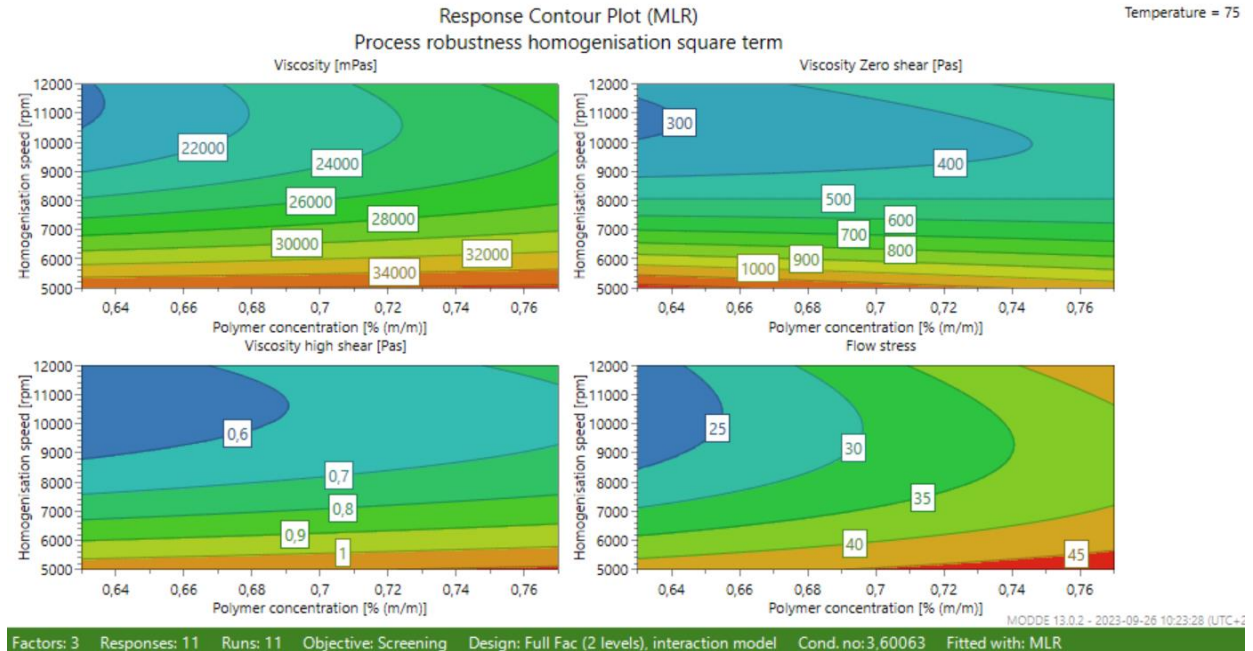


Figure 23: Response contour plot at 75°C for rheological models using a square term.

