LTH

Bridging the clinical claims of two nasal oil products using a physical chemical characterization

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Abstract

Bioglan AB is manufacturing a nasal water-in-oil microemulsion that is used to prevent allergic reactions in the nose. However some users have reported that the product feels irritating in the nose and throat. Another problem with the product is that it is not temperature stable, forming crystals at low temperature. Sometimes spontaneous crystallization occurs.

Therefore a new product has been developed. The aim of the new product is to correct the problems with the old product as well as adding another effect. It should moisturize a dry nasal mucosa.

The main goal of the diploma work is making a physical-chemical characterization of the two products and from the results be able to state that the two products are similar enough that no new clinical trials have to be performed.

The characterization was made with a variety of techniques. Differential scanning calorimetry was performed to evaluate the temperature stability; Surface tension and spreadability was measured to judge the spreading on the nasal mucosa. NMR spectroscopy was performed to elucidate the microstructure of the products. Mucoadhesion was measured with a tensile strength experiment. The rheological behavior was measured and the phase behavior was briefly studied.

The products were tested and evaluated by 20 volunteers per product to give more understanding of how the products are received and how well the physical chemical properties translated to real use.

The results indicate the products are similar enough to have the same effect and no new clinical trials have to be performed.

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Introduction

Suffering from allergy is a common health problem. It is estimated that around 15 % of the population suffers from pollen allergy alone. Most of these people suffer from nasal symptoms such as congestion and rhinitis.

Another nasal health problem that is especially prevalent among the elderly is having a dry mucosa in the nose. This can lead to various symptoms such as irritation, itching and a burning sensation.

Bioglan AB is manufacturer of a product called Blox4 Allergy. It is a nasal oil formulation that forms a protective barrier over the mucosa inside the nose to prevent allergens from causing a reaction which would happen if the allergens were to contact the mucosa and elicit a response from the immune system [5-7].

The product is classified as a medical device since it contains no active pharmaceutical ingredient. The product received some complaints because it was irritating in the nose and throat to some users. Another problem was that it was not temperature stable. Crystals could also spontaneously form inside the vials.

Therefore a new product has been developed. It aims to address the problems with the old formulation and at the same time also have the ability to moisturize a dry nasal mucosa.

The main goal of this diploma work is making a physical-chemical characterization of the two products and from the results be able to state that the products are similar enough that no new clinical trials have to be made. There are no well-defined demands from authorities for this type of bridging and for a certain amount of time the new product can be sold without a new set of clinical trials.

Theoretical background

Nasal physiology

The inner parts of the nose are lined with mucosal membranes. The surface of the membranes is covered with a sticky water rich material, mucus. The mucus consists of water, glycoproteins, lipids and salt. The water content is over 95 %. It is the mucin glycoproteins that give mucus its viscoelastic and gel like properties. The mucus catches particles, microorganisms and some gases [8,9].

There is a synchronized movement of cilia that propels the mucus towards the back of the throat were it is swallowed. Most harmful microorganisms and particles are thus hindered from entering the lungs. The turnover rate, how long it takes for the mucus to leave the nose once it has been secreted, is estimated to be about 10-15 minutes [8,9].

When air is inhaled through the nose it is warmed and moistened. If the nose is exposed to dry and hot or cold air it leads to drying up of the mucosal membrane that in normal conditions should be moist when contacting the air. If loss of water is too large it causes epithelial damage.

Anatomical changes in elderly and certain medications can lead to increased risk of suffering from dry nose. Some of the symptoms can be: Sensation of dryness, itching, a mild burning sensation, nasal obstruction and crusting. The condition can be treated by the application of a protective film to the inside of the nose to form a barrier against further contact with dry air. This allows it to heal without exposure to mechanical irritation [2].

Allergic rhinitis

Allergic rhinitis is a very common condition. Symptoms regularly associated with the disorder are sneezing, itching, rhinorrhea and nasal congestion. Oftentimes these symptoms are accompanied with conditions in the eyes, throat and ears.

About 50% of the total amount of rhinitis cases is caused by allergy. The most common allergens are pollens, molds, animal dander and dust mites. If a person suffering from allergic rhinitis comes into contact with a specific allergen that triggers the immune response of the body, a special type of cells called mast cells travel to the surface of the mucosa, where the allergens can be found, and release a number of compounds that trigger the allergic response. This is what causes the rhinitis symptoms [3].

Microemulsions

The definition of a microemulsion has changed over the years since the term was first introduced. Today a microemulsion is defined as an optically isotropic, thermodynamically stable mixture containing a hydrophobic compound, a hydrophilic compound and an amphiphilic compound and sometimes a cosurfactant. The term is a bit misleading since a microemulsion is not really an emulsion. A normal emulsion phase separates if given enough time while a microemulsion is always mixed homogeneously since it is thermodynamically stable. Because of the unique properties microemulsions exhibit, they are of great interest for use in various pharmaceutical products [4]. There are certain proportions of ingredients that enable the microemulsion to be thermodynamically stable at a constant temperature. This can be illustrated in a ternary phase diagram. In such a diagram there are different areas that correspond to various microstructures that the microemulsion can possess. In the most basic ternary phase diagram, like the one below in Figure 1, A,B and C would correspond to the hydrophilic, hydrophobic and amphiphilic compounds.

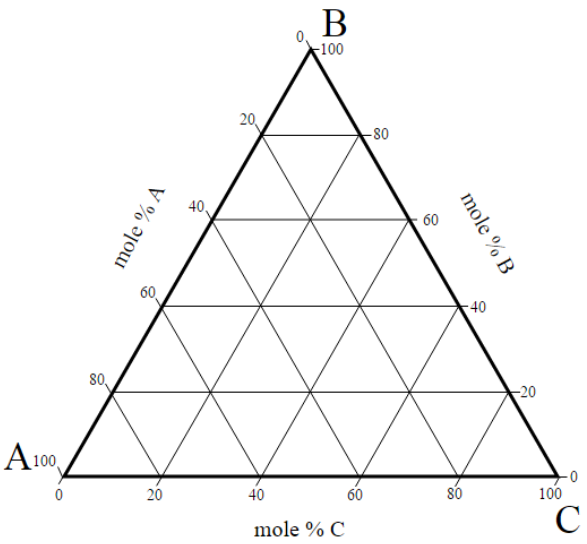

Figure 1. Example ternary phase diagram. The composition at a given point is derived by following the lines towards the borders [29].

The three basic structures of a microemulsion are: Oil in water (direct), water in oil (reversed) and bicontinuous (L_3, V_1? or sponge phase).

Furthermore the amphiphilic molecules will arrange themselves into different structures depending on their concentration, to minimize energetically unfavorable interactions. Figure 2 below shows an example of the different structures that can appear in a water rich environment. The structures depend on the concentrations and temperature of the mixture [31].

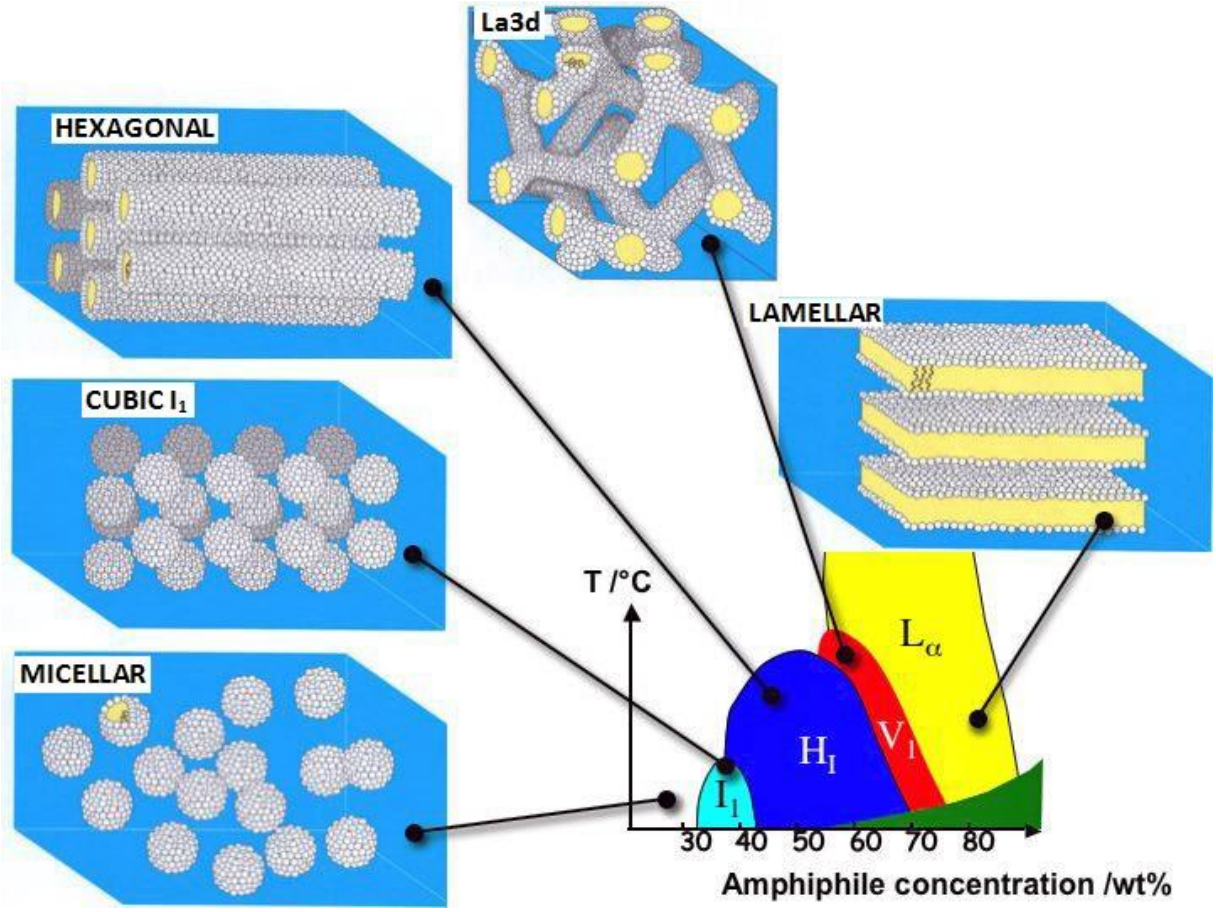

Figure 2. Illustration of amphiphile aggregation structures at different concentrations and temperatures [30].

The structures can be isotropic which means they look the same in all directions like the micellar and cubic structures or they can be anisotropic like the hexagonal and lamellar structures. When a sample with anisotropic properties is viewed in a microscope with a polarized light source will have a distinct texture.

Effect of Blox4 Allergy and the need for a new formula

The formulation of Blox4 Allergy is complicated since many demands, some of them a bit contradictory, are put on the product. Ideally it should be moisturizing, mucoadhesive, protective film forming, temperature stable, low irritating and sprayable.

The current Blox4 Allergy formulation has a few problems. It is not stable at lower temperatures. It has received complaints from users of being irritating inside the nose and throat. Some users also report a bad smell and taste of the product.

There is a constant transportation of mucus from the nose to the throat so the spray film is moved to the throat. It is in this stage that some people can detect the taste.

The product contains humectants that absorb water. This leads to better mucoadhesion and a longer duration in the nasal mucosa [34]. Too much water absorption leads to irritation and a burning sensation in the nose due to the water being pulled out of the underlying mucosa.

The product phase separates reversibly in a refrigerator (+5°C).

The aim of the new formulation is to address these issues, while still retaining similar properties. The old product is called the Glycerol MonoOleate GMO formulation in this report, and the new product is called the PolyGlycerolMonoOleate PGMO formulation. The two formulations should be compared to verify that the properties are not too dissimilar. This means that no new clinical studies have to be made since the products are similar enough to give the same effect.

The goal of the diploma work is to make a characterization of the formulations and from this verify that the products are similar enough to avoid making new clinical trials.

GMO and PGMO formulation differences

The ingredients differ somewhat between the GMO and the PGMO formulations.

An important difference between the two products is that the GMO formulation contains glycerolmonooleate (GMO) and the PGMO formulation has polyglycerol monooleate (PGMO) instead. GMO is an amphiphilic molecule with a glycerol group attached to a lipid hydrocarbon chain. PGMO instead has several glycerol groups linked in a chain to the same lipid molecule.

Both formulations contain polysorbate 80, also known as Tween 80. It is a commonly used solubilizing agent utilized in a variety of cosmetic and medical products [12]. The other shared ingredient is propylene glycol, with the systematic name propane-1,2-diol. It is a viscosity decreasing compound that can be used as a solvent. It is a humectant [13].

The GMO formulation contains Macrogol 400, which is a generic name for polyethylene glycol. It is often used as a solvent or co-solvent [17]. Menthol and eucalyptus are added to improve the taste and smell of the product while masking an unpleasant taste and smell that were experienced by some users.

Sesame oil has been exchanged with olive oil. Both oils are similar, with a melting point of -6°C; however sesame oil contains a higher percentage of polyunsaturated fats [18].

To lower the viscosity of the PGMO formulation, MCT oil has been added. MCT stands for medium chain triglycerides. It consists of small fatty acids, with 6-12 carbon molecules per hydrocarbon chain.

GMO Blox4 constituents:

GMO (Glycerol monooleate)

Propylene glycol (propane-1,2-diol)

Macrogol 400 (Polyethylene glycol)

Sesame oil

Polysorbate 80 (Tween 80)

Isotonic water solution

Menthol

Eucalyptus

PGMO Blox4 constituents:

MCT oil

PGMO (Polyglycerol Monooleate)

Olive oil

Propylene glycol (propane-1,2-diol)

Polysorbate 80 (Tween 80)

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a technique that is used to measure energy changes in a sample by heating it and recording the energy needed to raise the temperature of it on a thermal curve. The required power to heat the sample is then compared to the energy needed to heat an inert well defined reference. If the sample undergoes a phase transition or crystallizes, it can be detected as a peak in the thermal curve since more or less energy is required to heart the sample compared to the reference. The peak can be positive or negative depending on if the transition is exothermic or endothermic. The starting point of the peak, where the first crystals are formed is defines as the onset of the peak. At the end of the peak, where the last crystals are formed is defined as the endset of the peak. [1].

Rheology

Rheology deals with the flow and deformation properties of a material. If the resistance to flow is high when a force is applied to a sample, it corresponds to a high viscosity.

The groundwork for a mathematical description of rheology was laid by Newton. If the shear stress apply is proportional to the shear rate in a linear fashion at every point the fluid is a Newtonian fluid. Other relationships between shear stress and shear rate exist. For example if the viscosity of a fluid decreases the more force it is affected by it is known as a shear thinning fluid.

Some pharmaceutical materials are viscoelastic. This means that when stress is applied to a sample with this property, some of the energy is dissipating as heat in the viscous flow and some will be stored in the material and recovered after the stress has been removed. During a reasonable time frame, a perfectly viscous material will have all the energy from the stress converted to heat and no deformation will occur. Water is a good example of a material which exhibits this behavior. A perfectly elastic material will store all the energy in a structure deformation and will use this energy to return to its original structure after the stress is removed. An example of an elastic material is rubber.

The viscoelastic properties of a sample can be described with the storage modulus, G', that is a measurement of how elastic the material is. A higher number means a more elastic material. The loss modulus, G'', is a measure of how viscous the material is. A higher number means a more viscous material. The storage modulus and loss modulus are related to each other by the complex shear modulus G*.

$$G* = \sqrt{G'^2 + G''^2}$$

Another relationship is the so called loss angle δ. It is given in degrees.

$$\tan\delta = G''/G'$$

A value of 0° means that the material is perfectly elastic and a value of 90° means that it is perfectly viscous [10].

These properties can be measured by performing an oscillation test. The sample is exposed to a sinusoidal oscillation at increasing shear stress and G* is measured as a function of this increasing shear stress. As long as G* is constant it means that the structure of the sample is not disturbed and the stress applied is in the linear viscoelastic region. It is important to stay in this region when performing further measurements, such as varying the oscillation frequency to determine G', G'' and δ [11].

Nuclear magnetic resonance spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy uses the fact that atomic nuclei possess a spin magnetic moment. When the nuclei are placed in a strong external magnetic field inside an NMR spectrometer, the magnetic moments slightly align with the magnetic field and thus the sample becomes magnetized. Thereafter a series of radio frequency pulses excite the samples and cause the magnetization vector to move away from equilibrium temporarily. After the pulse, the vector precesses back to equilibrium, giving rise to a free induction decay (FID) signal. This signal is measured. It can be Fourier transformed and the result is a typical NMR spectrum with peaks that correspond to different types of nuclei in the sample [19].

With NMR spectroscopy it is possible to measure the self-diffusion constant of a molecule, both in a pure sample and in a mixture with other substances. The technique uses a gradient magnetization of the sample and a special pulse sequence that disperses and then refocuses the magnetization. Depending on where in the sample a molecule is, it will experience a certain strength from the magnetic field, due to this gradient. If molecules diffuse it means that they are not in the same position as they were at the start of the measurement. When the magnetization is refocused after the dispersion, the resulting signal will be weaker than it would have been if all molecules were stationary and experienced the same magnetic field as during the start. By varying the gradient strength of the magnet, the resulting signal becomes less and less refocused. The gradient strength can be plotted versus the signal intensity and an equation can be fitted to the data points, giving the diffusion coefficient [19,20-23].

$$I = I_0 * e^{-D(2\pi\gamma G_i\delta)^2 * \left(\Delta - \frac{\delta}{3}\right) * 10^4}$$

Where I and I_0 are intensities, $\gamma$is the gyromagnetic ratio, Δ and δ are delay times, G_i is the gradient strength and D is the diffusion constant.

Sometimes there are several compounds beneath the same peak in the NMR spectrum. It is still often possible to determine the self-diffusion constants of the molecules. In these cases a multiexponential fit is made to the equation:

$$I = I_{01} * e^{-D_1(2\pi\gamma G_i\delta)^2*\left(\Delta-\frac{\delta}{3}\right)*10^4} + I_{02} * e^{-D_2(2\pi\gamma G_i\delta)^2*\left(\Delta-\frac{\delta}{3}\right)*10^4}$$

A size comparison between I_{01} and I_{02} gives some information about how much of each type of molecule is part of the sample.

If the water in the sample is enclosed in micelles it will not have the usual self-diffusion coefficient of 2.299 $*10^{-9}$ m^2/s [26]. Instead it will be an order of magnitude larger due to the entrapment inside the micelles.

With NMR spectroscopy it is possible to detect what type of structure is in a liquid crystalline sample. By observing the peak of added deuterium in a ^2H NMR spectrum and noting the splitting of the peak it is possible to determine if the sample has isotropic phases, anisotropic phases or a mixture of both. A ^2H nucleus has a strong quadropolar moment, which can lead to a splitting of the peak in the spectrum. In an isotropic environment the interactions from the quadropolar moment is averaged out to zero and only one peak is observed [27,28].

Materials

Both formulations were obtained from Bioglan AB, that had made test solutions for use in lab work.

Mucin from porcine stomach type II (CAS number 84082-64-4) was obtained from Sigma-Aldrich.

Propylene Glycol was obtained from BASF, lot. S002531.

Olive oil was obtained from Gustav Heess, lot. GHM-0569.

Sesame oil was obtained from Henry Lamotte Oils.

MCT Oil was obtained from R2 Pharmactive, lot. S001960.

PGMO was obtained from Hydrior.

Gelatine was obtained from Tørsleffs, bought from a normal grocery store.

GMO was obtained from Danisco, batch no. 4011742134

A few different types of PGMO were also used, called PGMO1, PGMO2 PGMO2g, PGMO3 and PGMO4.

Methods

Phase behavior of the formulations

The phase behavior of the formulations was investigated at different temperatures and water content to determine the stability of the product at lower temperatures and at different water content. The initial water content of the formulations was determined by using Karl Fisher titration on a Metrohm 701 KF Titrino that was calibrated with Hydranal composite 5 and controlled with

sodium tartrate dehydrate. The tests were done in duplicates. The results were an initial amount of 5.16 % of water in the GMO formulation and 0.45 % in the PGMO one.

Nine vials with 3.0 g of the GMO were prepared. The water content was varied from 5.16 % to 9.16 % since the starting concentration of water was 5.16 %, with an increment of 0.5 percentage points each time.

The vials were stirred to allow the water to homogeneously spread out and mix properly with the microemulsion. They were put in a water bath (Julabo FS18, manufacturing number 15904360008, with HP Basis heating circulators, manufacturing number 17603320091) at 40°C and were left for one hour to allow the temperature of the vials to equilibrate with the bath. The same procedure was repeated for the temperatures 37°C, 35°C, 30°C and 25°C. The temperatures 20°C, 15°C and 10°C were done in another water bath since the Julabo FS18 required extra cooling pipes to run at these temperatures. The new water bath was cooled by a Huber ministat 240. The bath itself contained no internal thermometer.

When the Julabo FS18 bath was at 25°C an external uncalibrated thermometer measured 25.4°C. The same thermometer measured 20.2°C in the bath cooled by the Huber ministat 240 when the temperature set for the water in the cooling pipes was set to 19.4°C.

The samples were quickly taken from the water bath to maintain the temperature. Then they were shaken and visually inspected.

The PGMO formulation was mixed with water to produce samples with 4.45 %, 6.0 %, 7.0 %, 8.0 %, 10.0 % and 12.0 % water content. The vials were inspected at 40°C, 35°C, 30°C and 25°C using the same Julabo FS18 water bath, and at 20°C, 15°C and 10°C with the same Huber ministat 240. All the GMO and PGMO samples were also put in a refrigerator (5°C).

DSC measurements

First DSC mesasurements were made on the GMO and PGMO formulations to detect the peak at the highest temperature. The endset temperature of this peak gives some information on how temperature stable the sample is.

The DSC apparatus was a Perkin Elmer DSC 7 with Pyris version 3.03 software. It was controlled and calibrated each day with a reference sample of indium. The run should fulfill the requirements of an onset of 156.60 +/- 0.5 °C (melting point) and a ΔH of 28.45 +/- 0.20 J/g. Nitrogen gas was supplied at 2 bar pressure which corresponds to about 20 mL/min of flow to the sample compartment during all the measurements.

The samples for the DSC tests were weighed up on a calibrated Sartorius 23607401 scale and put into 40 µl BO14-3021 aluminum pans that were hermetically sealed with 0.1 mm BO14-3003 aluminum lids. The DSC chamber held room temperature when the pans were inserted. The samples were run with a program of first holding for 2 minutes at +40°C, then cooling to -40°C and finally reheating to +40°C. The cooling and heating rates were 1, 5 and 10 degrees. Duplicates were made for each run.

According to unpublished work by Soma Ghosh and Johan Engblom, Malmö University, there might be an overlapping of peaks if the temperature program going from +40°C to -40°C was used. Because of this a new set of measurements were made. These DSC measurements were started at +40°C with

a cooldown to -50°C where the temperature was held for ten minutes, after which it was raised back to +40°C. These new experiments were done on a Seiko instruments Exstar DSC6200 with the software Muse 7.0 U. The samples were weighed on a Cahn C-30 microbalance. Two measurements were made with both formulations to check if peaks were overlapping, one at 5°C/min and one at 1°C/min.

Another set of measurements was made on different types of Glycerol monooleates. They were performed on the Perkin Elmer DSC 7. Details can be found in appendix 1.

Water uptake experiment

To produce an atmosphere with a well-defined relative humidity a saturated salt solution can be used. A saturated solution with Potassium carbonate was put in an exicator to give a relative humidity of 43.16 % at 25°C. Another exicator was prepared with a saturated salt solution of potassium sulfate, giving a relative humidity of 97.30 %RH at 25°C [15]. A beaker of each Blox4 formulation was put in both exicators and left for 53 days. After 18 and 53 days the amount of water that the formulation had absorbed into the beakers was measured.

Figure 3. Picture of one of the exicators with the formulations in beakers inside.

Rheology

The rheology measurements were done on a Bohlin VOR rheometer.

The geometry was a C14 concentric cylinder with about 2 ml of sample to fill the sample cup, a gap of 0.15 mm and a measurement slit of 0.7 mm. All measurements were performed at 25°C.

First a 3.8 g cm torsion bar was used and a strain sweep from 0.0018 to 0.1946 s^{-1} was made to determine the linear viscoelastic region of both formulations by monitoring the G* value during the

sweep. The frequency was set to 10 Hz since the fluids seemed to have a low viscosity upon visual inspection.

With the same torsion bar a frequency sweep was performed from 10 Hz to 0.1 Hz to determine G', G'' and the loss angle.

Afterwards viscosity measurements on the formulations were performed with the same torsion bar. The shear rate was varied from 0.00927-233.2 s^{-1}.

Thereafter a high strain rate test was done with the 19 g cm torsion bar for the GMO formulation from 9.29-1470 s^{-1} and the same was done with the PGMO formulation. However at 1470 s^{-1} the measurement was stopped because of overload. The torsion bar was changed to 38.7 g cm and it allowed the full range to be measured. At all the measurements of the high shear rate, the value of 46.4 s^{-1} was avoided since it corresponded to the resonance frequency of the measurement system.

To get values that could be compared to the viscosities of the formulations, measurements were made on MCT oil, sesame oil, olive oil and PGMO. The measurements were divided into the same shear rate intervals as the Blox4 Allergy formulations. The low shear rate measurements for olive oil, MCT oil and sesame oil were made with a 3.8 g cm torsion bar and the high with a 19 g cm torsion bar. Since PGMO had a much higher viscosity, the low shear rate measurement was done with the 19 g cm torsion bar and the high with the 270 g cm torsion bar.

Mucoadhesive tensile strength

The method for measuring mucoadhesion was based on the method described in [24].

The mucoadhesive properties of the formulations were tested on a Stable Micro Systems TA-XT2i texture analyzer with a 36 mm diameter probe.

A dispersion of 4 wt % mucin and 96 wt % deionized water was mixed and heated to 30-35°C under stirring until it became homogenous.