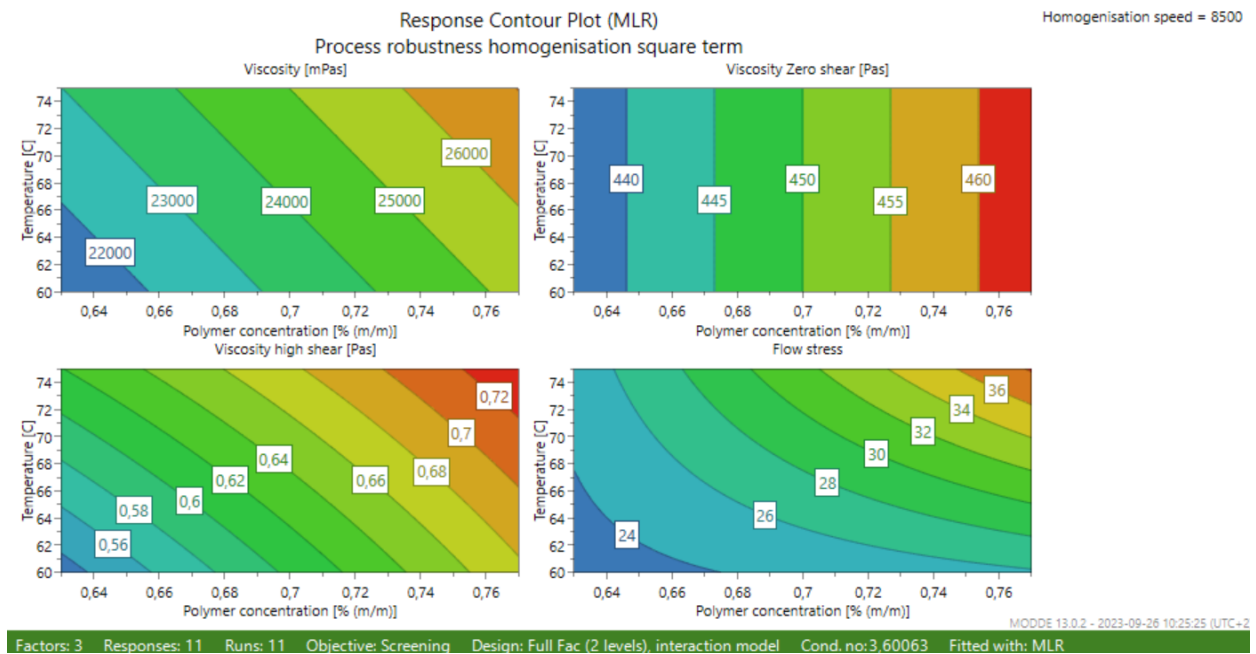


Figure 24: Response contour plot for rheological models using a square term displayed at a homogenization speed of 8500 rpm.

The full report from the DoE models created for rheological properties using a square term can be found in appendix VI.

Textural models using a square term

Implementing a square term immediately resulted in exemplary models for all four textural factors. The summary of fit model can be seen in Figure 25 where both R^2 and Q^2 is well above 0.9 which is considered to make an excellent model. On top of this, each model is acquired without disregarding any coefficients. Figure 26 display the effect of each coefficient, and here it can be seen that homogenization indeed is a CPP for each textural property. The polymer concentration does also have a significant effect on both cohesiveness and adhesiveness, which are textural properties relating to how sticky the substance is. Temperature does affect the adhesiveness outside of the margin of error but is still relatively small. These models describe that the homogenization speed is indeed a CPP, while polymer concentration and temperature does not have as large of an impact.

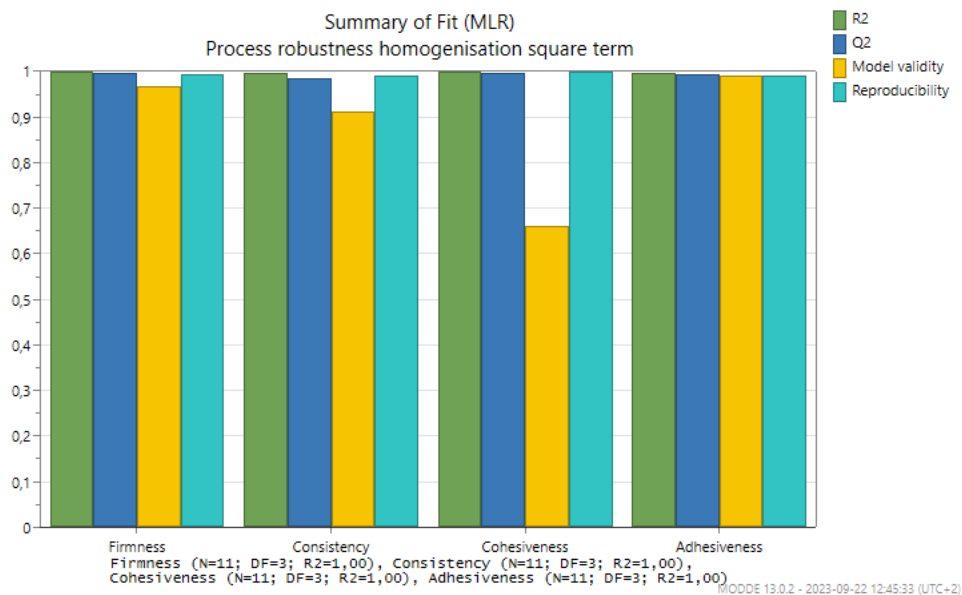


Figure 25: Summary of fit for textural models using a square term.

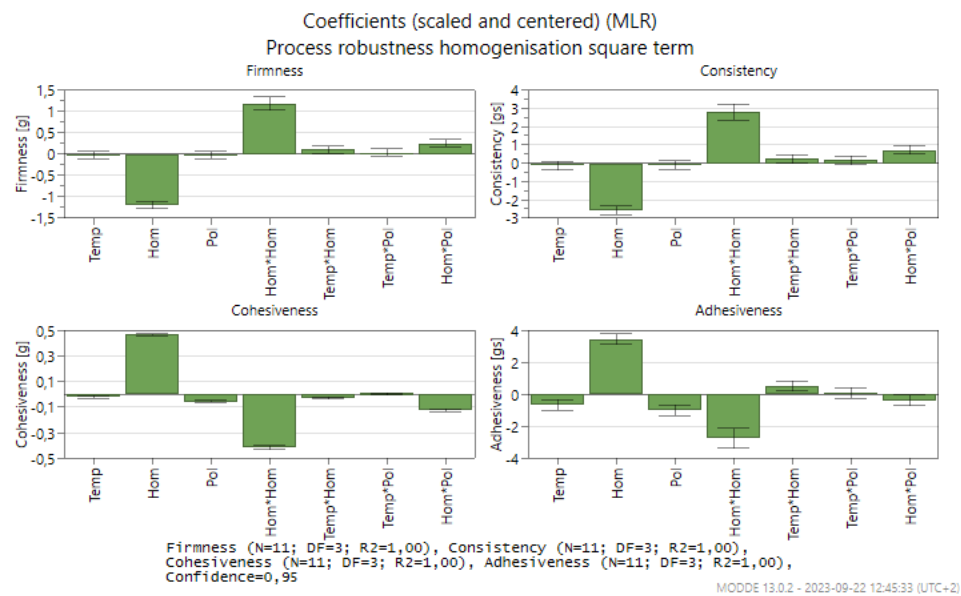


Figure 26: Coefficients of textural models created using linear terms.

The response contour plot with homogenization speed on the y-axis and polymer concentration on the x-axis for firmness, consistency, cohesiveness, and adhesiveness can be seen in Figure 27, Figure 28 and Figure 29, each depicting the same models and factors at a temperature of 60, 67.5 and 75°C respectively. Here it can be seen that at higher homogenization rates, the process is robust, with only minor changes from homogenization, polymer concentration and temperature. While these models are excellent, they still must be validated by performing 6 further experiments, and as a result, the models' appearance, relevant factors, and the summary of fit of the model might change.