Circular bits of filter papers with an inert backside were cut out to match the area of the probe. One paper was put on the probe and another one on the corresponding contact surface area below inside a petri dish using double-sided sticky tape. The top paper was smeared with 250 µl of the mucin dispersion in a thin smooth layer. The bottom paper was smeared with 250 µl of the Blox4 Allergy microemulsion. The papers were then left to soak and dry for 10 minutes before measurements began.

In the measurements, the probe was lowered with a speed of 1 mm/second until a force of 250 g was applied to the bottom sample. The force was applied for 180 seconds before detachment and measurement of the required force to separate the paper with mucus dispersion from the paper with Blox4 microemulsion. The petri dish was fastened on the bottom surface with double-sided sticky tape to hinder it from following the probe back up.

Another set of tests were made with the formulations and pure water to determine if the mucin had a special impact on adhesion.

To get some reference values, the mucoadhesion of olive oil, propylene glycol and PGMO were also tested. Propylene glycol was chosen to determine if humectant properties had any influence on the adhesion strength.

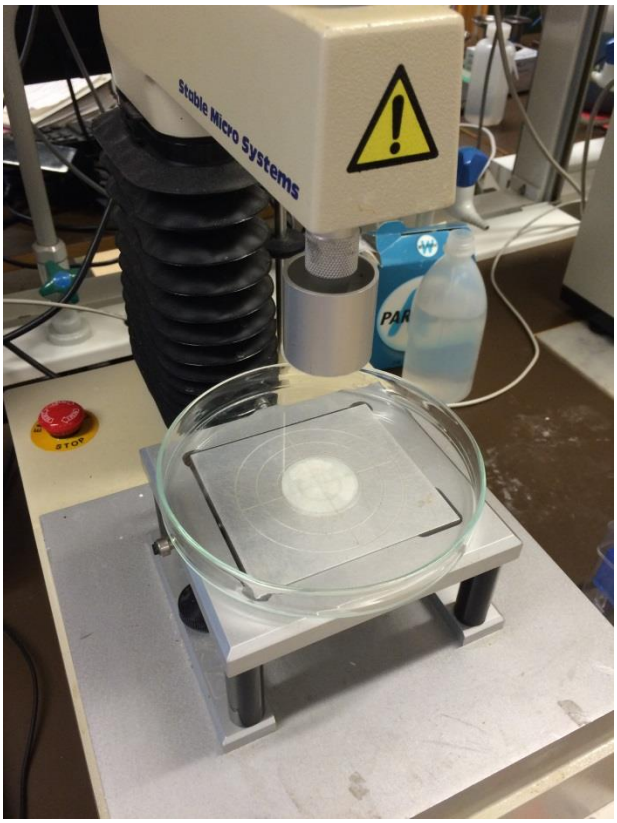

Figure 4. The texture analyzer with the parts for the tensile strength test assembled.

Surface tension

Surface tension comes from a less favorable interaction at an interface between a liquid and another material such as the one between water and air. A liquid will minimize its surface area to maintain as much of its internal more energetically favorable interactions as possible. The surface tension values give some information about how well the product will wet a specific surface, such as the hydrophilic mucosa inside the nose [33].

The surface tension of the two formulations was measured on a Tecklis Tracker tensiometer with Windrop for Windows XP as software.

All the beakers used were washed in beforehand in a mixture of sulfuric and hydrochloric acid (concentration?) to remove any contaminations such as surfactant residues. The needle of the syringe used to create the drop was washed with ethanol to prevent wetting of the liquids. The temperature was 24°C on the water calibration measurements and 25°C for the measurements on the formulations.

In the Milli-Q water calibration, two sets of 15 µl drops were formed and the surface tension was measured for 600 seconds.

When the two formulations were tested, the volumes of the drops were 6 µl. The density values used were 1.01 kg/dm^3 for the GMO formulation and 0.960 kg/dm^3 for the PGMO formulation.

Spreadability

A glass surface was put in a mixture of sulfuric and hydrochloric acid (concentration again?) for 1.5 hours, washed with deionized water and allowed to air dry. The glass surface thus becomes hydrophilic and can somewhat mimic the conditions of the nasal mucosa. The glass was put on a surface that was checked with a spirit level to be straight.

100 µl of sample was put on the plate and measured after spreading out for 30 minutes. The GMO formulation and PGMO formulation were each tested four times. The diameter of the drop on the plate was measured with a Vernier caliper.

Then the plate was washed with detergent and water, allowed to air dry and then put into the same bath of sulfuric and hydrochloric acid for 1.5 hours. It was allowed to air dry before 100 µl of deionized water and olive oil were put on the surface. Both the deionized water and the olive oil were tested four times in the same way as the two formulations.

Figure 5. 100 µl of each formulation was put on a glass plate.

Water Activity

Water activities of the formulations were measured on a Novasina Labmaster-aw at Malmö Högskola. The samples were measured at 25°C with a redox filter to protect the sensor from propylene glycol. A measurement was made on the 5.16 % water content GMO formulation and on a sample of the PGMO formulation with water added to give 5.16 % total water content.

Figure 6. The Novasina Labmaster-aw on which water activity measurements were made.

NMR spectroscopy - diffusion experiments

A sample of the PGMO formulation was mixed with water to give 3 % water content in total to keep it within the same region in the phase diagram. Disposable NMR tubes were filled with 300 µl of this mixture, the GMO formulation and pure PGMO. The PGMO was very viscous and had to stand overnight for the sample to settle at the bottom of the NMR tube.

The chemical shift scale was corrected with deuterated DMSO at 2.5 ppm, together with propylene glycol since it was present in both formulations. This was done on a Varian UNITY Inova 500 MHz, with the software VnmrJ 3.2.

Thereafter a total spectrum on both formulations was acquired. A PGSE type diffusion measurement was done on both formulations. Since the PMGO formulation had a lesser amount of excipients, its constituents were studied in diffusion experiments one by one to confirm that the water peak did not have too much of other compounds underneath at its chemical shift value. The exact value of water's chemical shift varies with temperature, pH and constituents of the sample. The peak should appear somewhere around 4.7 ppm [32].

The data was processed with the program DOSY Toolbox version 2.5 [25].

NMR spectroscopy - phase studies

A vial of each formulation was mixed with an equal amount of water inside a vial. It was allowed to phase separate for 5 days before the two phases that formed were put in separate vials at 4000 rpm for three hours, removing any residual content from the other phases from the vial.

540 µl of each of the four samples were put in NMR tubes and 60 µl of deuterated water was added to give a D_2O concentration of 10 % by weight, shortly before taking a ^1H and a ^2H spectrum of each sample on a Varian Unity Nova 500 MHz spectrometer at 298 K.

The PGMO bottom phase was also studied in a microscope under a polarized light to see if the phase showed any sign of anisotropic structures (Why this one?).

Consumer study

40 participants were given a questionnaire, which can be found in appendix 4, together with either a vial of the GMO formulation or the PGMO formulation. 20 people were given the GMO formulation and 20 people were given the PGMO formulation. The two formulations did not have the same type of pump for the vials. The GMO formulation had a one-hole pump and the PGMO formulation had a five-hole pump that spread the beam shot into smaller segments to achieve a less intense feeling when applying the product in the nose. Under supervision the participants were asked to test the product and fill in the questionnaire about how they experienced it. The answers were then collected, evaluated and scored in a series of diagrams, which can be found in appendix 5.

Results & Discussion

Phase behavior of the formulations

The results of the phase behavior study can be seen in Table 1 below. Phase separation means that an oil rich phase is forming at the bottom of the vial.

Table 1. Phase behavior for the GMO formulation at different water contents in percentage of total weight. ps = phase separated.

5°C	10°C	15°C	20°C	25°C	30°C	35°C	37°C	40°C
5.16 % ps	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %
5.66 % ps	5.66 %	5.66 %	5.66 %	5.66 %	5.66 %	5.66 %	5.66 %	5.66 %
6.16 % ps	6.16 % ps	6.16 %	6.16 %	6.16 %	6.16 %	6.16 %	6.16 %	6.16 %
6.66 % ps	6.66 % ps	6.66 % ps	6.66 % ps	6.66 %	6.66 %	6.66 %	6.66 %	6.66 %
7.16 % ps	7.16 % ps	7.16 % ps	7.16 % ps	7.16 % ps	7.16 % ps	7.16 %	7.16 %	7.16 %
7.66% ps	7.66% ps	7.66% ps	7.66% ps	7.66% ps	7.66% ps	7.66% ps	7.66% ps	7.66% ps
8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps	8.16 % ps
8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps	8.66 % ps
9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps	9.16 % ps

Phase separation in the GMO formulation was easy to notice since the liquid became turbid when shaken if phase separation had occurred. Phase separation in the PGMO formulation was not as easy to detect due to it having a higher viscosity and therefore not responding as much upon shaking.The GMO formulation phase separated at lower water content the lower the temperature. At 10°C even the 6.16 % water content sample was turbid. The original sample contains 5.16 % of water and phase separated at a typical refrigerator temperature of 5°C, after standing for several days even becoming completely solid.

The PGMO formulation with 4.45 % water content was stable at all temperatures. The 6 % water content sample was clear and in one phase at 25°C. At 20°C a small new phase was visible at the bottom of the vial.

Table 2. Phase behavior for the PGMO formulation at different water contents in percentage of total weight. ps = phase separated.

5°C	10°C	15°C	20°C	25°C	30°C	35°C	37°C	40°C
5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %	5.16 %
6.0 % ps	6.0 % ps	6.0 % ps	6.0 % ps	6.0 %	6.0 %	6.0 %	6.0 %	6.0 %
7.0 % ps	7.0 % ps	7.0 % ps	7.0 % ps	7.0 % ps	7.0 %	7.0 %	7.0 %	7.0 %
8.0 % ps	8.0 % ps	8.0 % ps	8.0 % ps	8.0 % ps	8.0 %	8.0 %	8.0 %	8.0 %
10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps	10.0 % ps
12 .0 % ps	12.0 % ps	12.0 % ps	12.0 % ps	12.0 % ps	12.0 % ps	12.0 % ps	12.0 % ps	12.0 % ps

The results indicate that the PGMO formulation, which is manufactured without containing water still can handle about as much water content as the GMO formulation without phase separating.

Results from DSC measurements

Below are example figures that show the heat flow as a function of time for some measurements. For the experiments done on the Perkin Elmer DSC at Bioglan AB, an upwards peak for the heating part of the measurement corresponds to an endothermic event. The peak of interest is the last peak found when reheating of the sample.

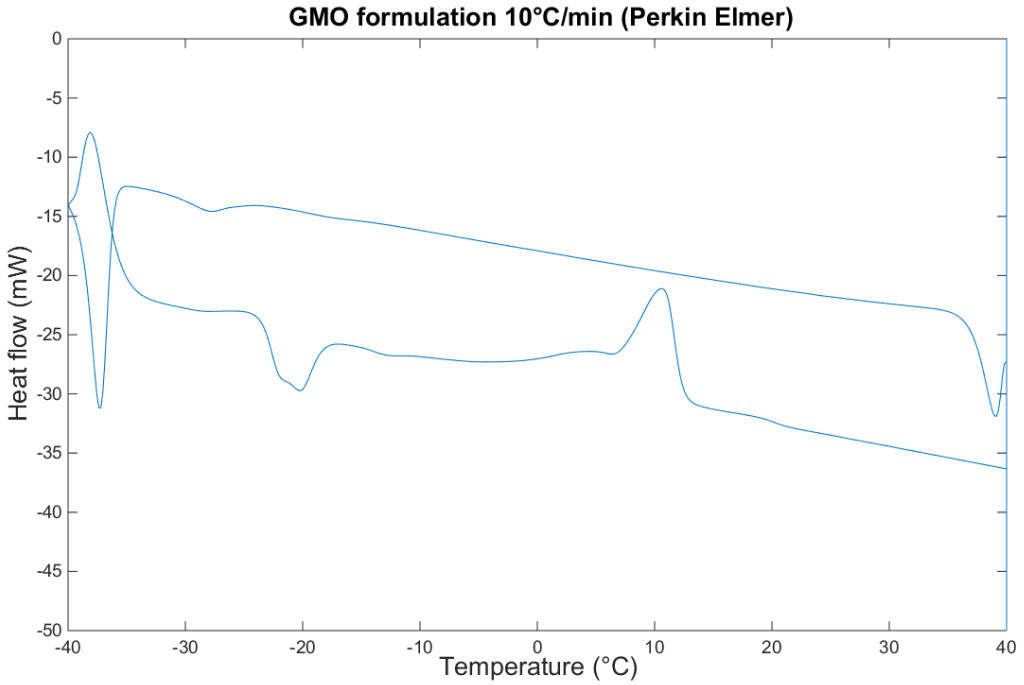

Figure 7. Plot of DSC data for the GMO formulation with a scan rate of 10°C/min, done at Bioglan AB.

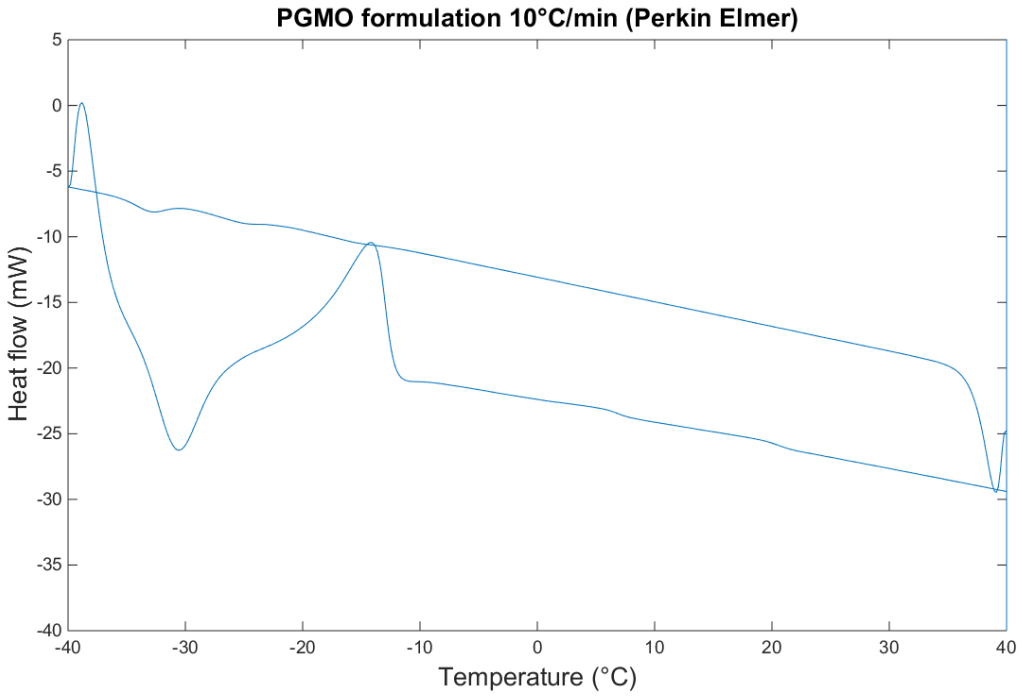

Figure 8. Plot of DSC data for the PGMO formulation with a scan rate of 10°C/min, done at Bioglan AB.

The average endset temperature of this peak was calculated for the different scan rates and extrapolated to zero scan rate. The mean extrapolated endset temperature was 10.789°C for the GMO formulation and -13.303°C for the PGMO formulation.

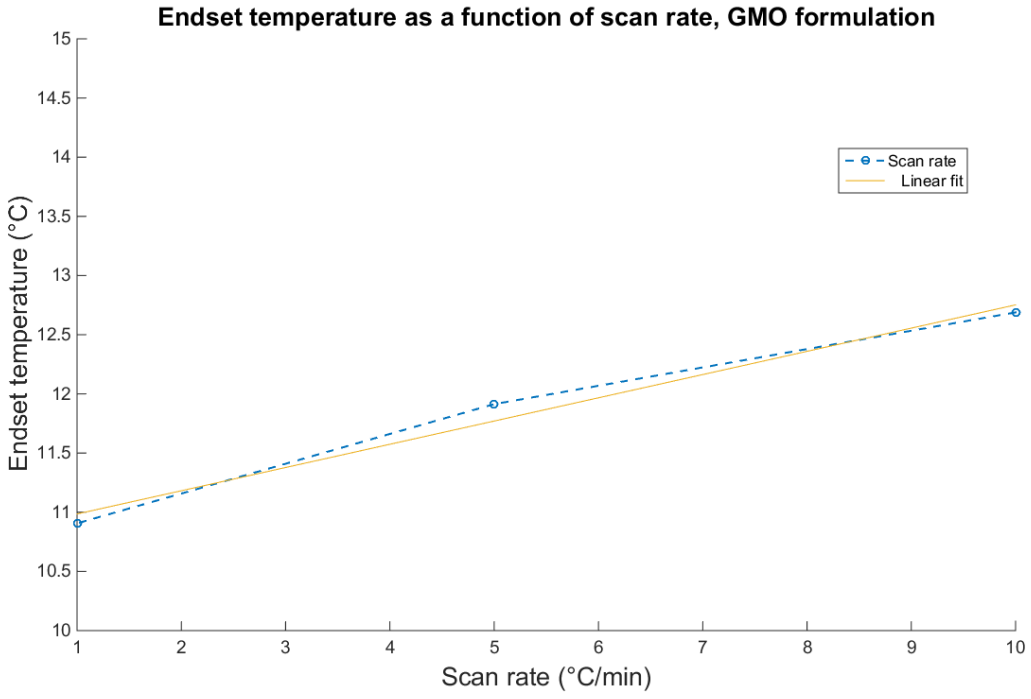

Figure 9. The endset temperature as a function of scan rate for the GMO formulation. Norm of residuals = 0.17541.

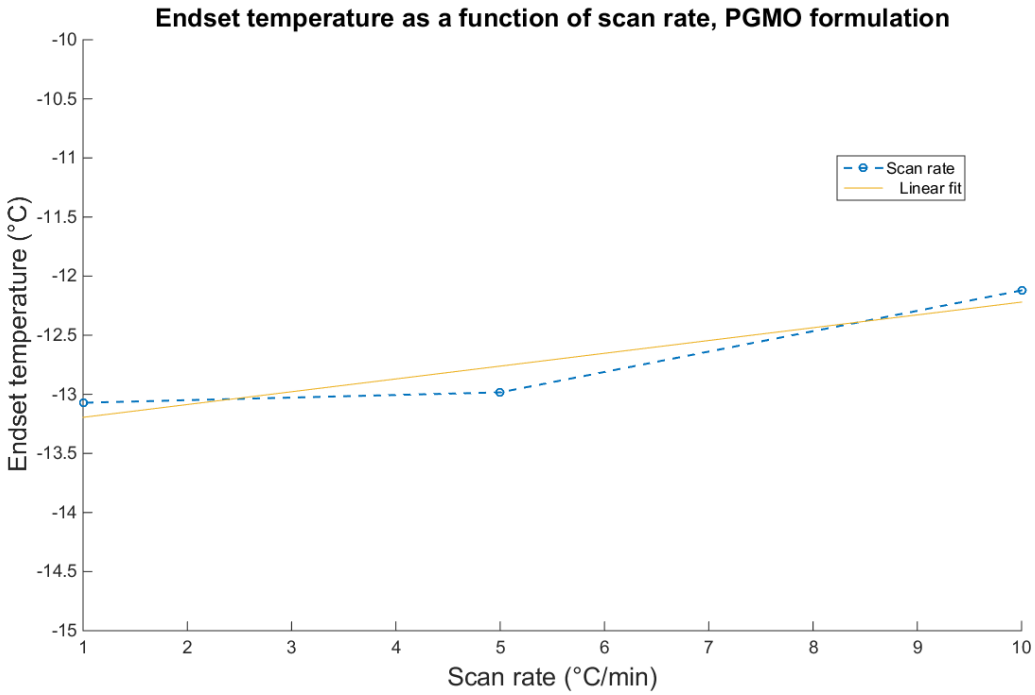

Figure 10. The endset temperature as a function of scan rate for the PGMO formulation. Norm of residuals = 0.27251.

There was a risk of peaks overlapping so a rerun was made with a cooldown to -50 °C with the samples holding this temperature for 10 minutes before going back up to 40°C to verify that the results were correct. The result is showed in the graphs below. There seemed to be no overlapping for the peak of interest. In these experiments, a downwards pointing peak corresponds to an endothermic event in the sample if the temperature is in the rising part of the measurement.

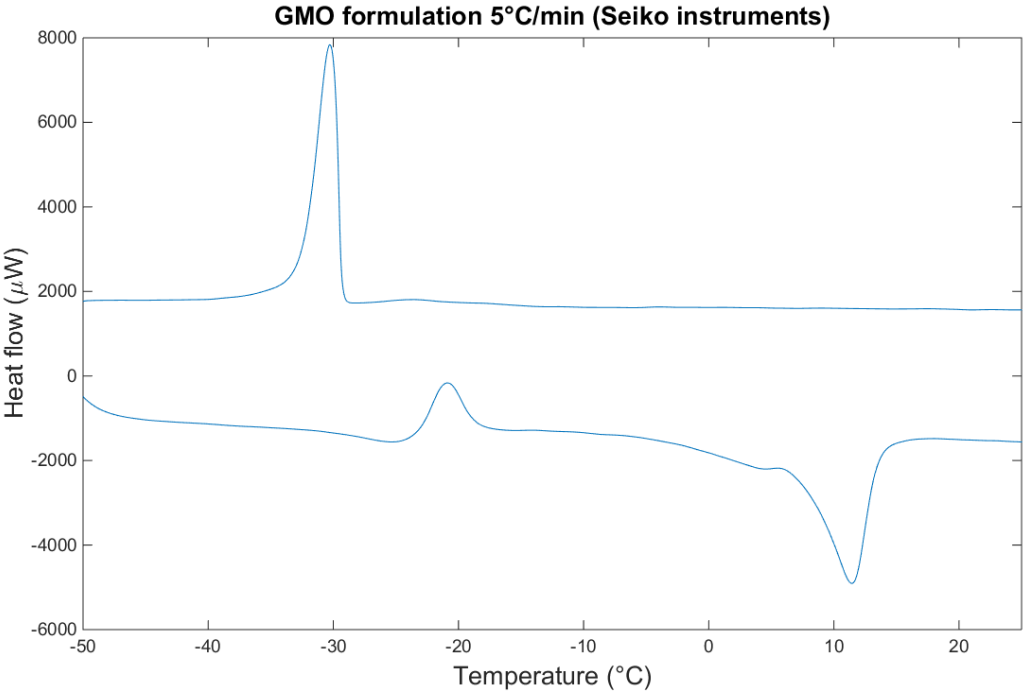

Figure 11. Plot of DSC data for the GMO formulation with a scan rate of 5°C/min, done at LTH.

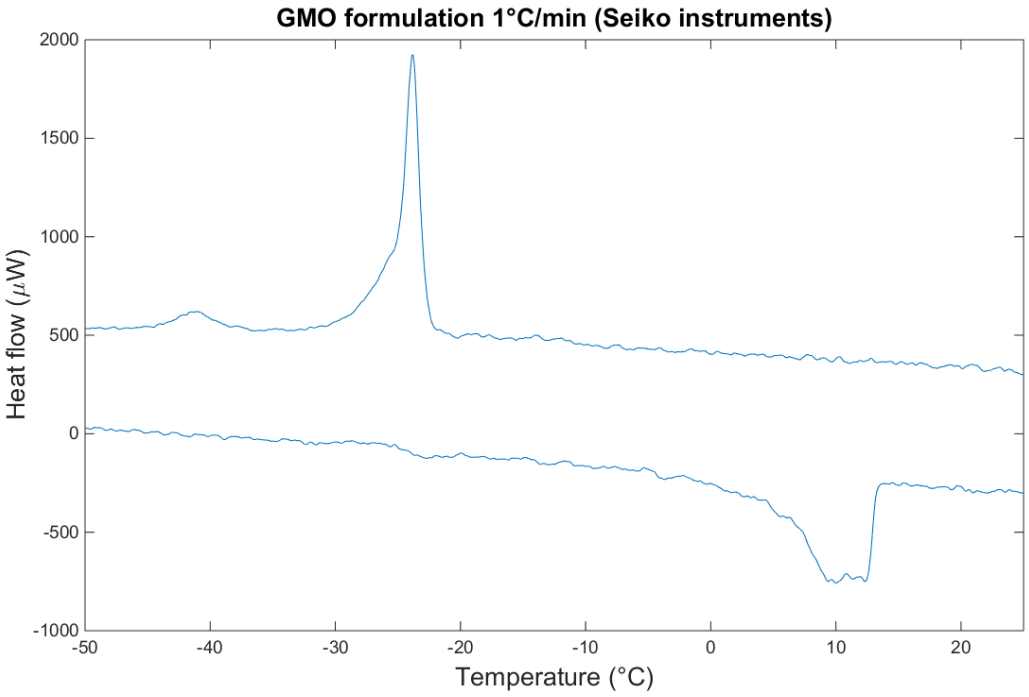

Figure 12. Plot of DSC data for the GMO formulation with a scan rate of 1°C/min, done at LTH.