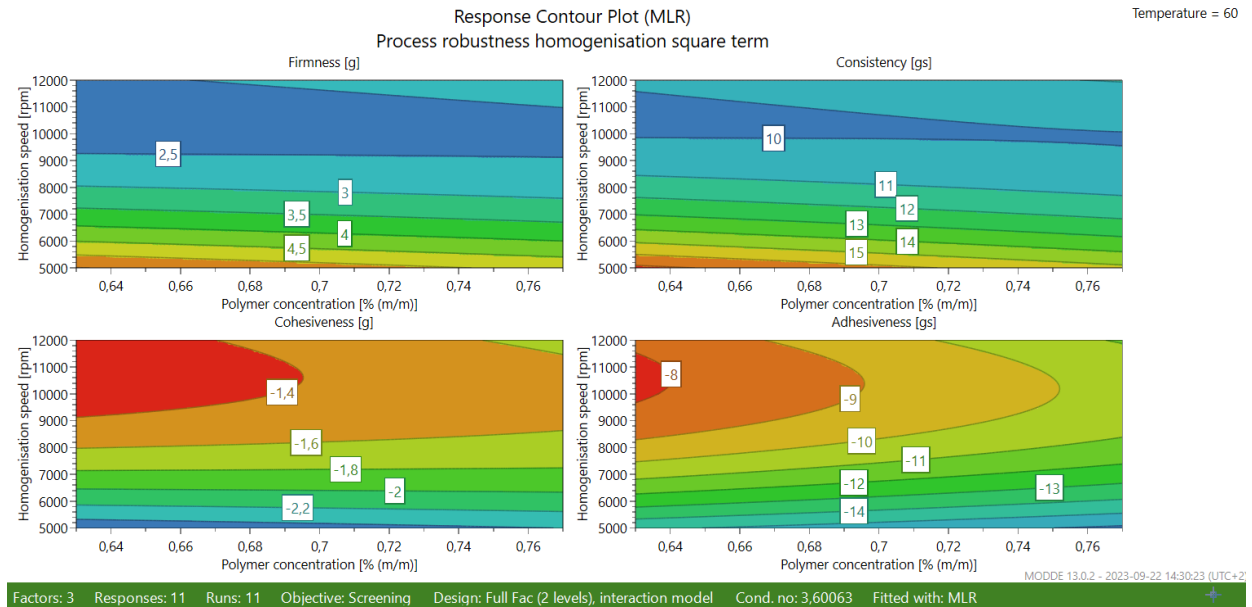


Figure 27: Response contour plot at 60°C for textural models using a square term.

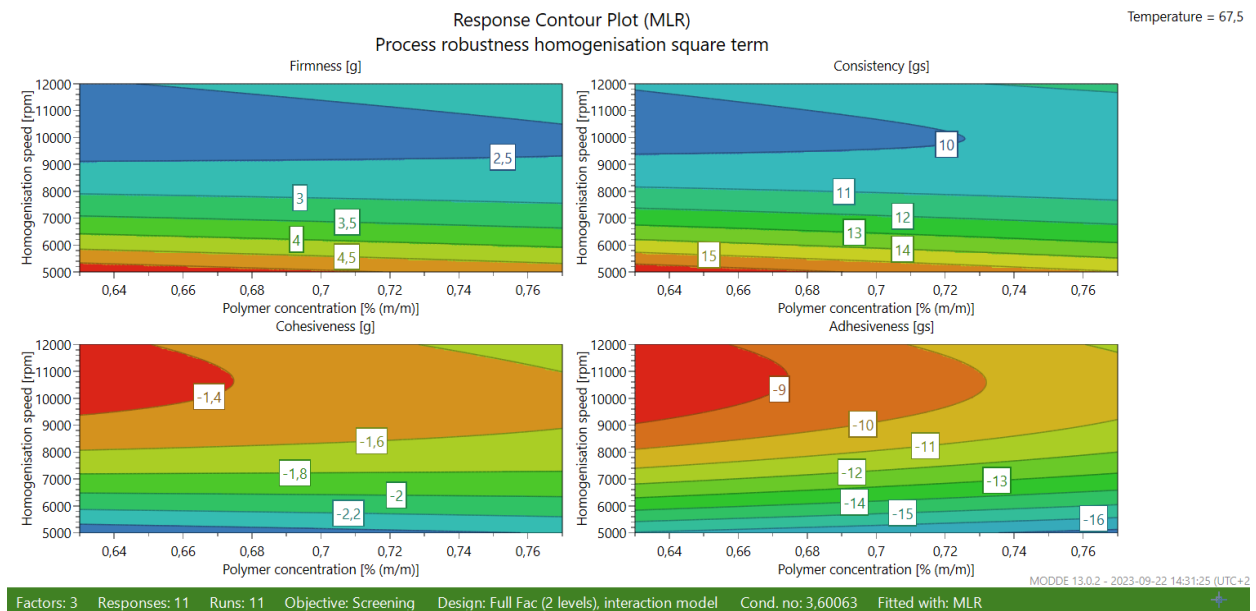


Figure 28: Response contour plot at 67.5°C for textural models using a square term.

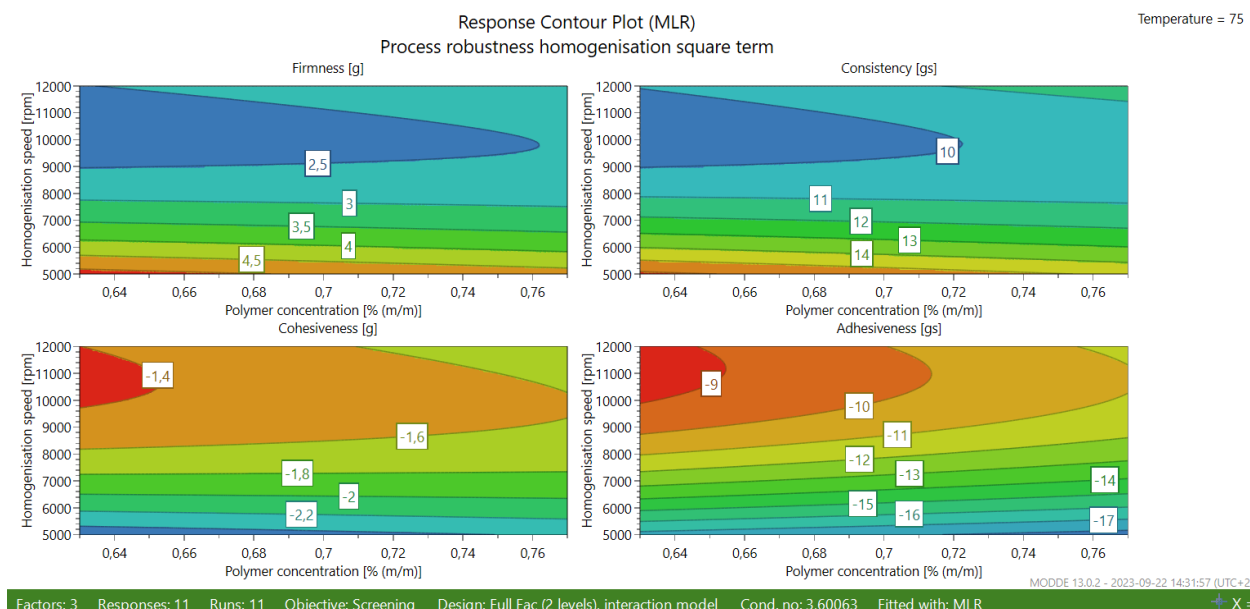


Figure 29: Response contour plot at 75°C for textural models using a square term.

The full report from the DoE models created for textural properties using a square term can be found in appendix VII.

5. Conclusions and Future Work

For the base formulation provided by Galenica, four different thickeners were evaluated. Hypromellose proved to be most suitable, and after further investigation, it was found to be stable to both heating and homogenization during production stages. During stress testing of the formulation with Hypromellose incorporated, no apparent stability issues were found, but the stability studies initiated during this project will need to be continued for months to come.

From the screening full factorial DoE that have been performed, it is possible to conclude that the homogenization is a CPP when examining the produced formulation's CQAs when using the linear terms. A simple description of how homogenization affects zero-shear viscosity was able to be developed, but no model to describe the full design space was acquired.

From the models developed by incorporating a square term much information was given of both relevant test factors as well as the appearance of the design space. The models were also found to be highly accurate, which promotes the hypothesis that homogenization does have a non-linear effect on CQAs. However, the models developed using a square term does require the performing of 6 more experiments to be validated, and it is possible that the accuracy of the models decreases

once more experimental data is added. For future work it is suggested to perform the final 6 experiments and evaluate the models once more to get a full description of the design space.

It is also suggested to further observe time dependent effects on the formulation, such as how time might affect the flow stress of the formulation as well as observe if the appearance of the frequency sweep measurements will change over time.

Finally, no definitive results were gathered regarding the droplet sizes for the different experiments. For future studies, it is suggested to determine the droplet size using more accurate analytical methods.

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