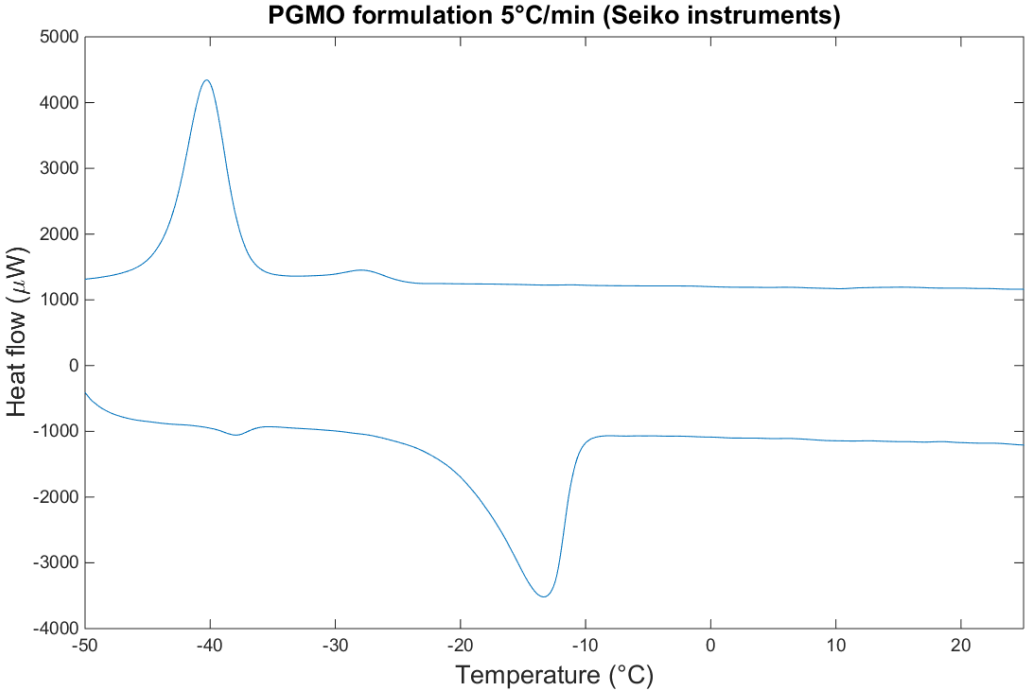

Figure 13. Plot of DSC data for the PGMO formulation with a scan rate of 5°C/min, done at LTH.

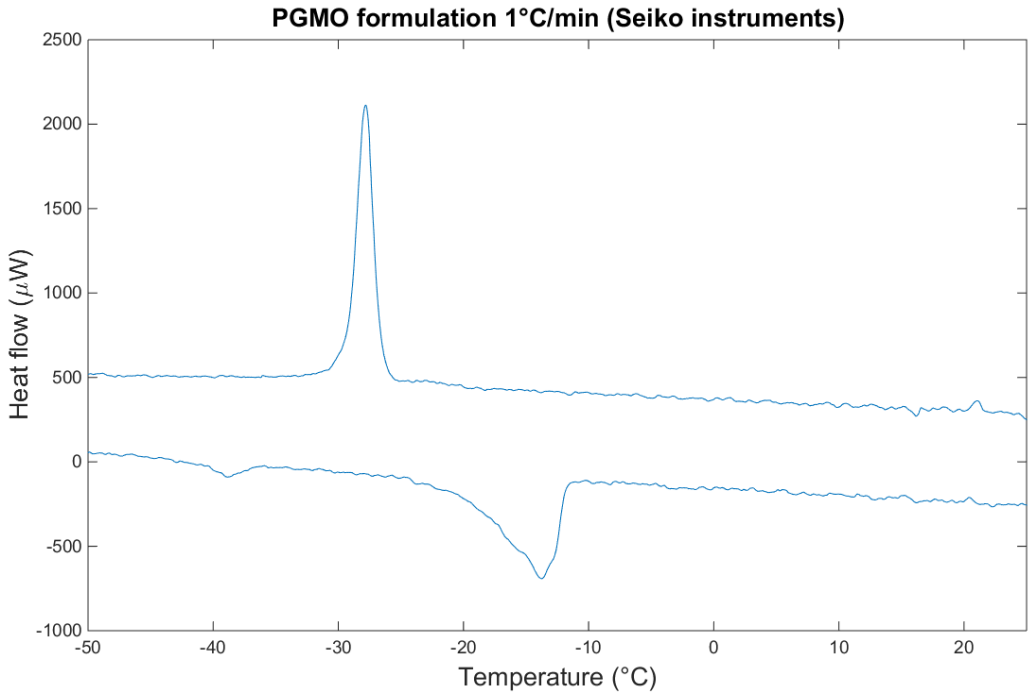

Figure 14. Plot of DSC data for the PGMO formulation with a scan rate of 1°C/min, done at LTH.

The results indicate that the PGMO formulation is much less sensitive to temperature changes. It will not phase separate and form crystals in the same degree as the GMO formulation does. This is probably linked to the PGMO. At room temperature it is liquid. GMO on the other hand is solid and quite hard.

Surface tension

The GMO formulation had a surface tension of 29.4 +/- 0.2 mN/m and the PGMO formulation had a surface tension of 29.7 +/- 0.1 mN/m.

This can be compared to some values from the literature. Mueloliva extra virgin olive oil has a surface tension of 28.499 mN/m at 26.94°C and pure sesame oil from Zhangxingbang has a surface tension of 28.106 mN/m at 28.31°C [14]. Pure water has a surface tension of 71.99 mN/m at 25°C. Propylene glycol has a surface tension of 45.6 mN/m at 25°C [16].

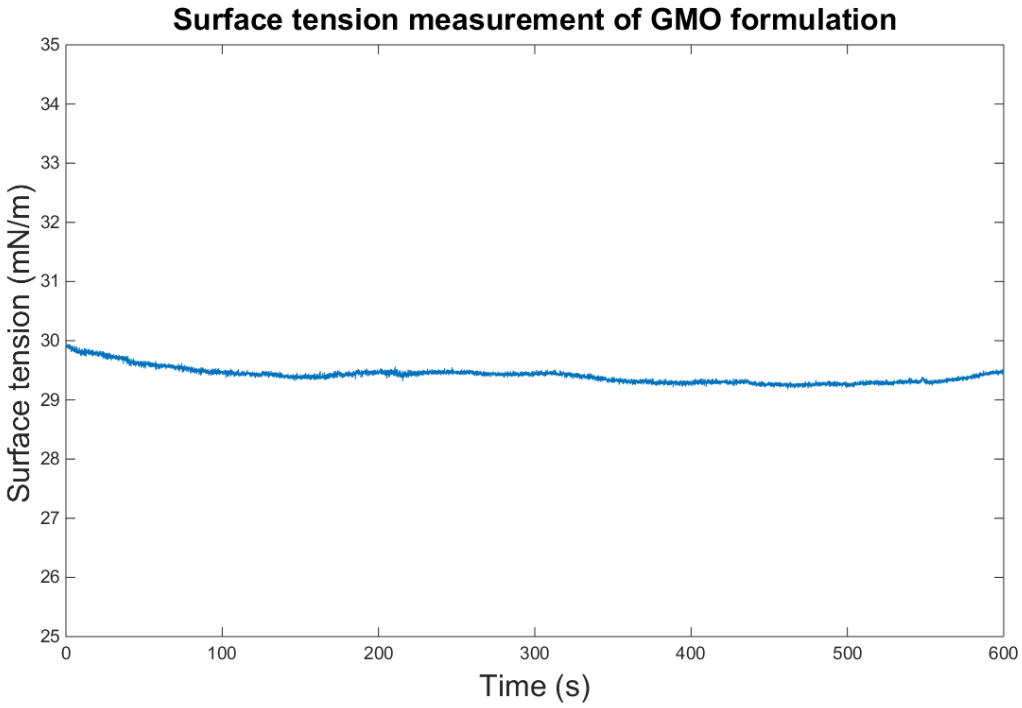

Figure 15. Graph showing the surface tension of the PGMO formulation over 600 seconds.

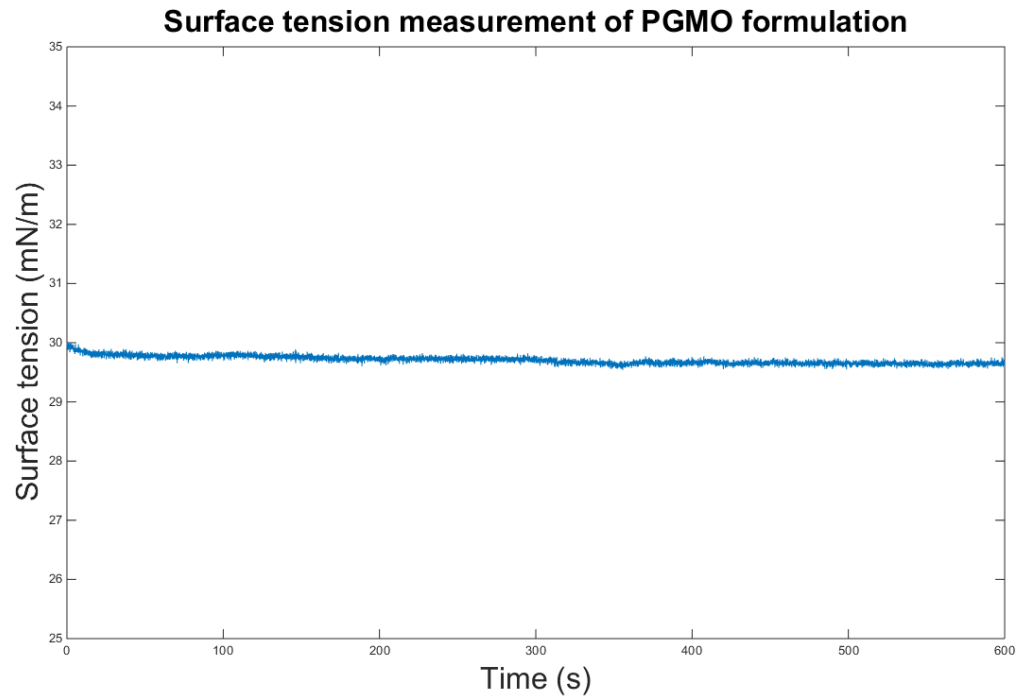

Figure 16. Graph showing the surface tension of the PGMO formulation over 600 seconds.

The results are very similar for the two formulations. The values are close to pure olive oil or sesame oil and much lower than water. This indicates that spreadability of the PGMO formulation should be similar to the GMO formulation.

Spreadability

The mean diameter of the four 100 µl drops from the spreadability testing were:

23.2 +/- 2.3 mm for the GMO formulation

19.6 +/- 0.5 mm for the PGMO formulation

11.9 +/- 0.3 mm for the deionized water

15.3 +/- 0.4 mm for the olive oil

The GMO formulation was observed to spread significantly faster than the PGMO formulation within the first 10 seconds after applying the product. The PGMO formulation probably spreads more slowly partly because of its higher viscosity value.

Despite having the same surface tension the PGMO formulation shows slightly less spreadability on a hydrophilic surface. The values are still fairly similar compared to the values for water and olive oil.

Water does not spread much despite being put on a hydrophilic surface due to the high surface tension of water. However the result might have been affected by some of the water evaporating during the 30 minutes.

Olive oil is very hydrophobic and does not spread well on a hydrophilic surface, despite having a low surface tension.

Mucoadhesive tensile strength

Figure 17 below illustrates how the work needed to separate the layers from each other is calculated as well as the peak measured force.

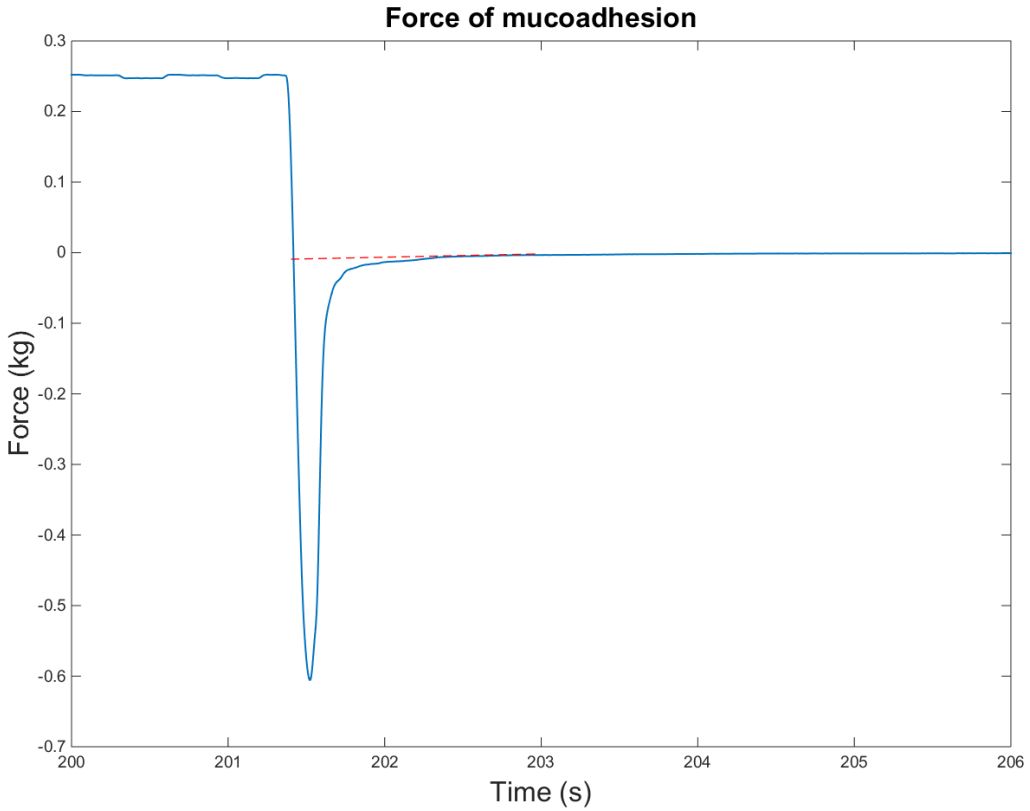

Figure 17. The figure shows the force applied as a function of time. The work needed to separate the layers from each other is the area calculated from integration of the negative peak. The maximum negative force is the minimum value of the negative peak.

Both the GMO and the PGMO formulations showed quite strong mucoadhesion, with similar tensile strength values. Compared to adhesion to pure water it was higher as well.

The comparison results on olive oil and propylene glycol showed significantly lower values than the two formulations. PGMO is intrinsically very sticky and attaches strongly to most surfaces.

Table 3. Summary of the results from the mucoadhesion measurements.

Upper sample	Lower sample	Mucoadhesion (kg s)	Peak force (kg)
Mucin	GMO formulation	0.18 +/- 0.18	0.89 +/- 0.11
Mucin	PGMO formulation	0.21 +/- 0.16	0.88 +/- 0.21
Water	GMO formulation	0.067 +/- 0.033	0.65 +/- 0.24
Water	PGMO formulation	0.090 +/- 0.034	0.76 +/- 0.20
Water	Water	0.017 +/- 0.00	0.12+/- 0.011
Mucin	Olive oil	0.089 +/- 0.016	0.79 +/- 0.14
Mucin	Propylene Glycol	0.051 +/- 0.0089	0.50 +/- 0.051
Mucin	PGMO	0.75 +/- 0.31	2.4 +/- 0.26
Dry paper	Dry paper	0	0.006

There is a large fluctuation in the values which leads to a high uncertainty. A better method for measuring mucoadhesion would be desirable. The results seem conclusive however. The PGMO formulation has a value that is a little bit higher. This could be linked to the viscosity which is also higher, or that it contains PGMO which is so sticky in itself.

Rheology

The strain sweep measurements showed initial fluctuations probably due to insufficient sensitivity in the torsion bar and thereafter a linear response, indicating that if the products show any viscoelastic behavior the whole measured shear rate is in the linear viscoelastic region. From this data, the maximum available shear rate was chosen (0.1946 s^{-1}) since this probably gave the most accurate measurements.

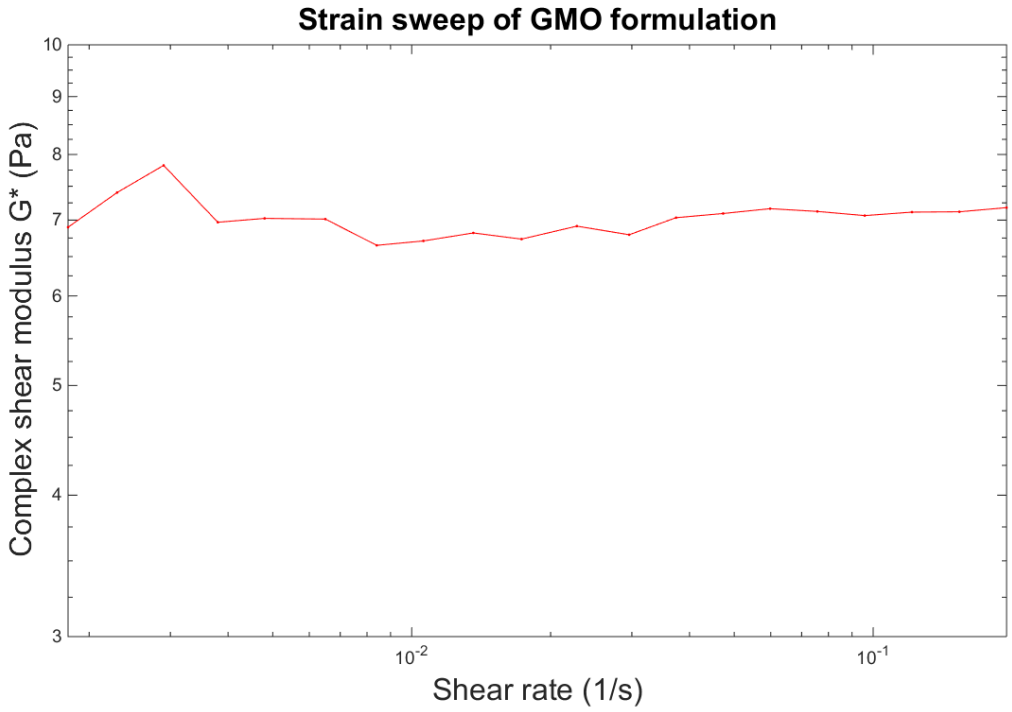

Figure 18. The shear rate was varied at a constant frequency of 10 Hz to determine the viscoelastic region of the GMO formulation.

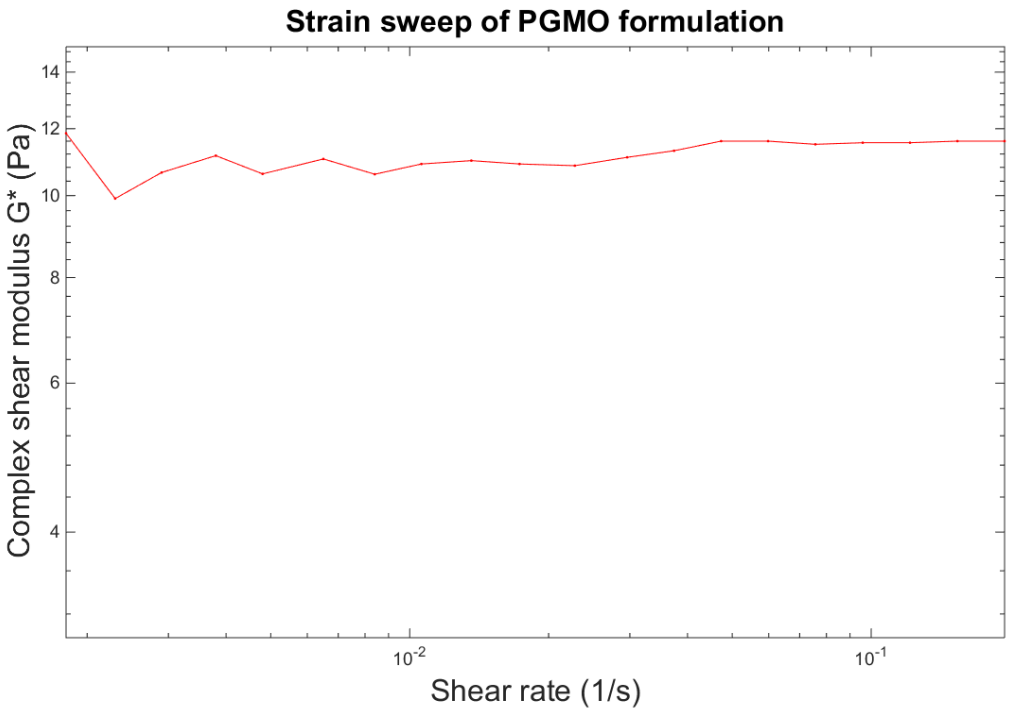

Figure 19. The shear rate was varied at a constant frequency of 10 Hz to determine the viscoelastic region of the PGMO formulation.

The determination of the storage and loss modules as well as the phase angle showed that both formulations had an almost ideal viscous behavior, showing almost no elastic properties.

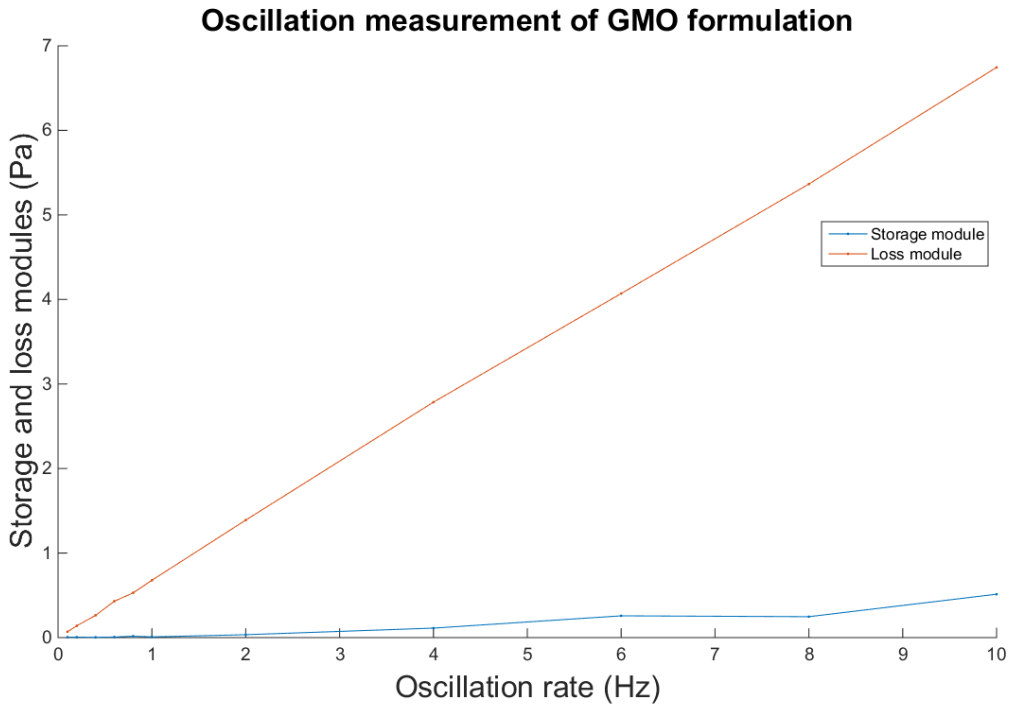

Figure 20. Frequency sweep of the GMO formulation. At all times the loss module is significantly higher than the storage module indicating a viscous behavior.

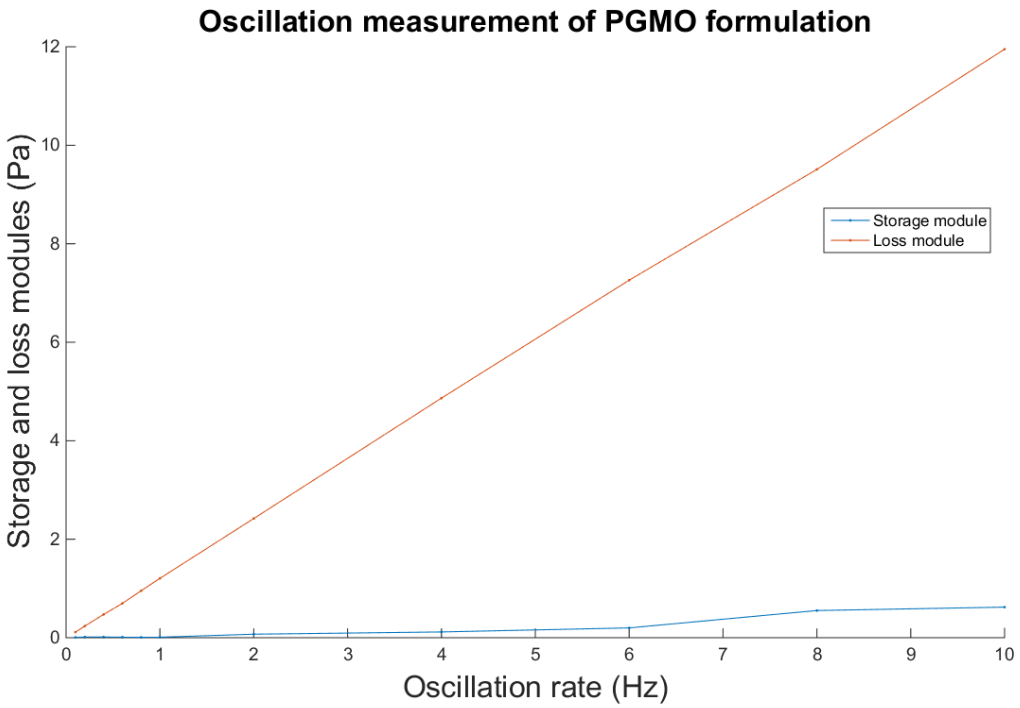

Figure 21. Frequency sweep of the PGMO formulation. At all times the loss module is significantly higher than the storage module indicating a viscous behavior.

The phase angles for the GMO and PGMO formulations were 87.6014° and 88.0459° respectively indicating an almost completely ideal viscous behavior.

The results from the Blox4 allergy viscosity measurements are presented in the figures below. The PGMO formulation had a higher viscosity at all times compared to the GMO one. Both formulations display a Newtonian behavior.

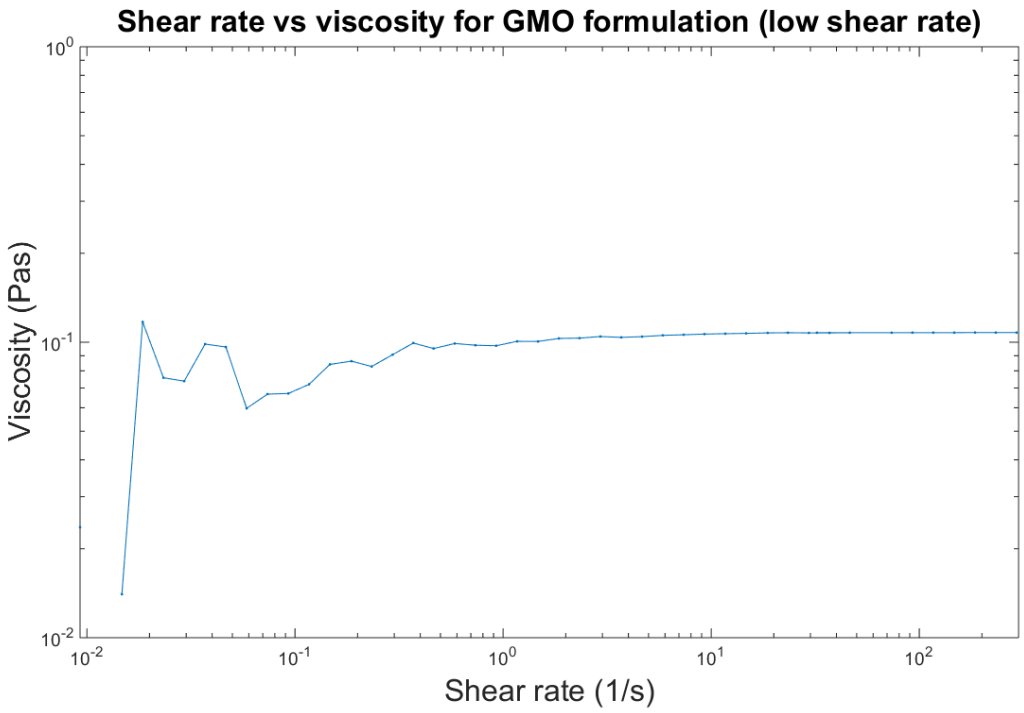

Figure 22. The figure shows the viscosity of the GMO formulation as a function of shear rate at low shear rates.

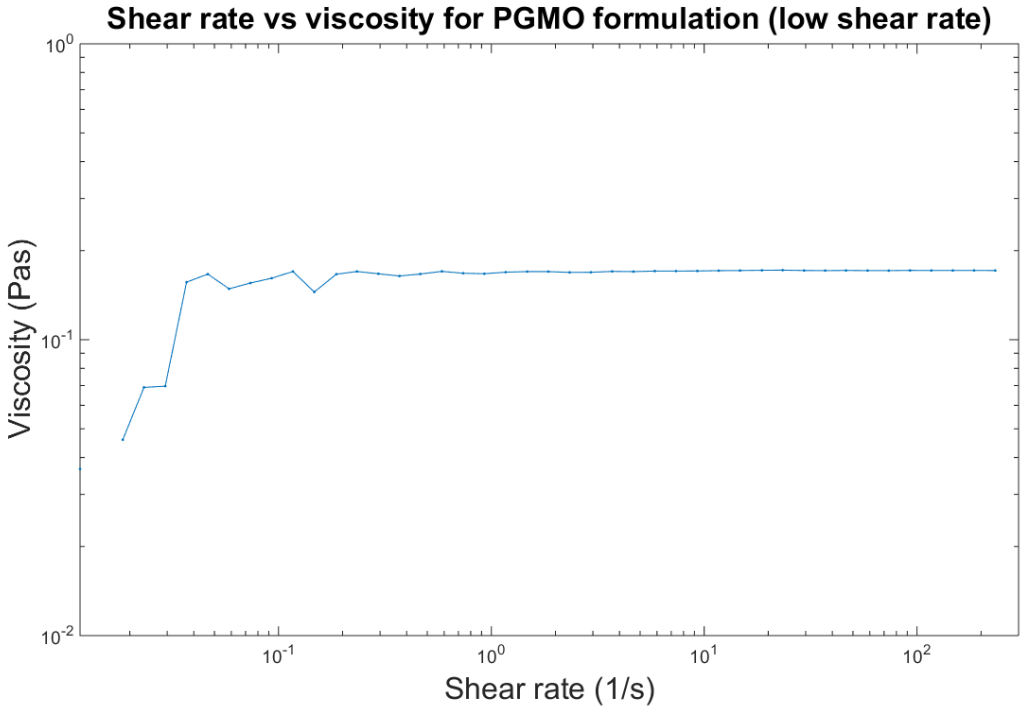

Figure 23. The figure shows the viscosity of the PGMO formulation as a function of shear rate at low shear rates.

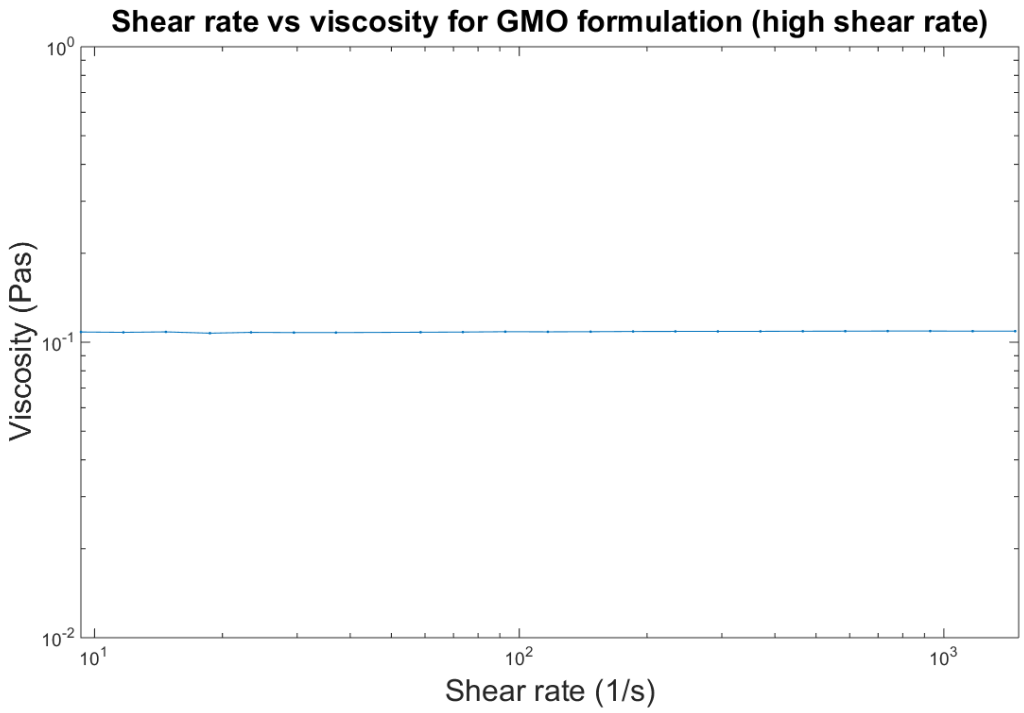

Figure 24. The figure shows the viscosity of the GMO formulation as a function of shear rate at high shear rates.

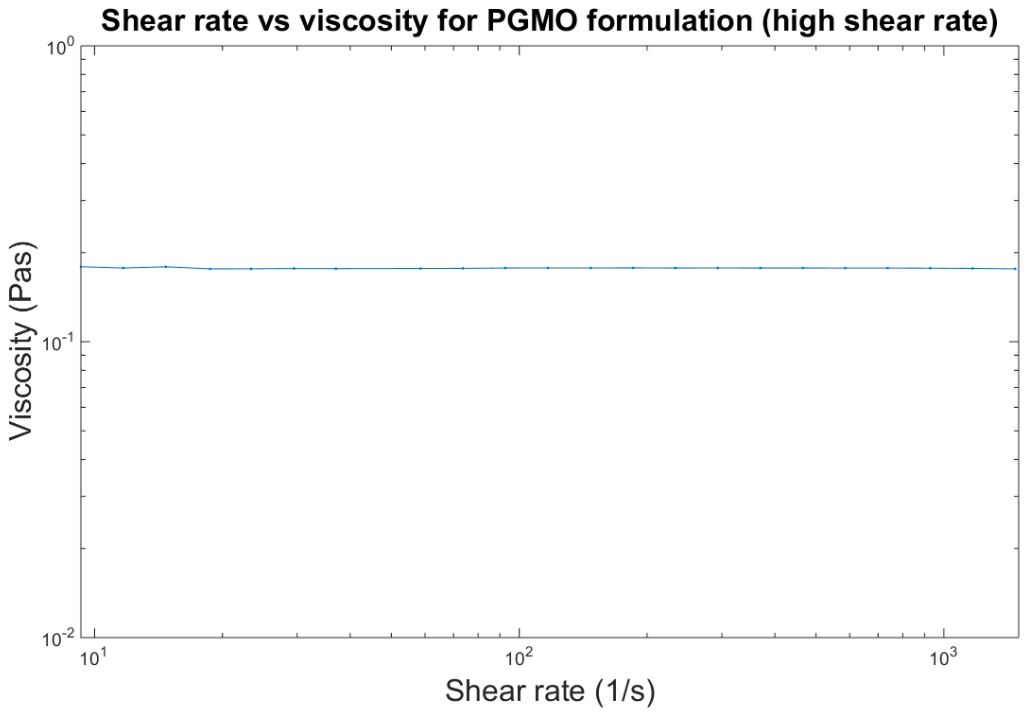

Figure 25. The figure shows the viscosity of the PGMO formulation as a function of shear rate at high shear rates.

The plots of viscosity values for the vegetable oils can be found in the appendix.

Table X below contains a summary of the apparent viscosity at 116.8 s^{-1} shear rate. Both Blox4 formulations have a higher value than for example olive oil. The PGMO formulation has a significantly higher value than the GMO one however both values are eclipsed by the value of PGMO.

Table 4. Summary of apparent viscosities at 116.8 s-1 shear rate.

Substance	**Viscosity (Pas)**
MCT oil	0.0203
Sesame oil	0.0514
Olive oil	0.0599
PGMO	4.497
GMO formulation	0.1085
PGMO formulation	0.1768

The higher value for the PGMO formulation seems positive in the sense that the product does not drip out of the nostrils as easily as the GMO formulation. A disadvantage though is that it makes the pump more difficult to initially get started.

Water Activity

The water activity was 0.365 for the GMO formulation at a water content of 5.16 % and 0.562 for the PGMO formulation with the same water content. This is relative to pure water which has an activity of 1.0. The results indicate that the GMO formulation has less free water available at the surface when both formulations have the same water content. However the water activity for the PGMO formulation at 0.45 % water content was 0.072.

Having too high water activity in the product can potentially lead to decomposition of the olive oil or sesame oil in the product due to microbial activity, which is highly affected by water activity.

A low water activity also means that the water is more tightly bound to the sample. Having a low water activity might lead to a higher water uptake, resulting in a slight burning sensation as water is pulled from the underlying tissue when the product is applied.

Water uptake experiment

The initial and final weights of the contents in the beakers is summarized in table 5 and 6 below:

Table 5. Summary of water uptake of the GMO sample.

GMO formulation	43.16 %RH	97.30 %RH	Relative increase 43.16 %RH	Relative increase 97.30 %RH
Initial weight	7.073 g	8.775 g	-	-
Weight after 18 days	7.145 g	9.981 g	1.0 %	13.7 %
Weight after 53 days	7.074 g	11.003 g	0 %	25.4 %

Table 6. Summary of water uptake of the PGMO sample.

PGMO formulation	43.16 %RH	97.30 %RH	Relative increase 43.16 % RH	Relative increase 97.30 %RH
Initial weight	7.495 g	10.002 g	-	-
Weight after 18 days	7.572 g	10.764 g	1.0%	7.6 %
Weight after 53 days	7.518 g	11.111 g	0.3 %	11.1 %

Both formulations absorbed a large amount of water into the beaker at 97.30 %RH. The microemulsions phase separated in both beakers into two phases. One on top of the other with a clear separation. This was apparent already at the first weighing after 18 days. However this did not stop the formulations from keeping to absorb water. The GMO formulations seemed to pull more water into the beaker compared to the PGMO formulation.

A liquid that is hypertonic can cause a burning sensation when applied in the nose since it pulls water from the tissue. The PGMO formulation seems to pull a lesser amount of water into the beaker and should therefore be less prone to pull water out of the nasal mucosa.

Diffusion NMR spectroscopy of the two formulations

The ^{1}H spectra of the GMO formulation, the PGMO formulation and all the parts of the PGMO formulation are presented below.

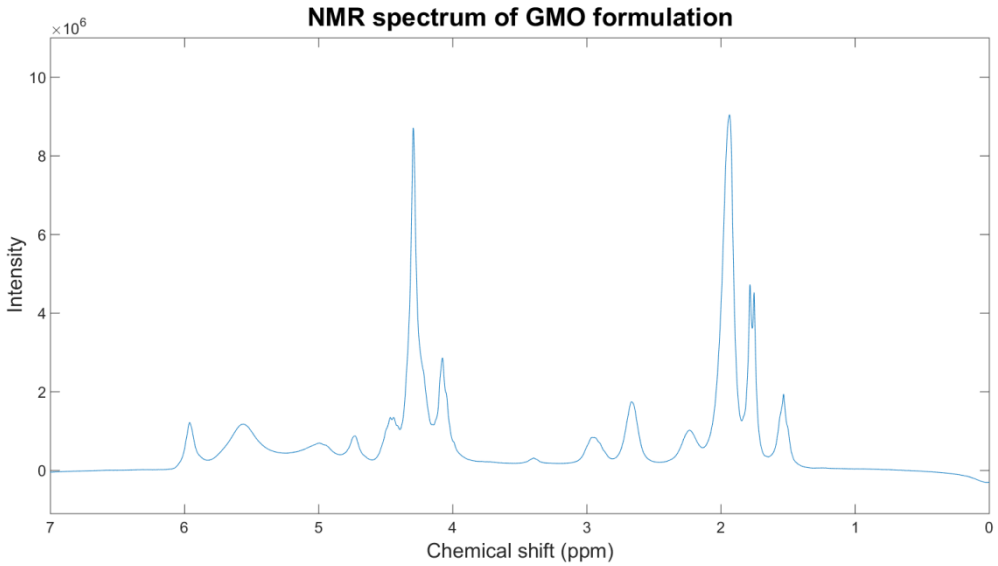

Figure 26. NMR spectrum of GMO formulation.

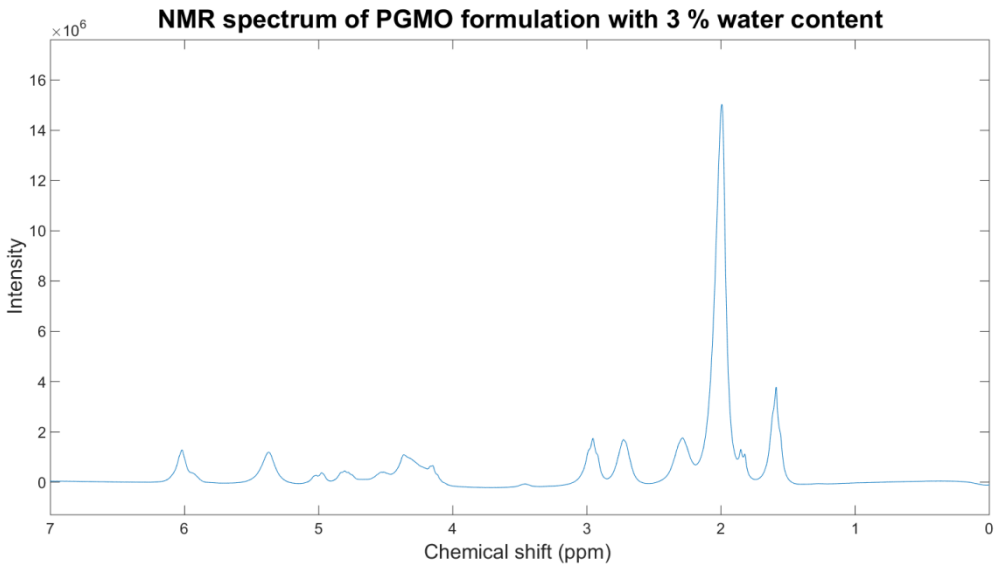

Figure 27. NMR spectrum of PGMO formulation.

In figure 28 below, at 4 to 6 ppm, the smeared out signal indicates that the PGMO sample contains various different compounds.

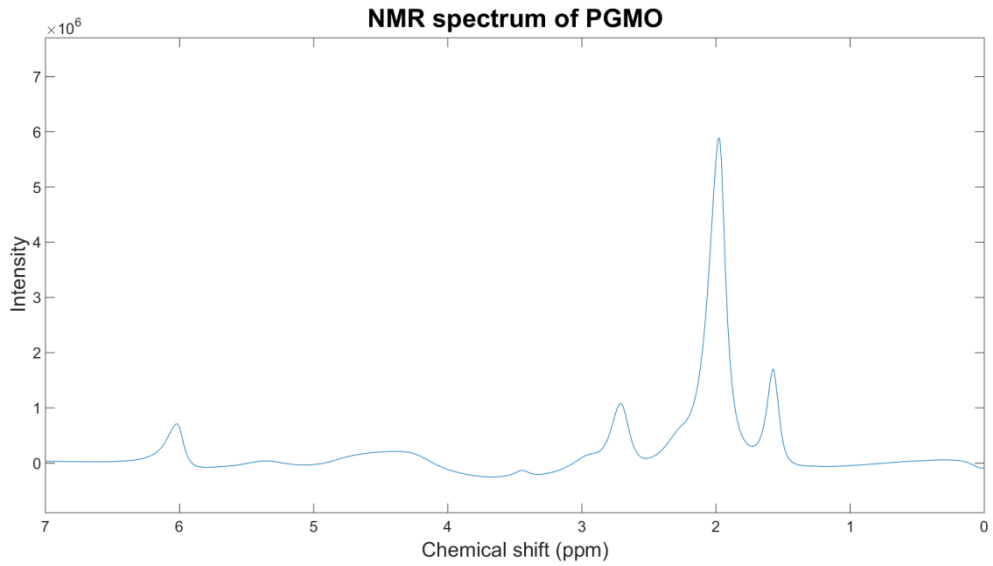

Figure 28. NMR spectrum of PGMO.

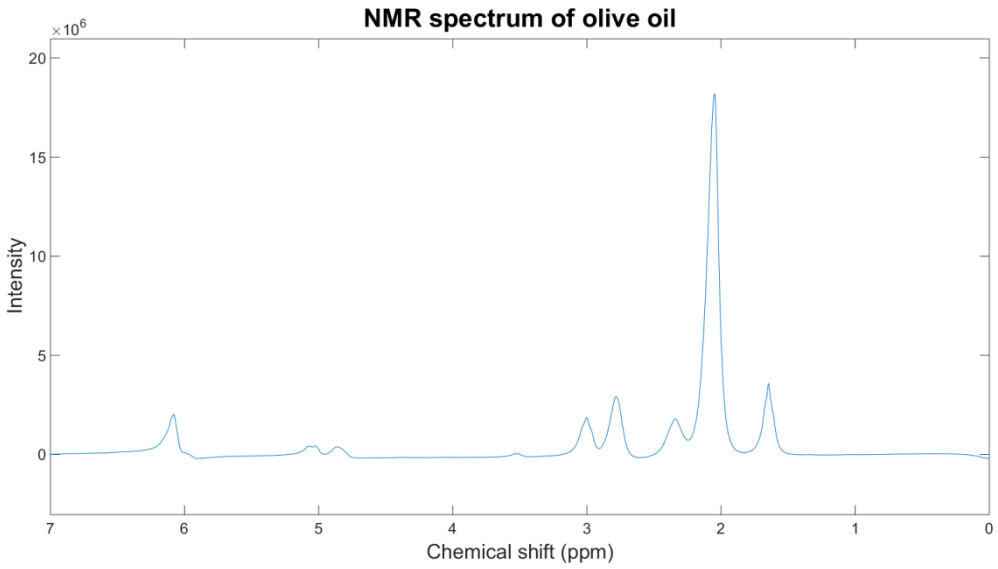

Figure 29. NMR spectrum of olive oil.

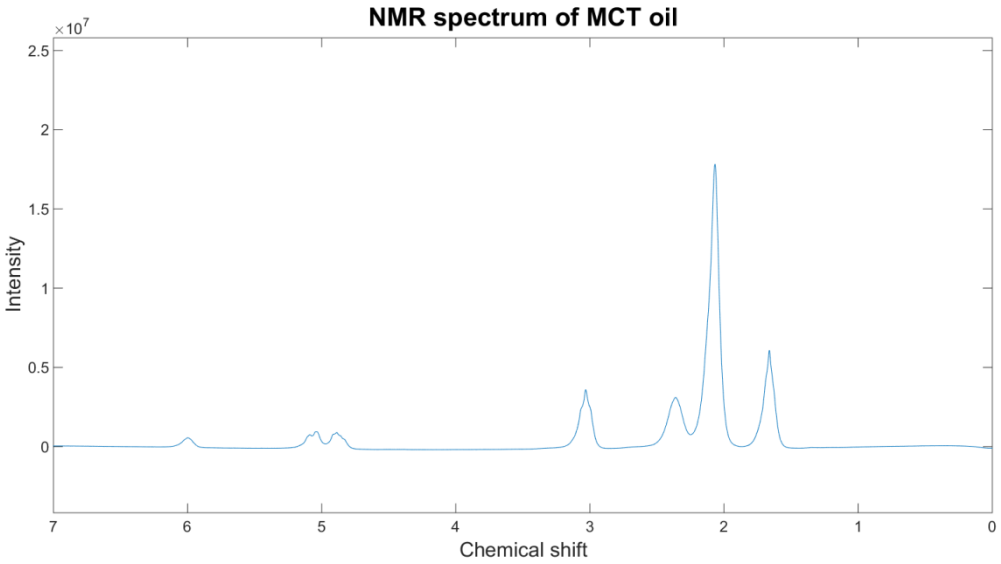

Figure 30. NMR spectrum of MCT oil.

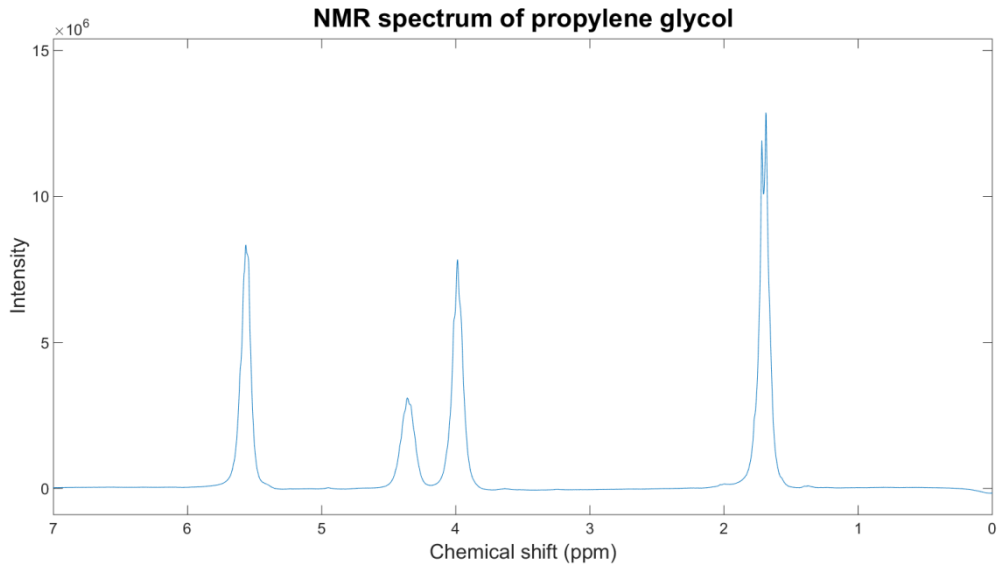

Figure 31. NMR spectrum of propylene glycol.

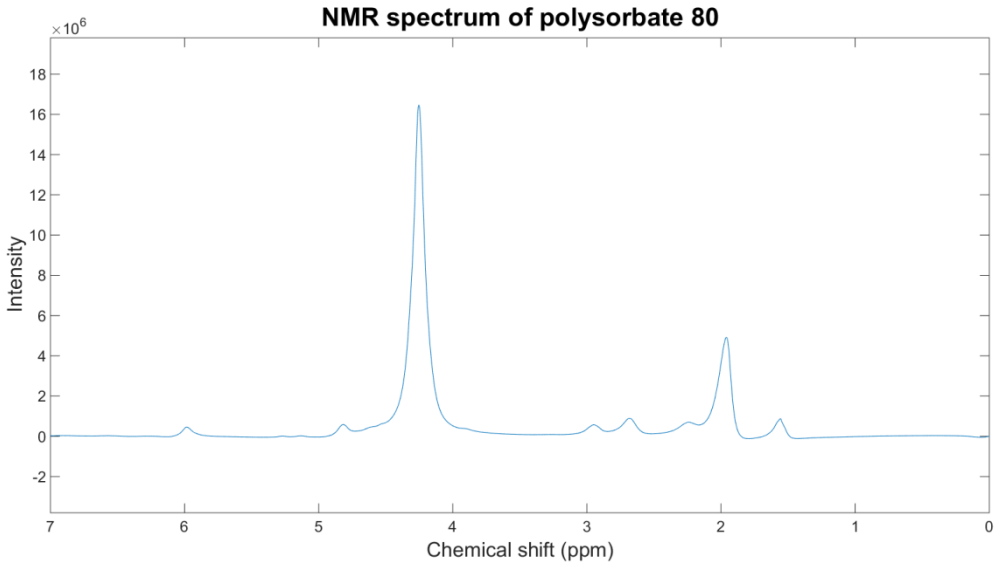

Figure 32. NMR spectrum of polysorbate 80.

The diffusion constants were calculated for each significant peak in each sample. When several compounds appeared to be found within the same peak a biexponential fit with two different diffusion constants was made. Example figures with an equation fitted to the measured points can be found below.

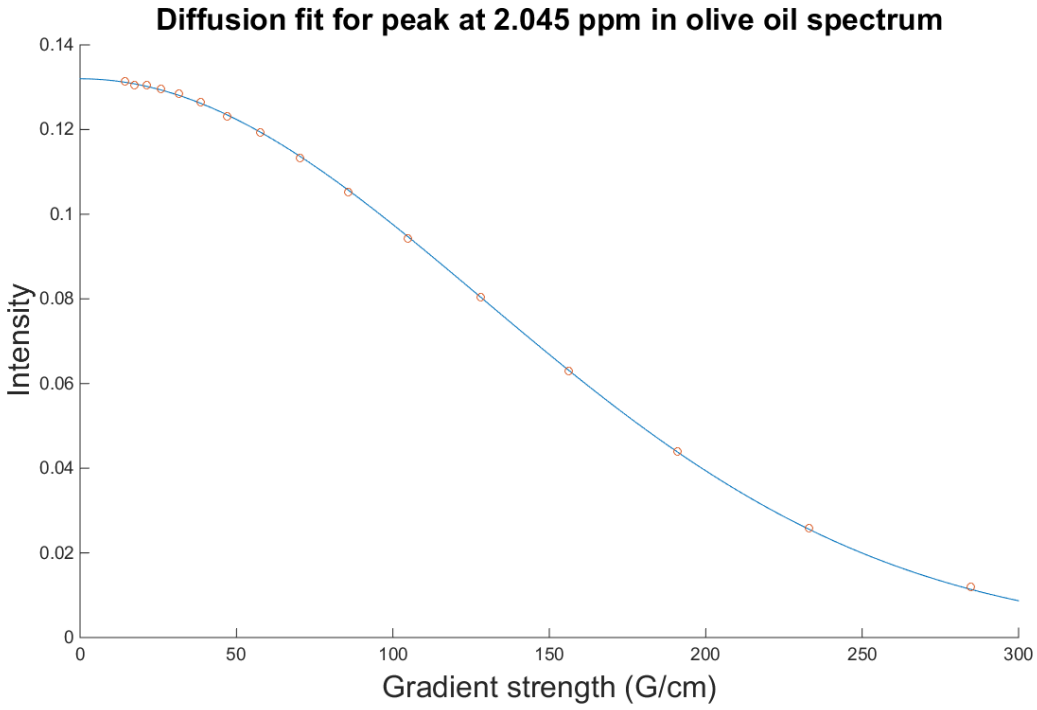

Figure 33. Exponential fit to the first peak at 2.045 ppm in the olive oil sample. An exponential equation with a single diffusion coefficient gives a good fit to the data points in this case. This is because olive oil contains molecules of the same size.

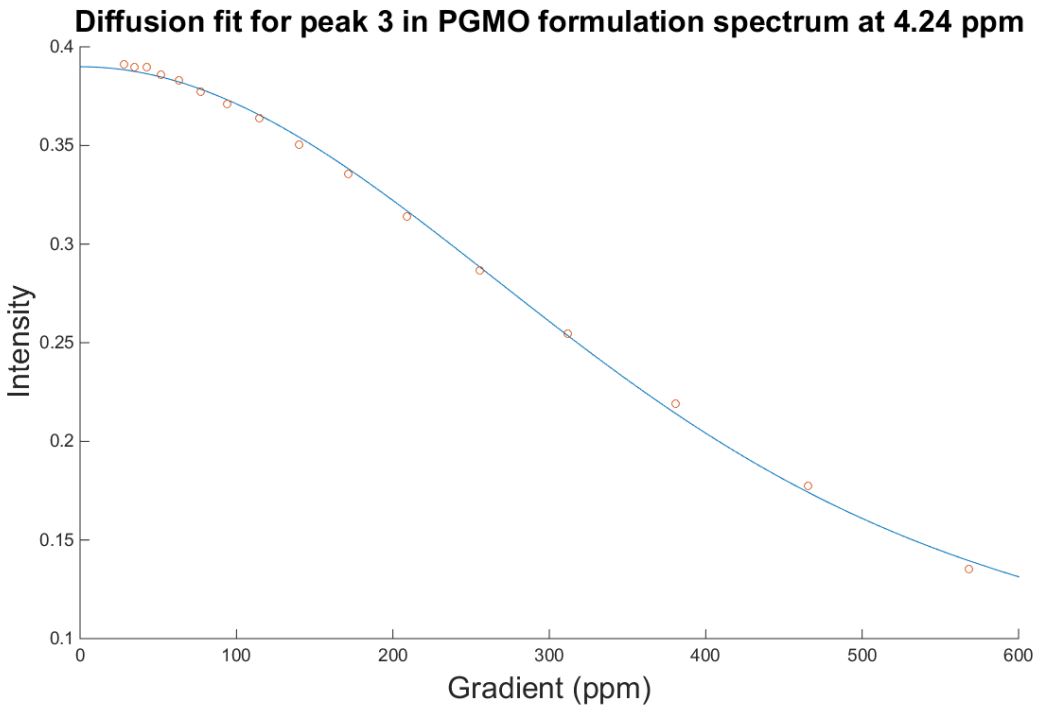

Figure 34. The PGMO sample seems to include various different compounds and a better fit is achieved with a biexponential equation.

When derived self-diffusion coefficients were evaluated for every significant peak in each sample it was found that no compound had a diffusion coefficient that was not an order of magnitude bigger than 2.299 $*10^{-9}$ m^2/s. Thus it is highly probable that the structure of the sample is not bicontinuous. Since the samples are isotropic the structures where the water is encapsueld are probably micelles.

Product behavior on gelatin interface

If the product was put on a gelatin surface, which is water rich, the product phase separated into two layers, one water rich and one oil rich layer. This somewhat resembles the conditions on the nasal mucosa.

If the GMO formulation is mixed with water, the temperature rises within the sample, as the GMO precipitates out of the solution.

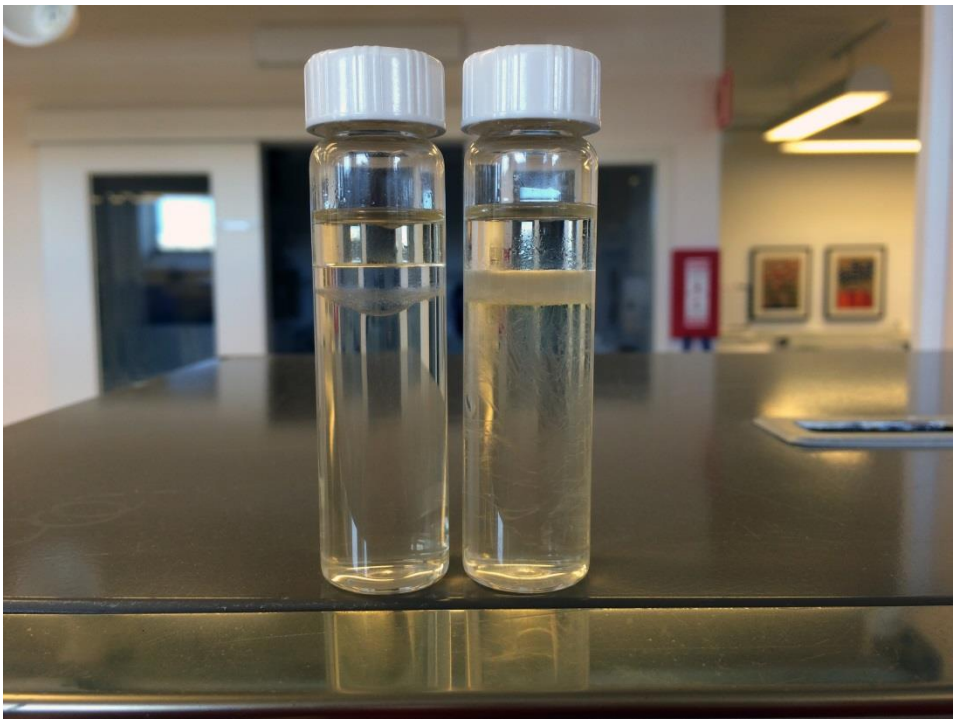

Figure 35. The formulations phase separates when put in a gelatine surface. A water rich phase forms closest the the gelatine surface and an oil rich phase floats on top.

NMR spectroscopy of phase separated layers

The ^{1}H spectra are presented in the Figures below:

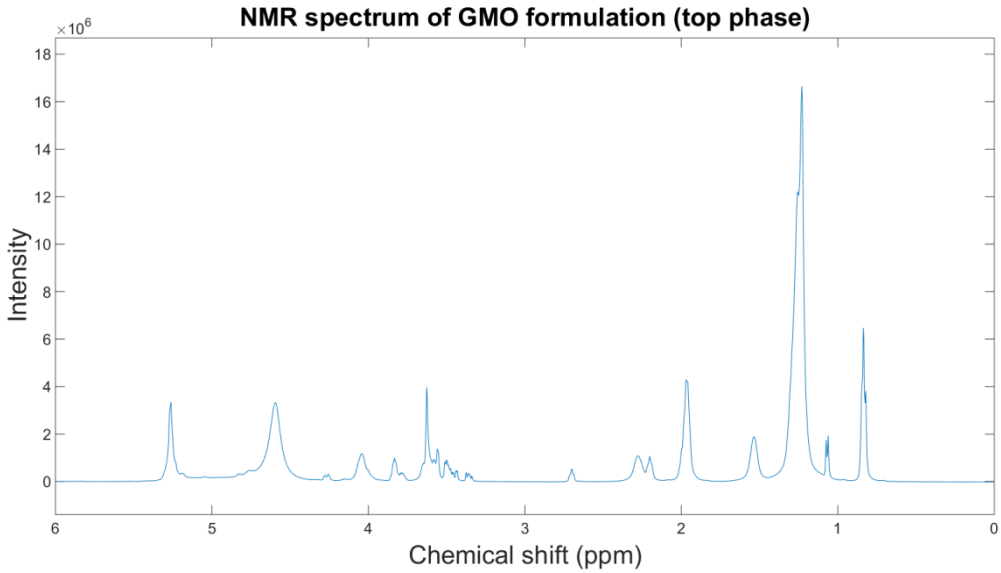

Figure 36. The figure shows a 1H NMR spectrum of the hydrophobic top phase of the GMO formulation.

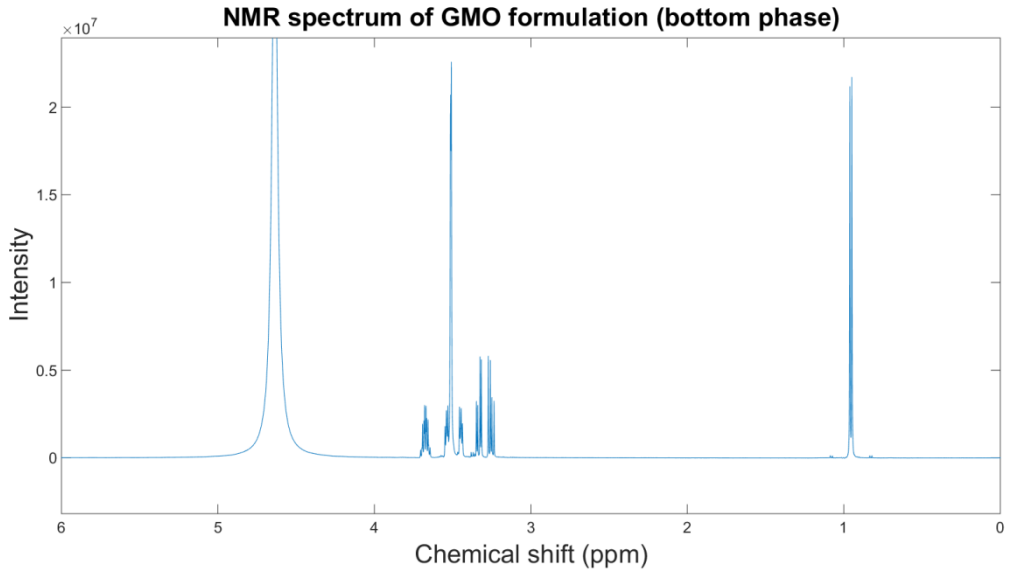

Figure 37. NMR spectrum of the hydrophilic bottom phase of the GMO formulation. The water peak at 4.7 ppm has been cut off.

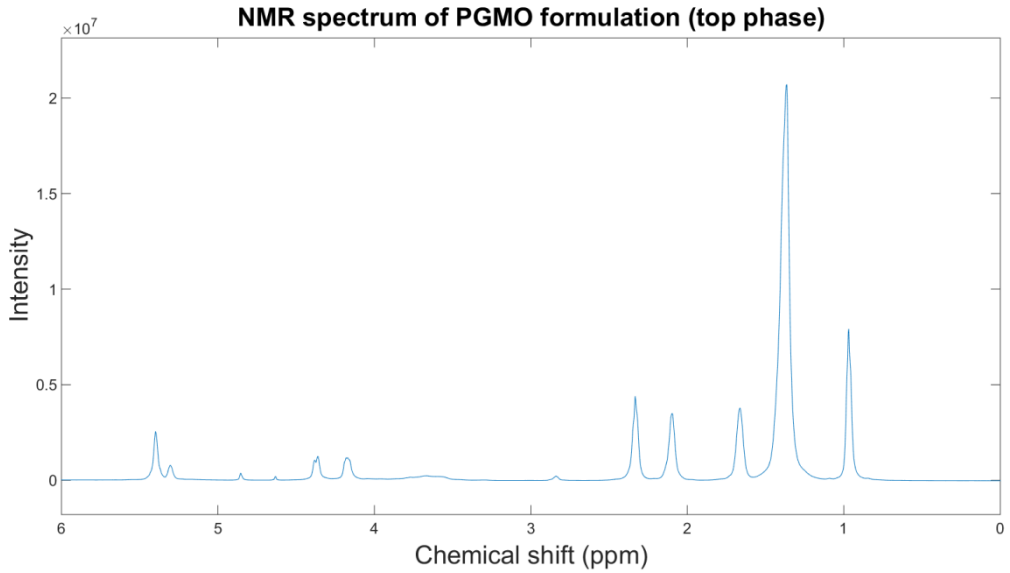

Figure 38. The figure shows a ^{1}H NMR spectrum of the hydrophobic top phase of the PGMO formulation.

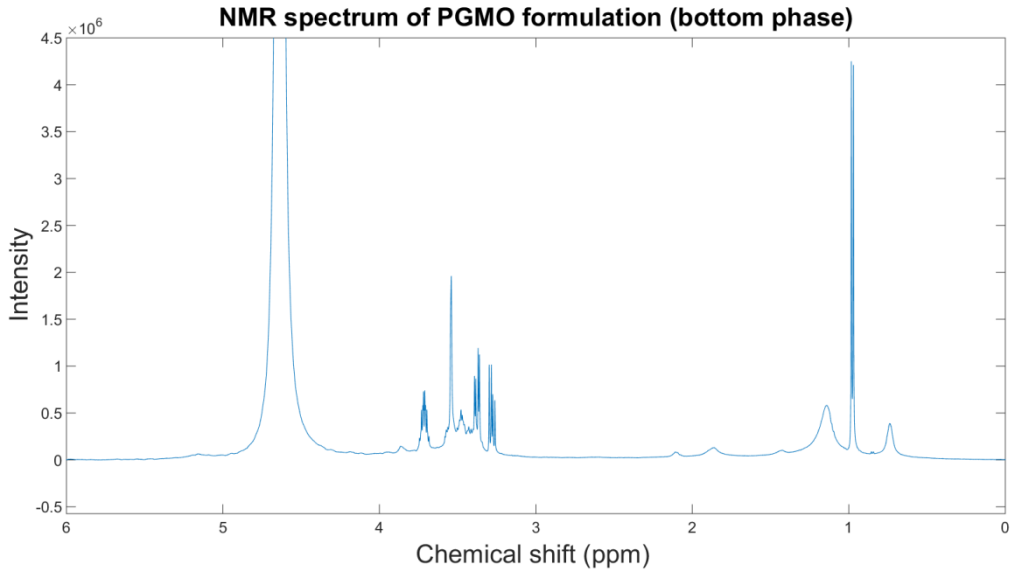

Figure 39. The figure shows a ^{1}H NMR spectrum of the hydrophilic top phase of the PGMO formulation. The water peak at 4.7 ppm has been cut off.

The spectra indicate that the top phases of both samples contain almost no water and consist mostly of hydrophobic compounds such as the olive, MCT and sesame oil. The bottom phase contains water, propylene glycol and polysorbate 80.

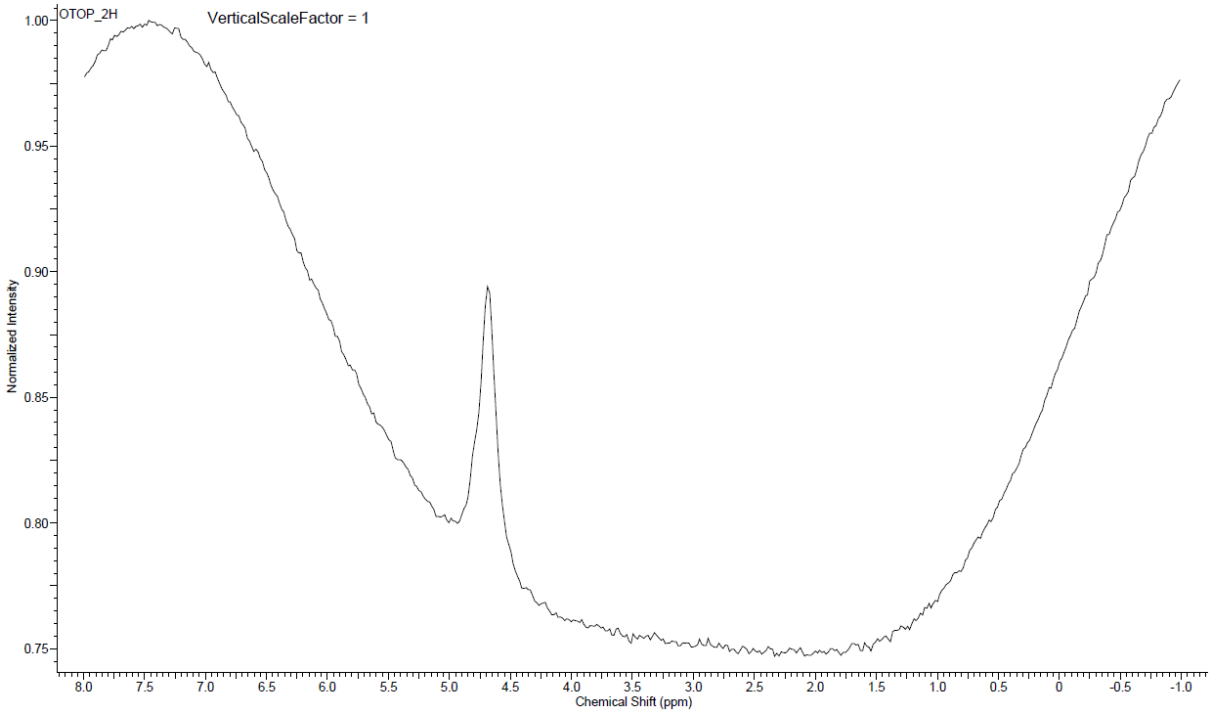

Figure 40. The figure shows the 2H spectrum of the GMO formulation top phase.

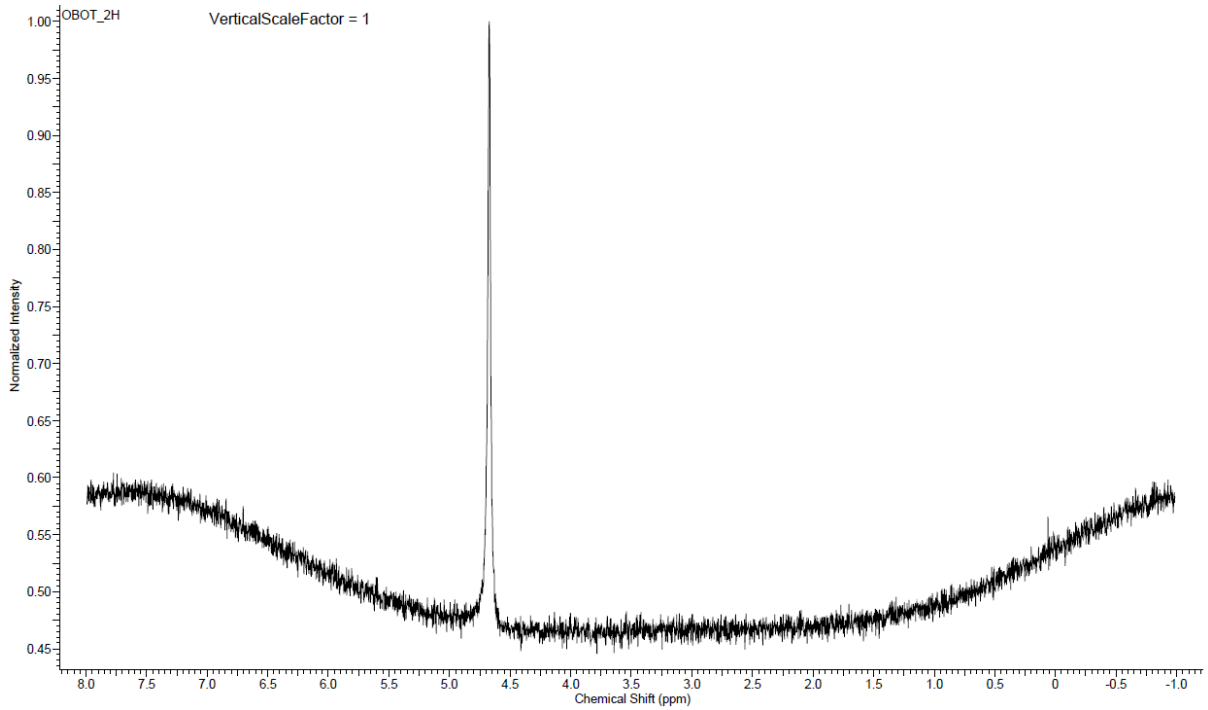

Figure 41. The figure shows the 2H spectrum of the GMO formulation bottom phase.

The hydrophobic top phase of the PGMO formulation could not dissolve the added deuterium. It became distributed into different compartments that gave rise to the peaks at varying ppm values in the spectrum since the D_2O encountered different chemical environments.

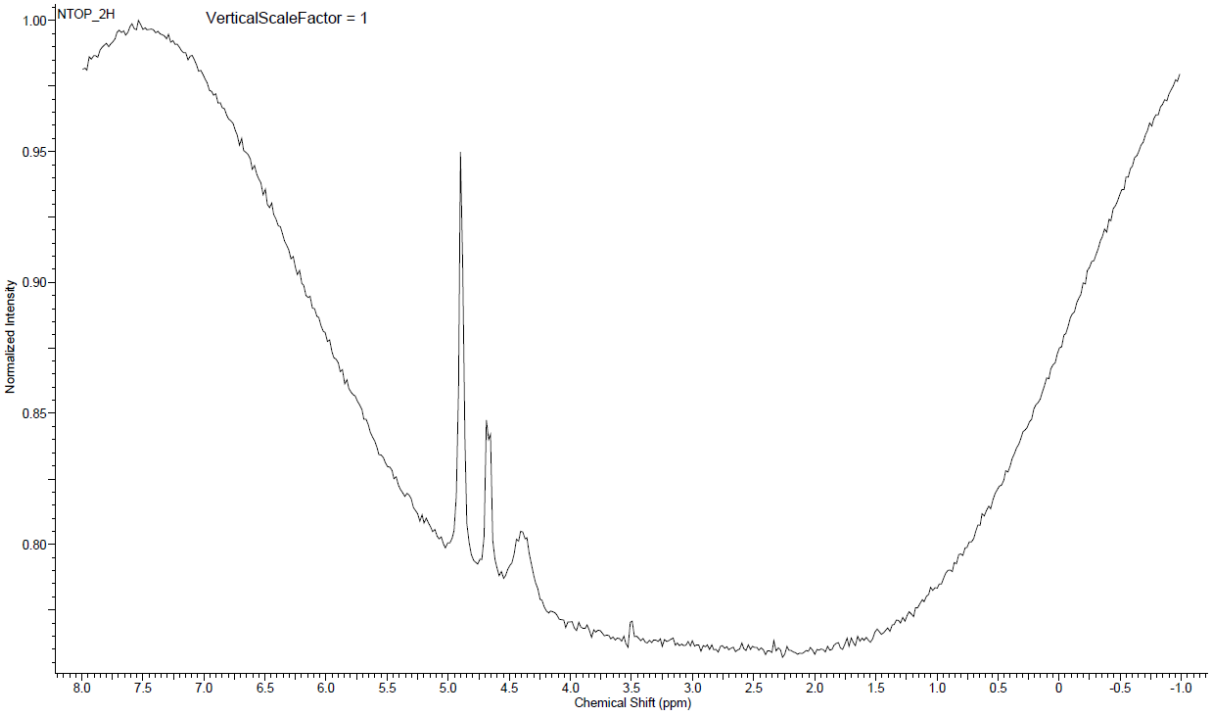

Figure 42. The figure shows the ^{2}H spectrum of the PGMO formulation top phase.

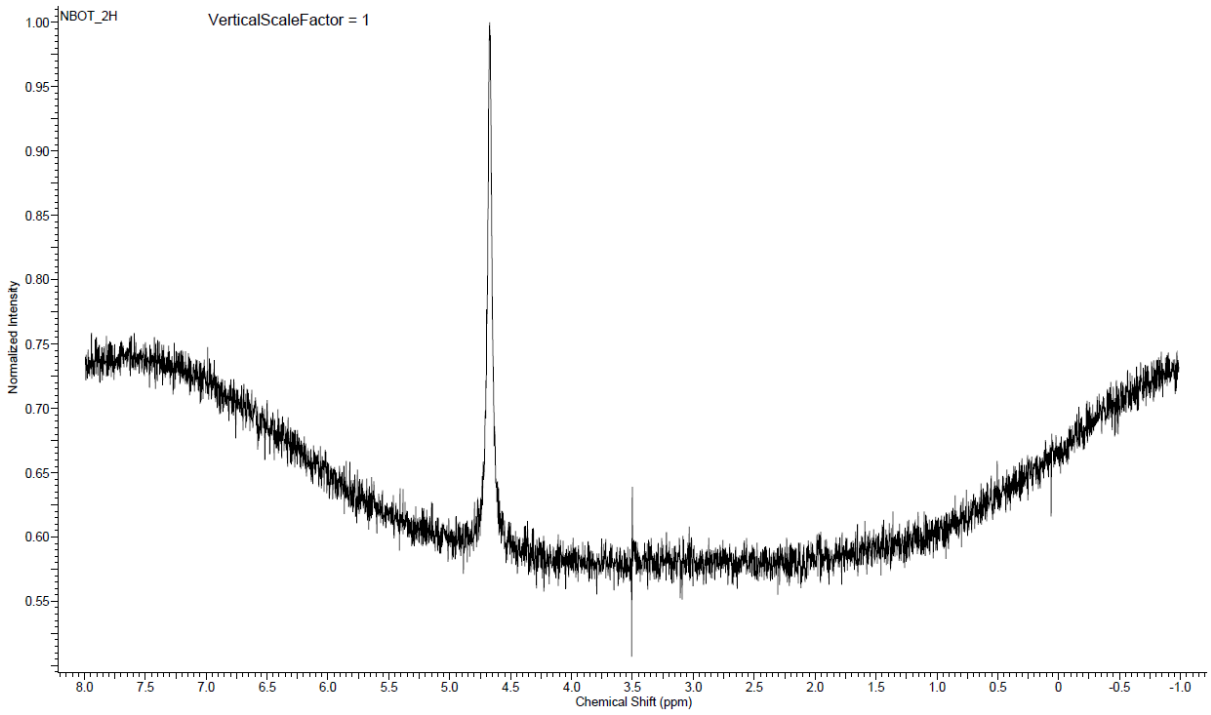

Figure 43. The figure shows the 2H spectrum of the PGMO formulation bottom phase.

When viewed in a microscope with a polarized light source, the bottom phase of the PGMO formulation shows anisotropic phase structure upon shearing, which disappears shortly afterwards. After less than one minute without stress the sample shows no anisotropic structure. Figure below shows the sample at 10x magnification. The white shining parts of the sample indicate that the structure is anisotropic.

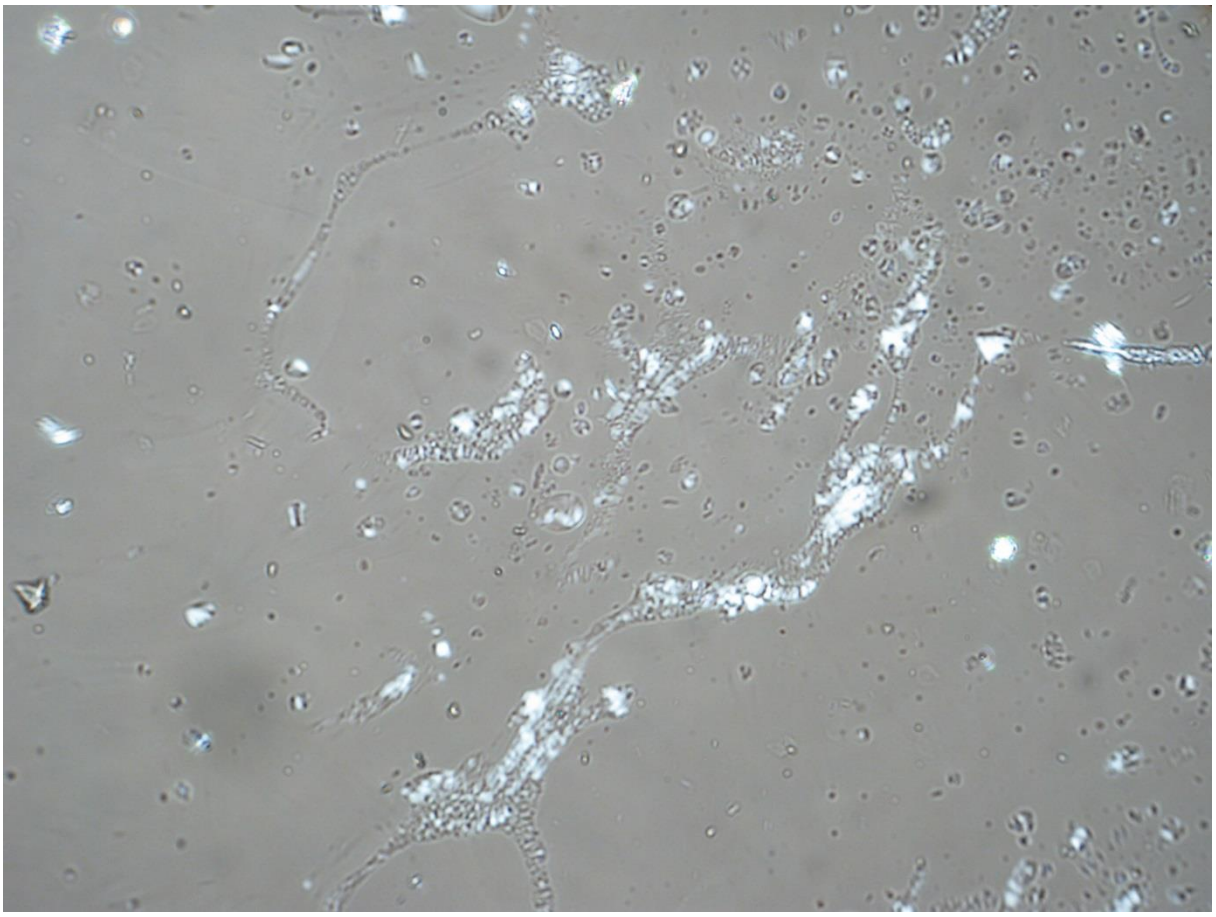

Figure 44. Picture of the PGMO formulation bottom phase sample viewed under polarized light at 10x magnification.

It appears that all the samples are isotropic and the bottom phase of the PGMO formulation becomes anisotropic for a short while upon shearing using a single stroke from a spatula.

Results from consumer study

The results from the consumer study can be found in table 7 below. A more detailed evaluation can be found in appendix 5.

Table 7. Summary of the results from the graded questions in the questionnaire handed out to the participants.

Question	Mean score of GMO formulation	Mean score of PGMO formulation
Ease of staring the pump (5 is the most difficult)	3.1	3.4
Ease of administering to nose (5 is the most difficult)	1.4	2
Beam shot feeling (5 is the most pleasant)	3.85	4.47
Product smell (5 is the most pleasant)	3.33	3.5
Product taste (5 is the most pleasant)	2.69	2.5
Irritating to nose (5 is the most irritating)	1.5	1.25
Irritating to throat (5 is the	2.0	1.1

most irritating)		
Caring to the nose (5 is the most caring feeling)	3.65	3.5
Barrier forming (5 is the strongest barrier feeling)	2.8	2.65
Dripping out of nose (5 is the most dripping out of nose)	2.9	2.55
Likelihood to start using (5 is the most likely to start using	2.3	2.5
Likelihood to recommend (5 is the most likely to recommend)	3.1	2.85

Most of the scores for the two formulations are fairly similar. The pump is more difficult to get going initially with the PGMO formulation compared to the GMO formulation. This is probably due to the PGMO formulation having a higher viscosity than the GMO formulation.

It appears that the problem with irritation in the throat has been solved with the PGMO formulation.

The barrier and caring feeling is less prevalent for the PGMO formulation. This might be because the total feeling of having a product in the nose is less prevalent with the PGMO formulation.

A slightly less amount of the PGMO formulation is dripping out of the nose compared to the GMO formulation. This is likely because the PGMO formulation has a higher viscosity than the GMO formulation.

Conclusions

The results indicate that the GMO and PGMO formulations have very similar physical chemical properties in most cases.

The problems with crystallization and phase separation at lower temperatures with the GMO formulation have been eliminated, probably due to the polydispersity of PGMO, which makes it a much less crystalline material that GMO. According to the product evaluation, the PGMO formulation is significantly less irritating to the throat compared to the GMO formulation. This is probably linked to the fact that it absorbs less water from the surrounding environment than the GMO formulation.

The PGMO formulation can tolerate a higher water content before phase separating than the GMO formulation.

The PGMO formulation has a higher viscosity, which in combination with the new five-hole pump leads to a difficulty in getting the pump to start. However it seems to lead to less dripping out from the nose.

Both products show quite strong mucoadhesion. The PGMO formulation shows slightly less spreadability on a hydrophilic surface. It spreads more slowly due to having a higher viscosity.

Future work

In the future it would be interesting to study for how long the nasal oils stay in the nose. For an effective product the duration should be at least a few hours. The turnover rate in the nose is only 10-15 minutes.

When put on a water-rich surface, the PGMO formulation phase separates, and a thick hydrophilic phase that becomes anisotropic upon shearing forms closest to the surface. It could be of interest to study how this affects staying power inside the nose.

The method for measuring mucoadhesion seems a bit unpredictable. A better method for measuring this property in vitro would be desirable.

Looking more specifically at what interactions constitute mucoadhesion could be of use when designing products that should adhere to a mucus rich surface.

Making a full clinical study where the effect against allergy and how the product is experienced is evaluated for the two formulations is a good way to verify that the physical chemical properties can translate to how the product is received by real consumers.

Acknowledgements

I would like to thank my Supervisor Birgitta Svensson for her support and guidance, all the personnel at Bioglan AB, Göran Carlström for helping me with the NMR spectroscopy experiments, Johan Engblom at Malmö Högskola for assisting me with the water activity measurements and Marie Wahlgren for showing me how to make mucoadhesion and surface tension measurements as well as giving valuable advice.

I would like to thank the 40 volunteers who tried the products out and filled out the questionnaire.

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Appendix

1. DSC run conditions

Table 8. Summary for the run configurations of the DSC measurements done at Bioglan AB.

Sample name	Cooling/heating rate	Sample weight	Temperature program
Blox4 GMO run 1	10 °C / min	9.94 mg	+40°C to -40 °C to +40 °C
Blox4 GMO run 2	10 °C / min	9.07 mg	+40°C to -40 °C to +40 °C
Blox4 GMO run 3	5 °C / min	11.19 mg	+40°C to -40 °C to +40 °C
Blox4 GMO run 4	5 °C / min	11.48 mg	+40°C to -40 °C to +40 °C
Blox4 GMO run 5	1 °C / min	12.33 mg	+40°C to -40 °C to +40 °C
Blox4 GMO run 6	1 °C / min	10.34 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 1	10 °C / min	11.73 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 2	10 °C / min	10.33 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 3	5 °C / min	12.34 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 4	5 °C / min	12.33 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 5	1 °C / min	11.28 mg	+40°C to -40 °C to +40 °C
Blox4 PGMO run 6	1 °C / min	11.21 mg	+40°C to -40 °C to +40 °C

Table 9. Summary for the run configurations of the DSC measurements done at LTH.

Sample name	Cooling/heating rate	Sample weight	Temperature program
GMO Blox4 test run 1	5 °C / min	7.06 mg	+40°C to -50 °C to +40 °C
GMO Blox4 test run 2	1 °C / min	7.06 mg	+40°C to -50 °C to +40 °C
PGMO Blox4 test run 1	5 °C / min	6.519 mg	+40°C to -50 °C to +40 °C
PGMO Blox4 test run 2	1 °C / min	5.874 mg	+40°C to -50 °C to +40 °C

2. Vegetable oil viscosity measurements

All the vegetable oil viscosity measurements show that the samples exhibit a Newtonian behavior.

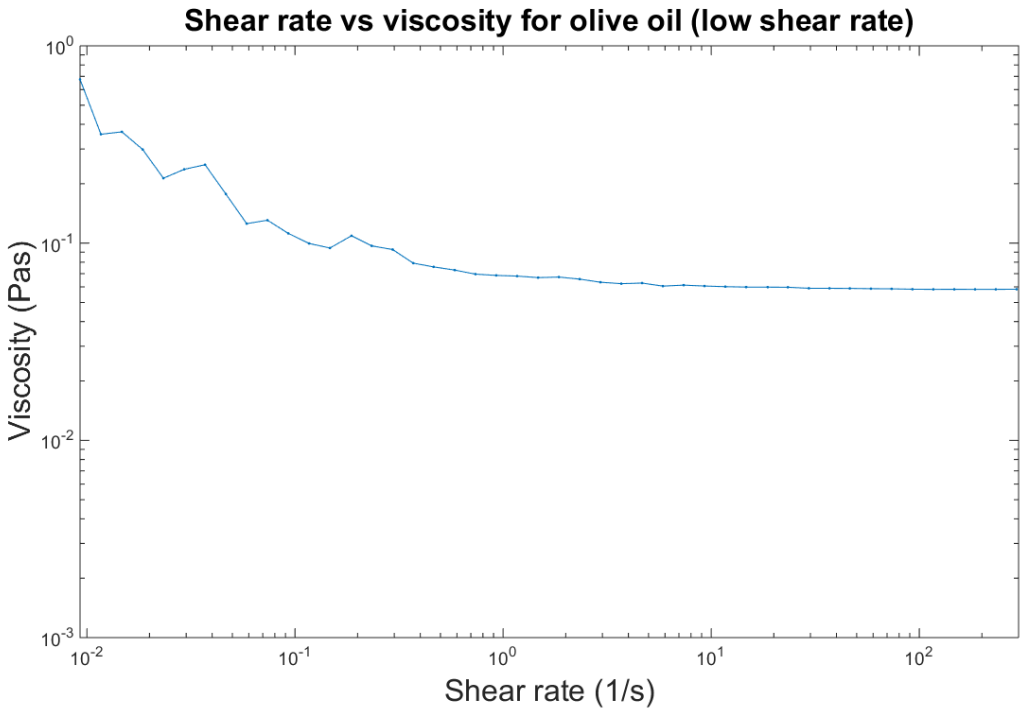

Figure 45. The figure shows the viscosity of olive oil as a function of shear rate at low shear rates.

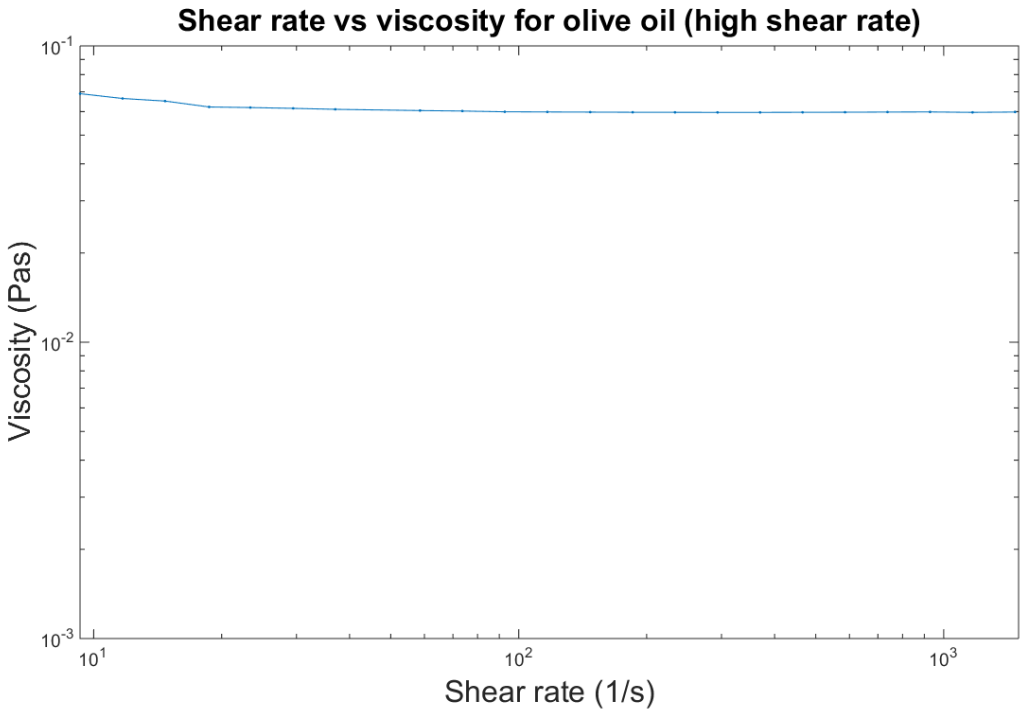

Figure 46. The figure shows the viscosity of olive oil as a function of shear rate at high shear rates.

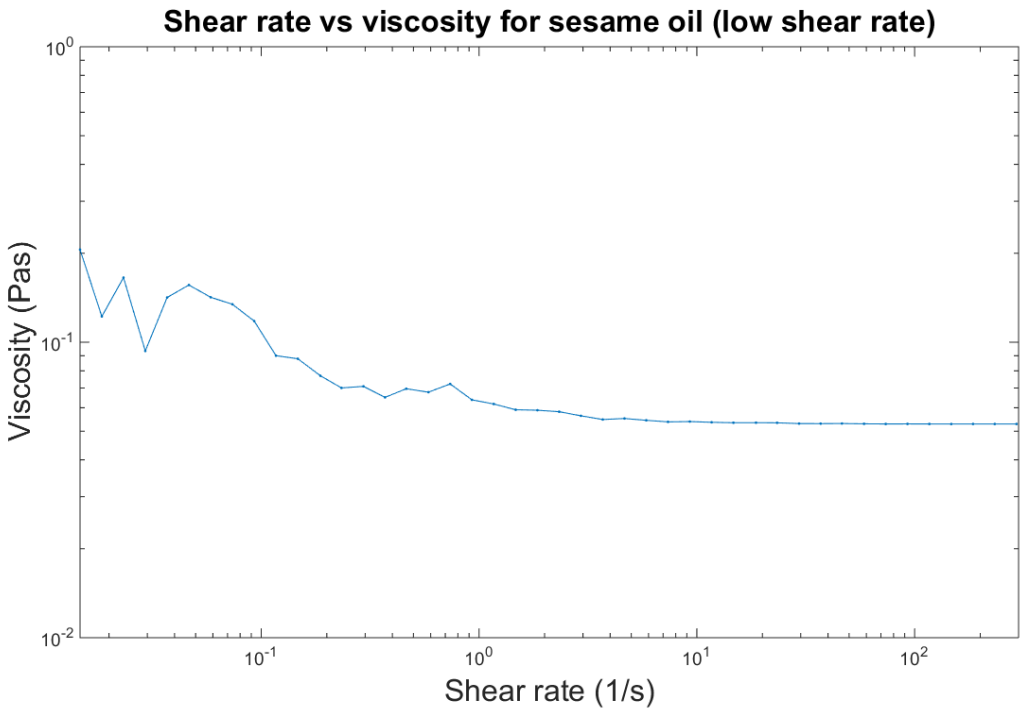

Figure 47. The figure shows the viscosity of sesame oil as a function of shear rate at low shear rates.

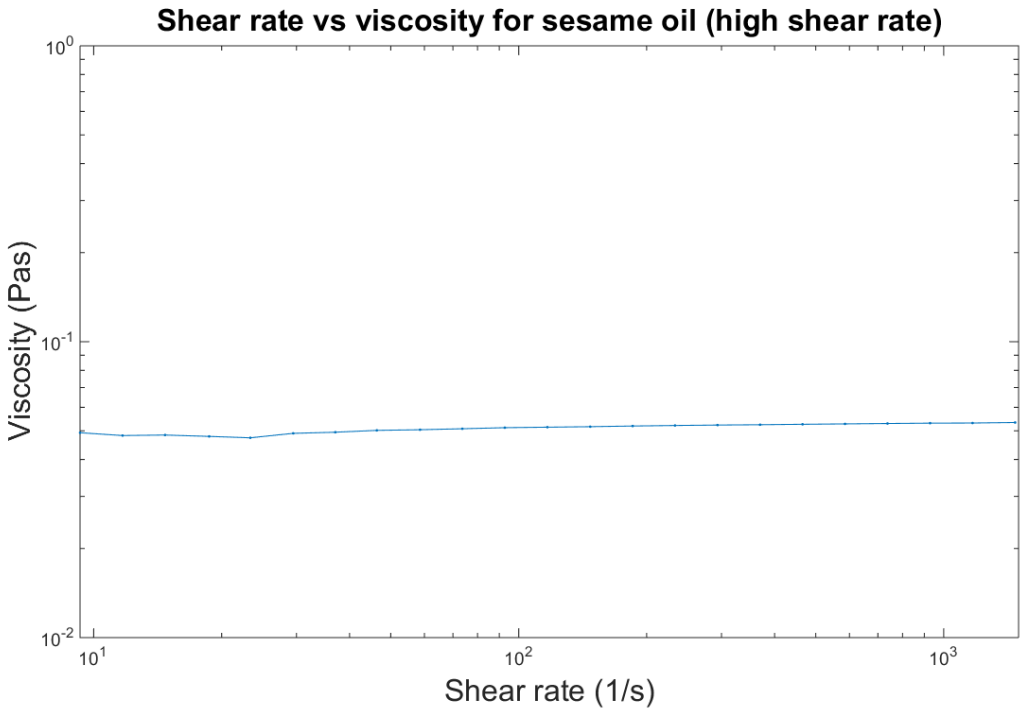

Figure 48. . The figure shows the viscosity of sesame oil as a function of shear rate at high shear rates.

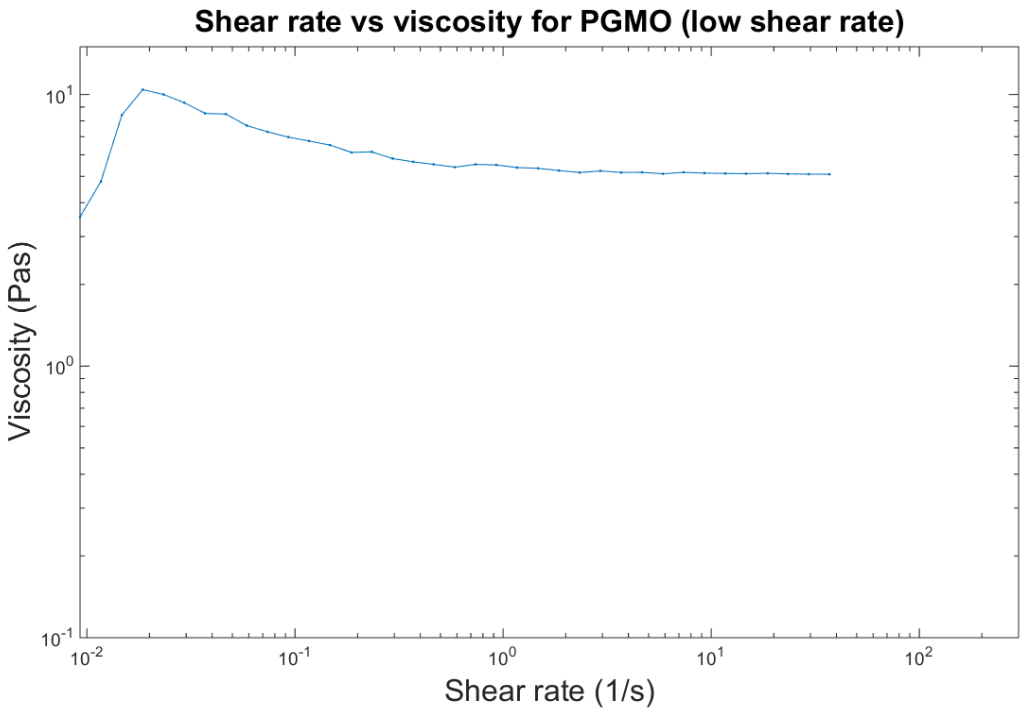

Figure 49. . The figure shows the viscosity of PGMO as a function of shear rate at low shear rates.

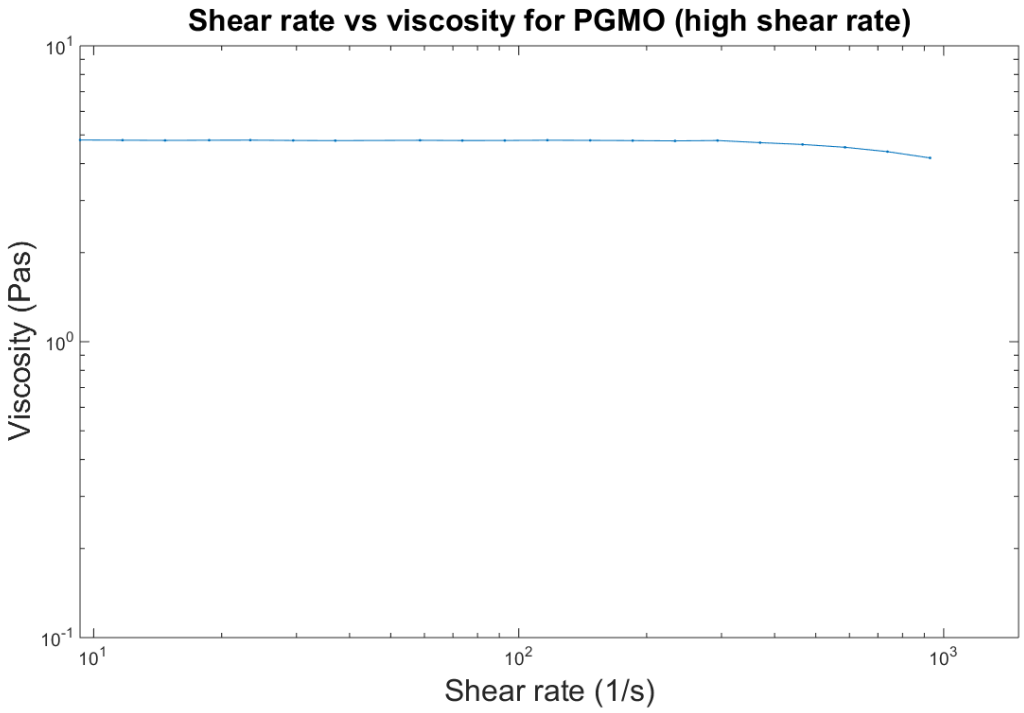

Figure 50. The figure shows the viscosity of PGMO as a function of shear rate at high shear rates.

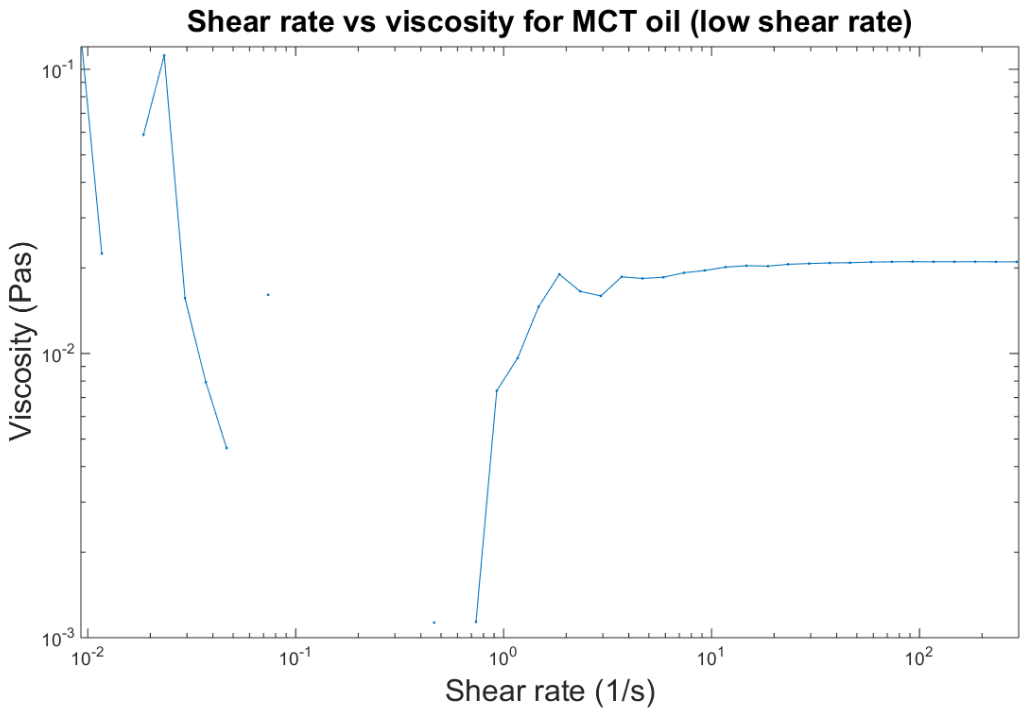

Figure 51. The figure shows the viscosity of MCT oil as a function of shear rate at low shear rates. The low points did not give a stable value due to the low viscosity of MCT oil. The missing points are due to negative received values, which have been discarded.

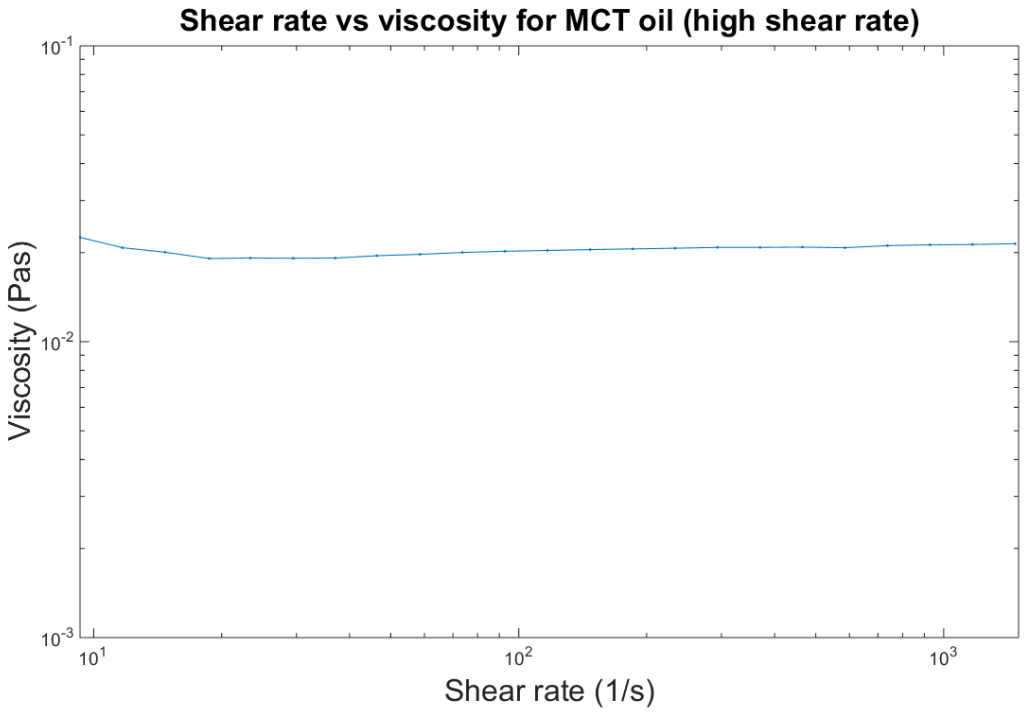

Figure 52. The figure shows the viscosity of MCT oil as a function of shear rate at high shear rates.

3. DSC of different glycerol monooleates

DSC runs were made on different types of glycerol monooleates to examine if a large crystallization peaks might lead to a product that is not as temperature stable. Below is a table that summarizes what compounds were tested and at what conditions. Depending on the state of the compounds at room temperature, the temperature program went either to 40 or 50°C.

Table 10. Summary of the run conditions for the DSC excipient analysis done at Bioglan AB.

Sample name	Cooling/heating rate	Sample weight	Temperature program
PGMO	5°C / min	10.50 mg	+40°C to -50°C to +40°C
GMO	5°C / min	11.79 mg	+50°C to -50°C to +50°C
PGMO3	5°C / min	9.12 mg	+40°C to -50°C to +40°C
PGMO2	5°C / min	13.42 mg	+50°C to -50°C to +50°C
PGMO2g	5°C / min	10.04 mg	+50°C to -50°C to +50°C
PGMO4	5°C / min	9.79 mg	+40°C to -50°C to +40°C

Below are figures that display the results from the DSC measurements.

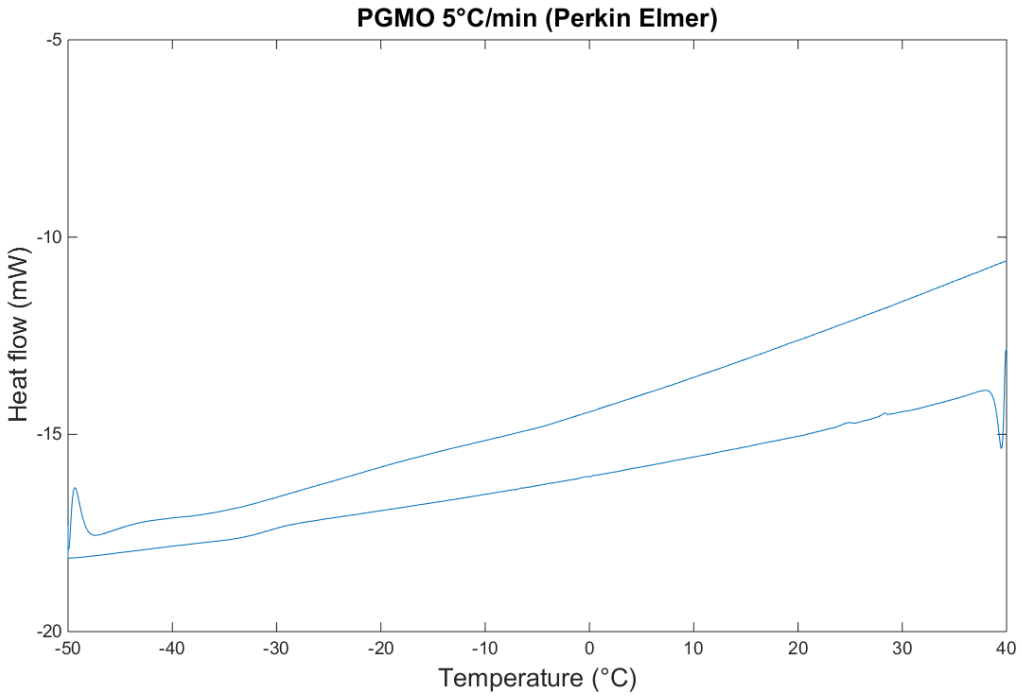

Figure 53. Plot of DSC data from the PGMO measurement, with a scan rate of 5°C/min, done at Bioglan AB.

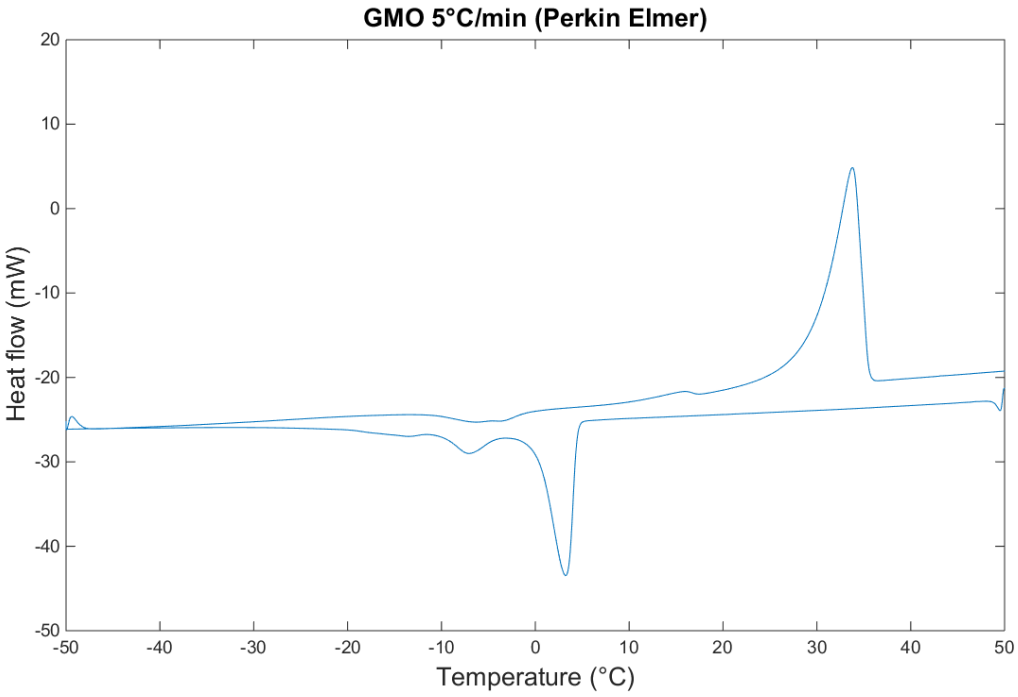

Figure 54. Plot of DSC data from the GMO measurement, with a scan rate of 5°C/min, done at Bioglan AB.

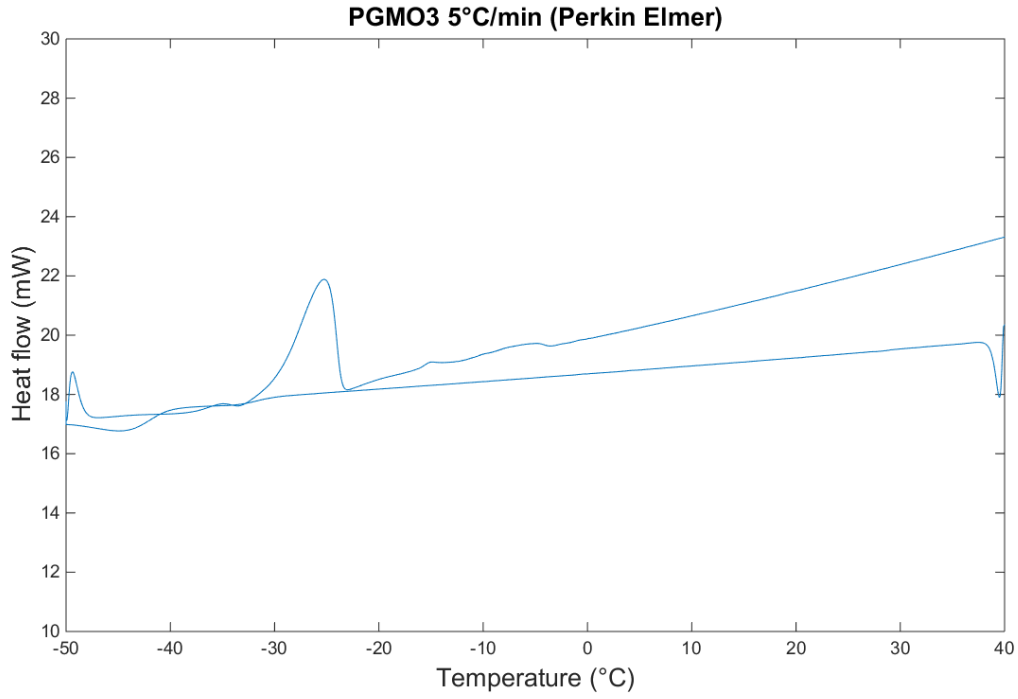

Figure 55. Plot of DSC data from the PGMO3 measurement, with a scan rate of 5°C/min, done at Bioglan AB.

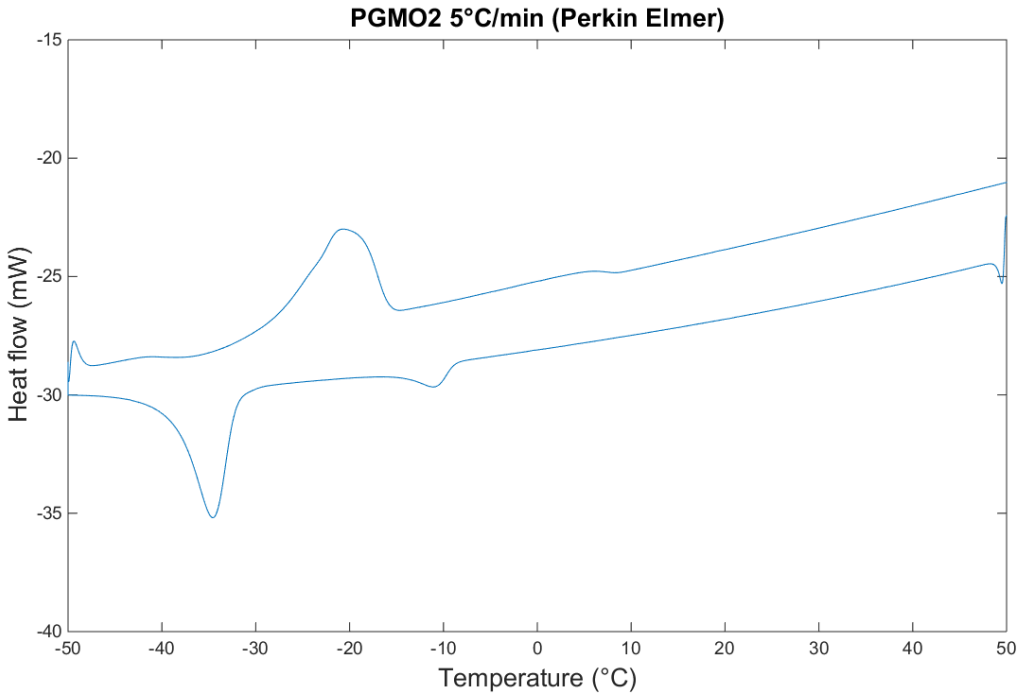

Figure 56. Plot of DSC data from the PGMO2 measurement, with a scan rate of 5°C/min, done at Bioglan AB.

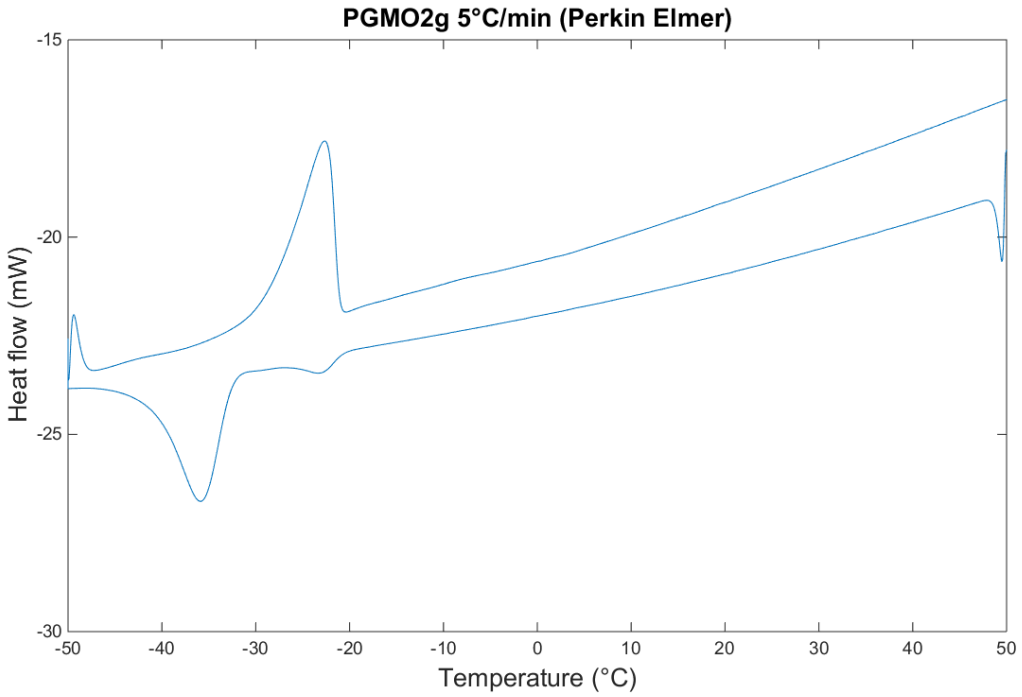

Figure 57. Plot of DSC data from the PGMO2g measurement, with a scan rate of 5°C/min, done at Bioglan AB.

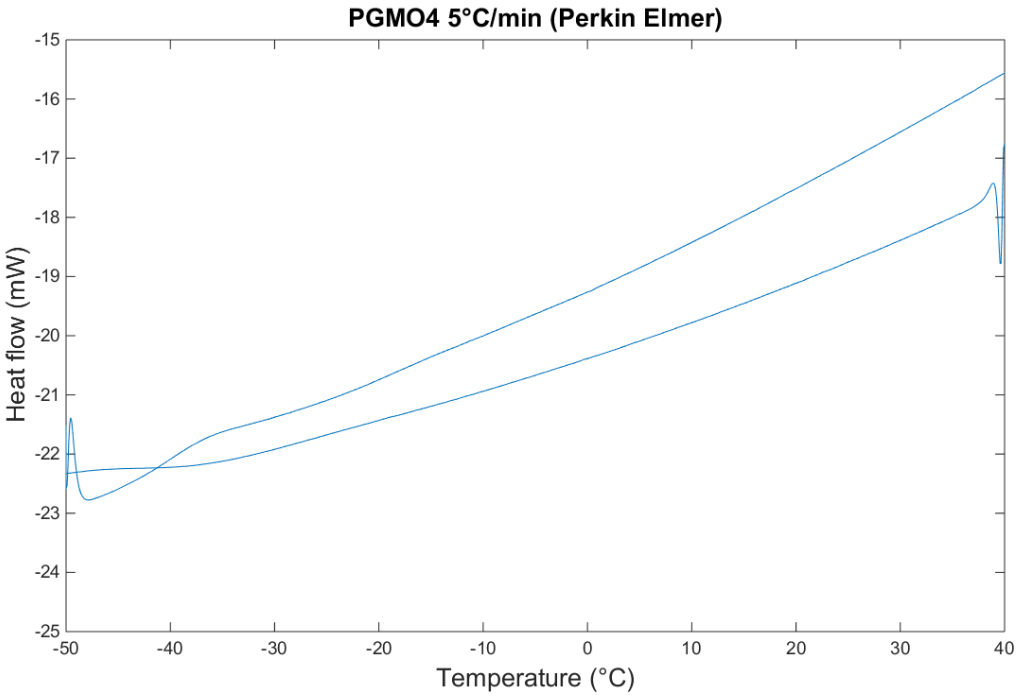

Figure 58. Plot of DSC data from PGMO4 measurement, with a scan rate of 5°C/min, done at Bioglan AB.

It appears that the variants with many glycerol groups attached to the carbohydrate chain make the compounds less liable to crystallize. PGMO and Hexaglyn 1-0V show no clear peaks from -50 to 40°C.

The endset temperatures for the peak occurring at the highest temperature can be seen in Table X below.

Table 11. Endset temperature for different glycerol monooleate variants.

PGMO	No clear peaks
GMO	35.506°C
PGMO3	-23.6°C
PGMO	-15.9°C
PGMO2g	-21.1°C
PGMO4	No clear peaks

4. Consumer study questionnaire

A product evaluation was performed with 40 participants. They received one of the formulations without knowing any details about what variant they were given. Below is the questionnaire that was handed out to each participant in the product evaluation together with a vial:

Nasal product evaluation

Welcome to a product evaluation of a nasal liquid that is used to form a barrier on the nasal mucosa to protect against pollen and house dust mites as well as to moisturize a dry nose. Please read the instructions and fill in the questionnaire.

First, fill in some background information about yourself:

Sex:

◯ Male

◯ Female

◯ Other:_____

Age: _____ years

Do you suffer from eucalyptus or menthol allergy? If so it is unsuitable to participate in this study.

Do you suffer from any allergies (if so, what allergies)?

Do you use nasal products at other times (If so, what kind and how often)?

Have you ever experienced problems (not caused by allergies or having a cold) with your nasal mucosa due to having a dry nose (dryness, itching and/or irritation in the nose)?

◯ Never

◯ Sometimes (when?)

_____

◯ Often (when?)

_____

Have you ever thought about the concept of dry nose symptoms before?

◯ Yes

◯ No

Now it is time for the practical evaluation to start!

Ensure that your nostrils are clear. If not, blow your nose.

Keep the nasal product vial upright.

Pump a few times into a paper or in the hand.

Now take the nasal product, after you have ensured that it is pumping out a consistent amount of product, aim diagonally up the nostrils and pump once in each nostril. Try to avoid sniffing! Press gently on the outside of the nostril with your hand if it feels better.

With the product in your nose, wait for ten minutes and then fill in the questionnaire by circling the number that corresponds to your experience of the product.

How easy was it to pump compared to other nasal products you have tried?

(Easy) 1 2 3 4 5 (Difficult)

How easy was it to administer to the nose?

(Easy) 1 2 3 4 5 (Difficult)

How did the product beam shot feel?

(Unpleasant) 1 2 3 4 5 (Pleasant)

Can you smell the product?

◯ No, I can't

◯ Yes, and it is:

(Unpleasant) 1 2 3 4 5 (Pleasant)

And it smells like:_____

Can you taste the product?

◯ No, I can't

◯ Yes, and it is:

(Unpleasant) 1 2 3 4 5 (Pleasant)

And it tastes like:_____

Do you feel any sensation in your nose from the product?

◯ No, I don't

◯ Yes

It feels like: _____

Does the product feel irritating in the nose?

(No irritation) 1 2 3 4 5 (Strong irritation)

Does the product feel irritating in the throat?

(No irritation) 1 2 3 4 5 (Strong irritation)

Do you experience the product as caring to a dry mucosa?

(Not caring at all) 1 2 3 4 5 (Very caring)

Do you experience the product as forming a protective barrier in the nose?

(No barrier feeling) 1 2 3 4 5 (Strong barrier feeling)

Is the product leaving the nostrils (dripping out of your nose)?

(Not leaving the nose at all) 1 2 3 4 5 (A lot of product dripping out)

Would you consider starting to use this product?

(Unlikely) 1 2 3 4 5 (Likely)

If you do, in what circumstances?

_____

_____

Would you recommend this product to someone else?

(Unlikely) 1 2 3 4 5 (Likely)

Why/why not?

_____

_____

Good job answering the questions!

If you have any comments you are very welcome to write them down below. We really appreciate your input.

_____

_____

_____

_____

_____

5. Assessment of product evaluation answers

The answers are presented in the figures below. In all the diagrams in this section, the Y axis represents the number of people and the X axis the answer to the question or a grade from 1 to 5.

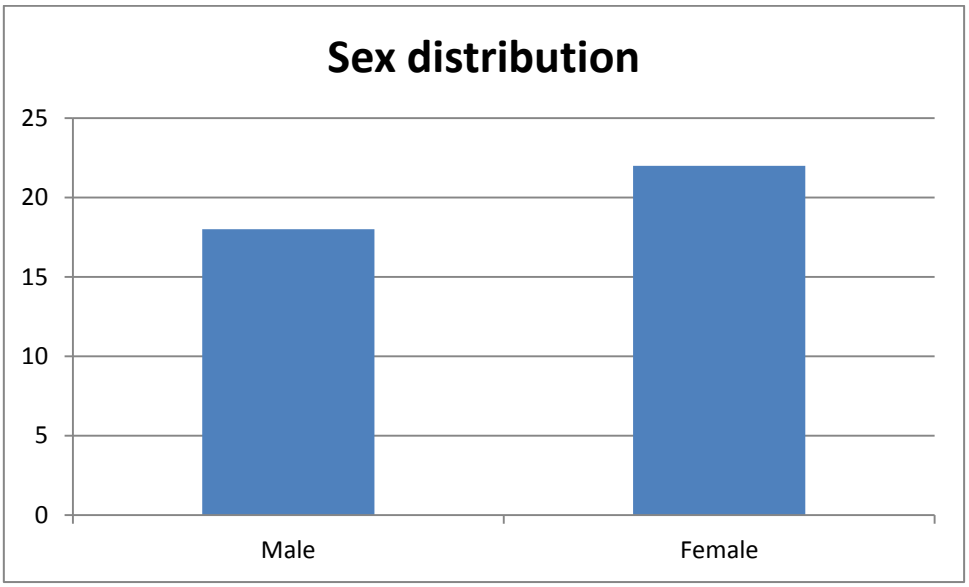

Figure 59. The diagram shows the sex distribution of the participants.

Most of the people participating in the evaluation were students aged 18-25. Among elderly it was more common to having heard about or suffering from a dry nasal mucosa.

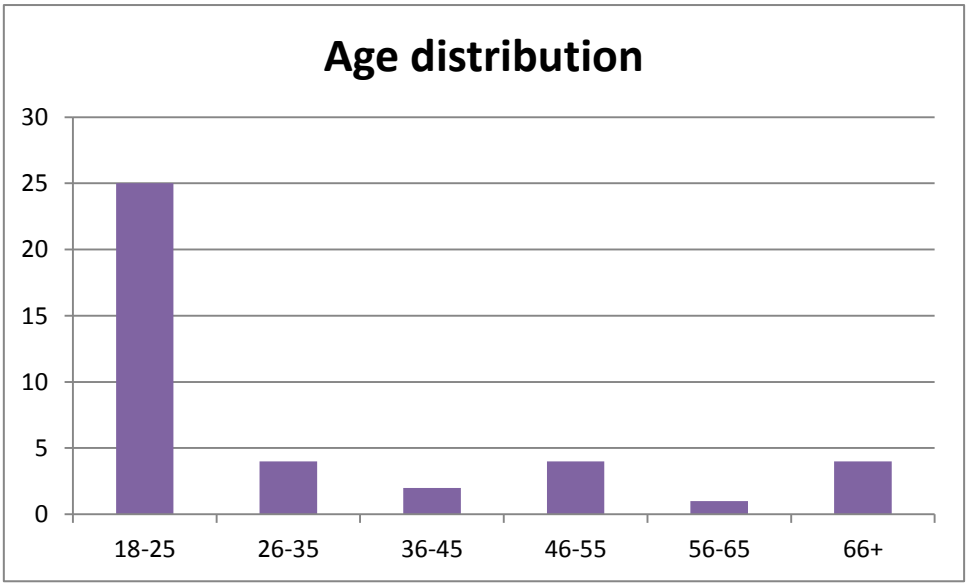

Figure 60. The diagram shows the age distribution of the participants.

Many of the participants used nasal products as illustrated in the diagram below.

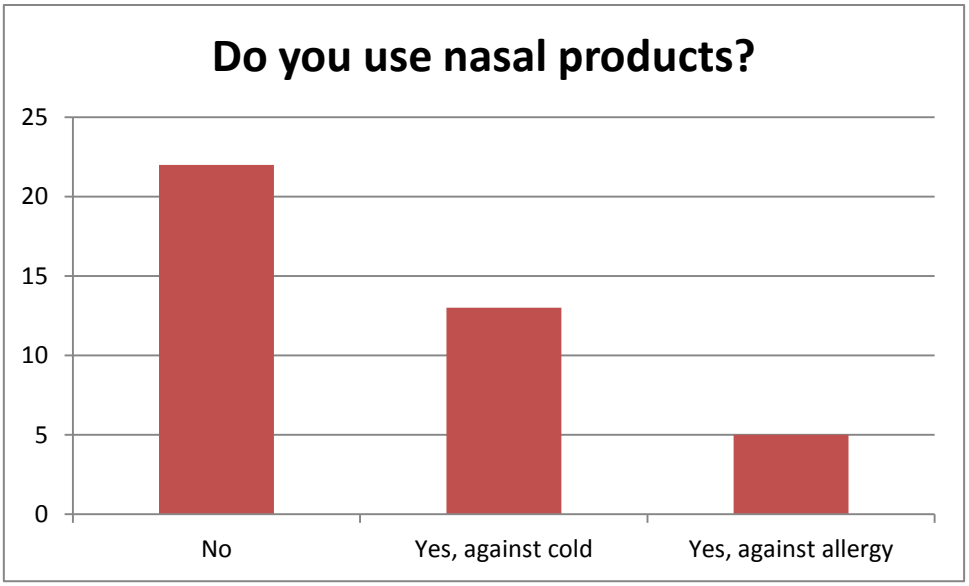

Figure 61. The diagram shows how many of the participants uses nasal products.

45 % of the participants suffered from allergies caused by pollen or animals.

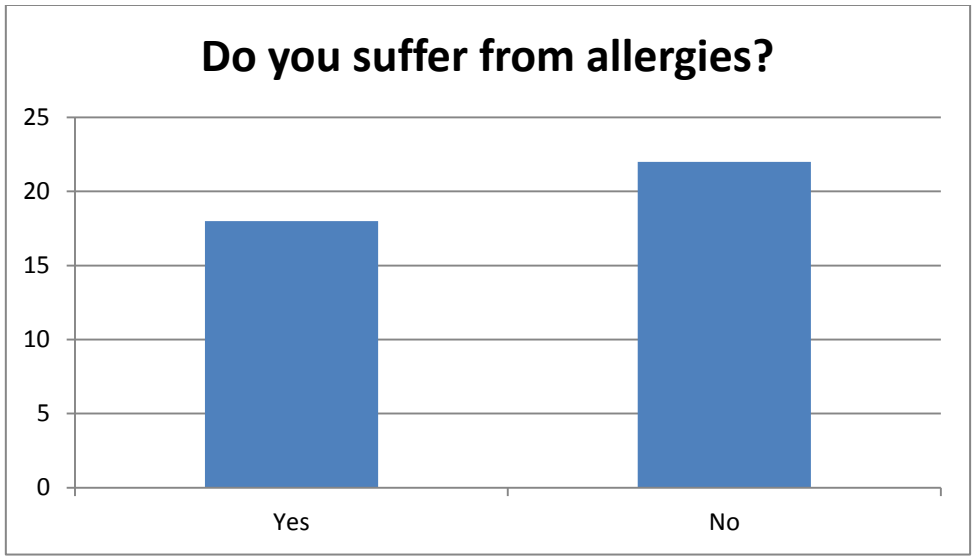

Figure 62. The diagram shows how many of the participants that suffer from allergies.

Most participants had never thought about the concept of a dry nose before. Only 28 percent had thought about it.

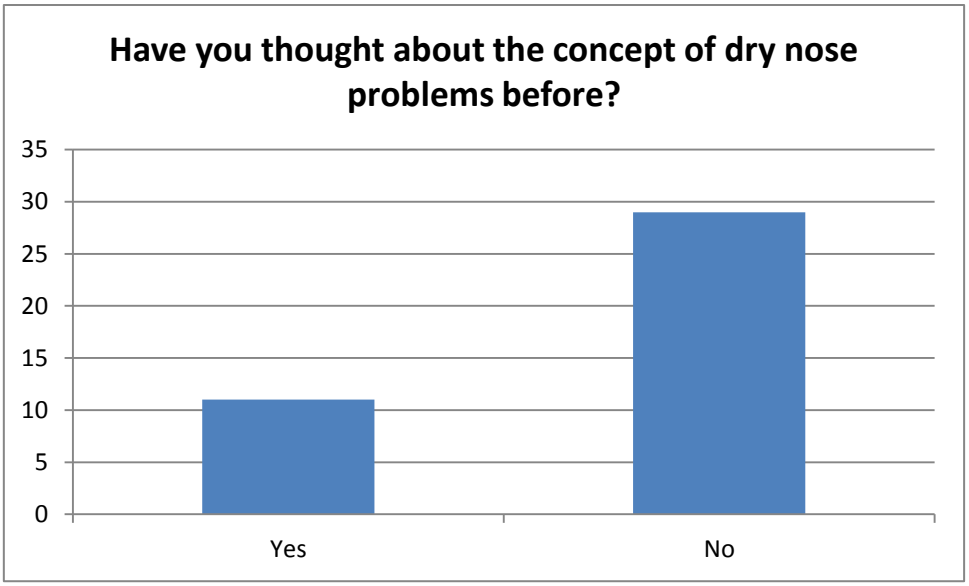

Figure 63. The diagram shows how many people have thought about the concept of dry nose problems before.

In some cases it was so hard to get the PGMO formulation pump going that the participants gave up thinking they had gotten a faulty device.

The participants started the pumps by spraying a few times into a paper and then judging how difficult it was to get the pump started. A higher number means more difficult. The mean score for the GMO formulation in the evalutation was 3.1.

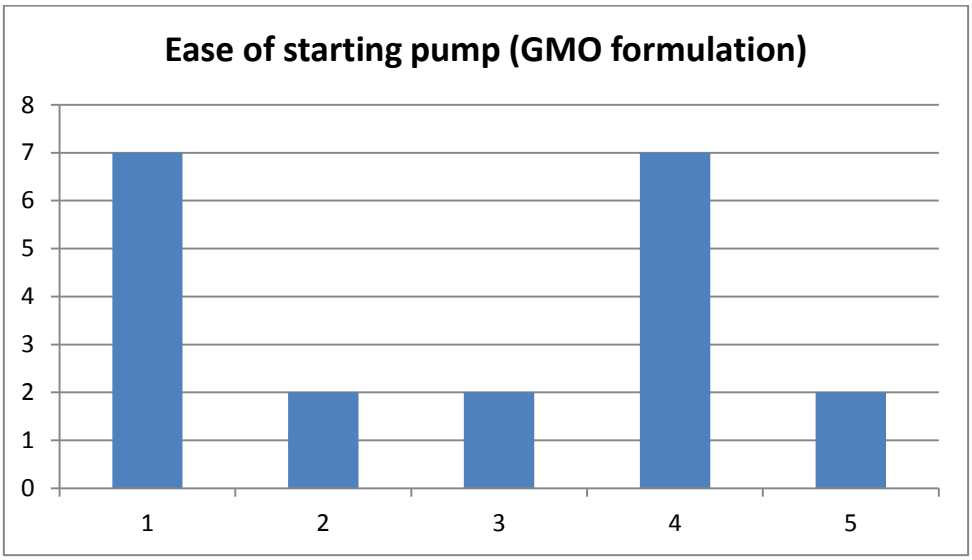

Figure 64. The diagram shows how difficult it was to get the pump started.

The mean score for starting the PGMO formulation pump was 3.4.

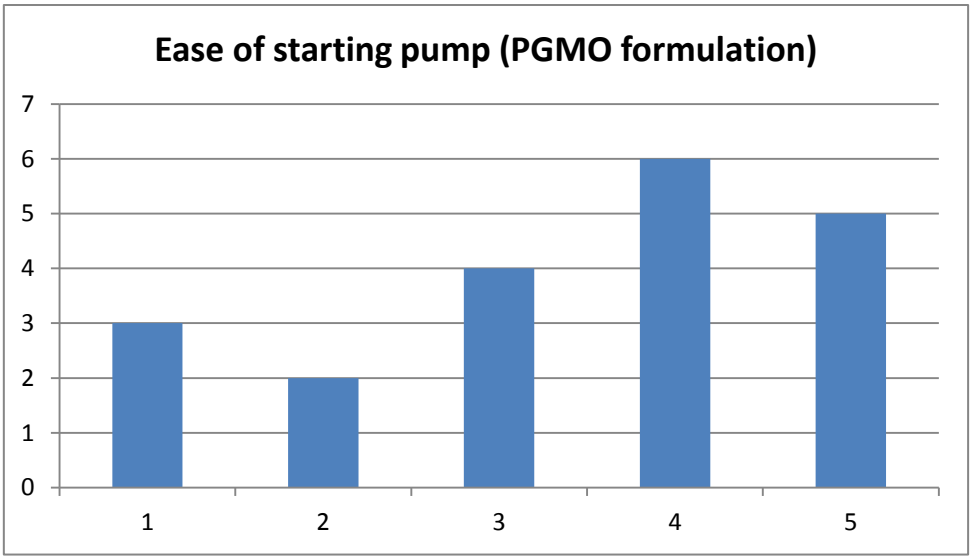

Figure 65. The diagram shows how difficult it was to get the pump started

Ease of administering the product to the nose was not a clear question according to the participants. The figure below shows how easy it was to administer the product to the nose. 5 is the most difficult. The mean score for the GMO formulation was 1.4.

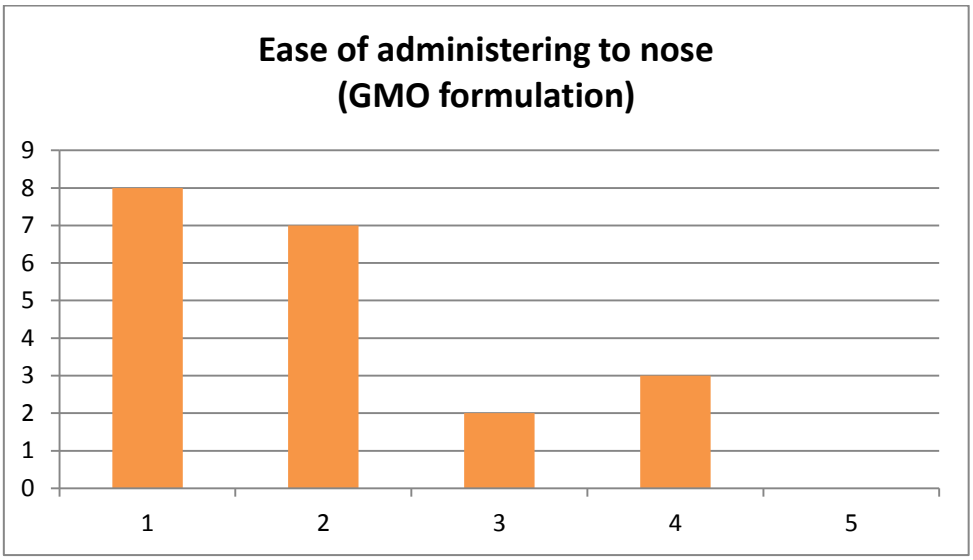

Figure 66. The diagram shows how difficult it was to administer the GMO formulation to the nose.

The mean score for ease of administering the PGMO formulation to the nose was 2.0.

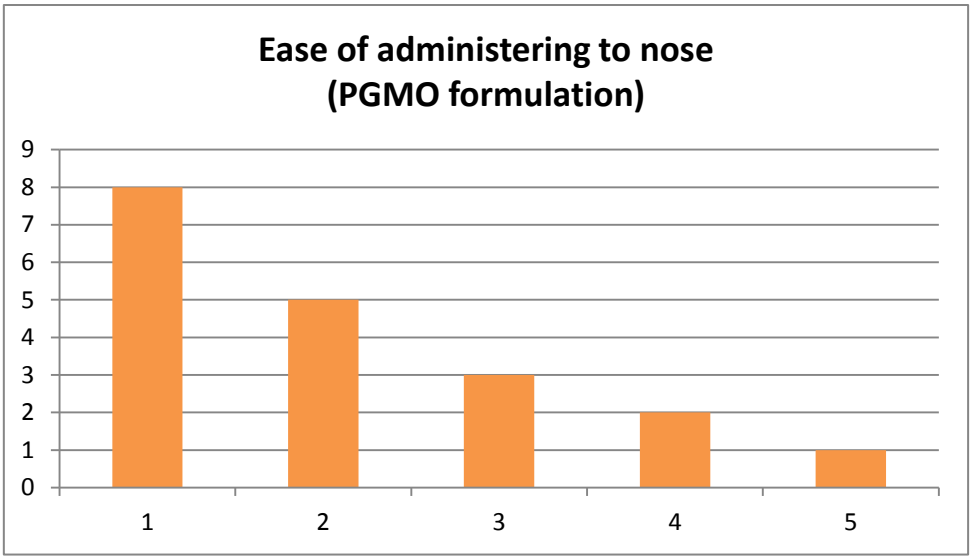

Figure 67. The diagram shows how difficult it was to administer the PGMO formulation to the nose.

The participants were asked how the beam shot felt. The GMO formulation nozzle had one centered hole giving a consistent hard jet. The PGMO formulation nozzle had five small holes leading to a more dispersed shot. The mean score for the GMO formulation beam shot feeling was 3.9.

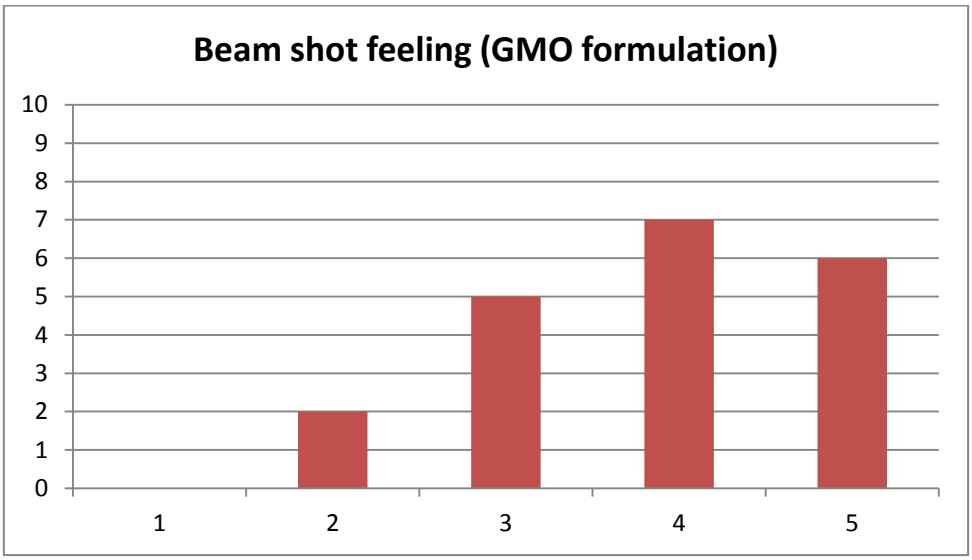

Figure 68. The diagram shows how the participants experienced the beam shot feeling of the GMO formulation.

The mean score for the PGMO formulation was 4.5.

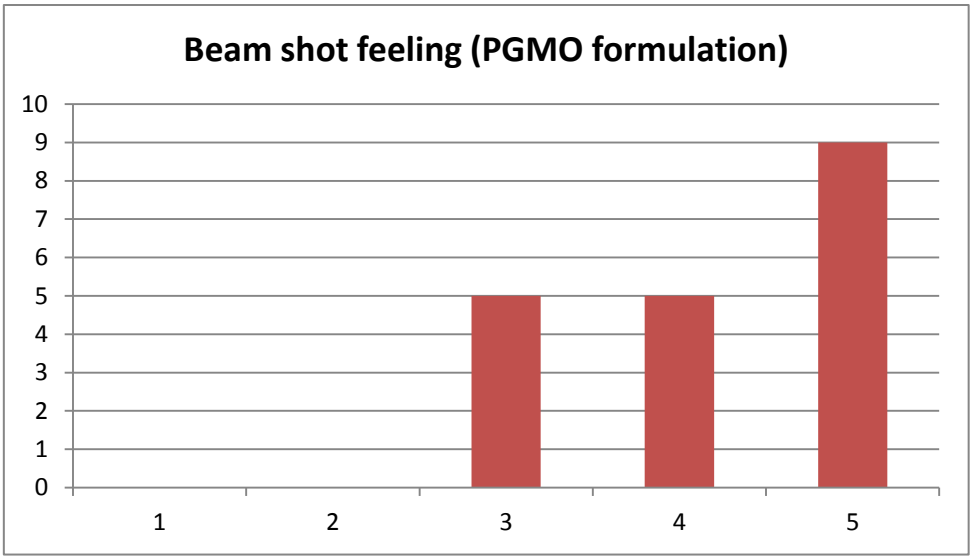

Figure 69. The diagram shows how the participants experienced the beam shot feeling of the PGMO formulation.

The participants were asked if they could sense any smell from the nasal oil. 75 % of the participants could smell the GMO formulation. 30 % of the participants could smell the PGMO formulation.

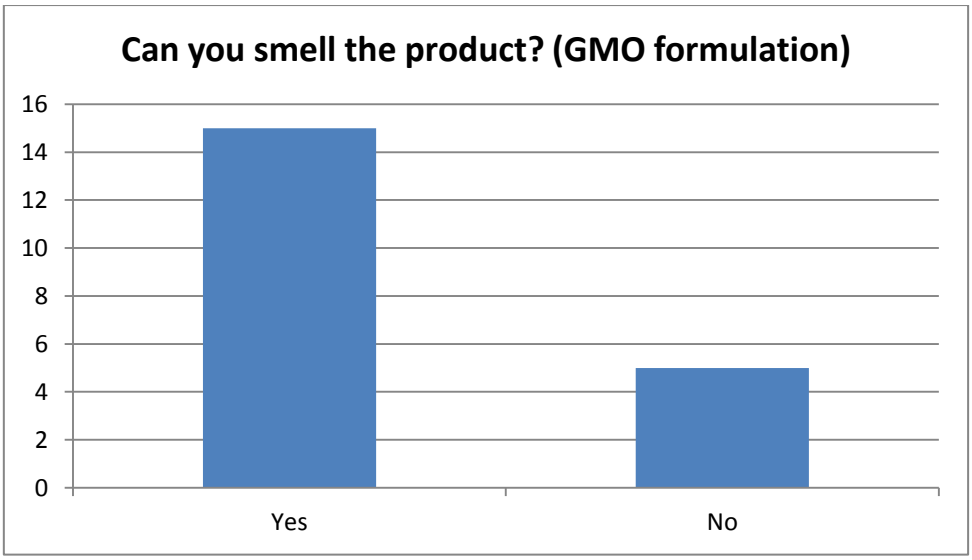

Figure 70. The diagram shows how many of the participants could smell the GMO formulation.

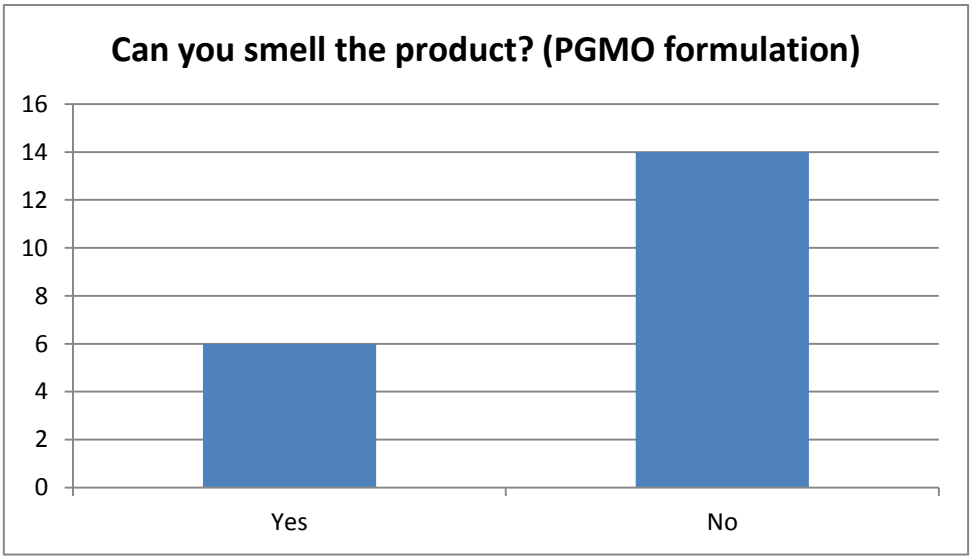

Figure 71. The diagram shows how many of the participants could smell the GMO formulation.

The participants were asked how they experienced the smell. The GMO formulation smelt like linseed oil, lavender, menthol, eucalyptus, woody, härsken symaskinsolja.

When asked how pleasant the smell was the participants rated the GMO formulation smell as 3.33 where 5 is the most pleasant. The PGMO formulation was rated with the score 3.50.

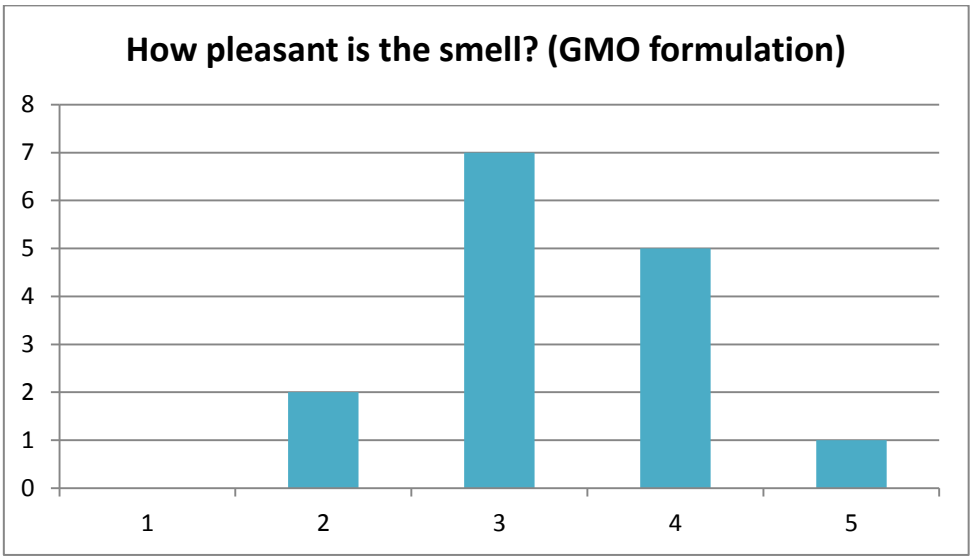

Figure 72. The diagram illustrates how pleasant the smell of the GMO formulation was.

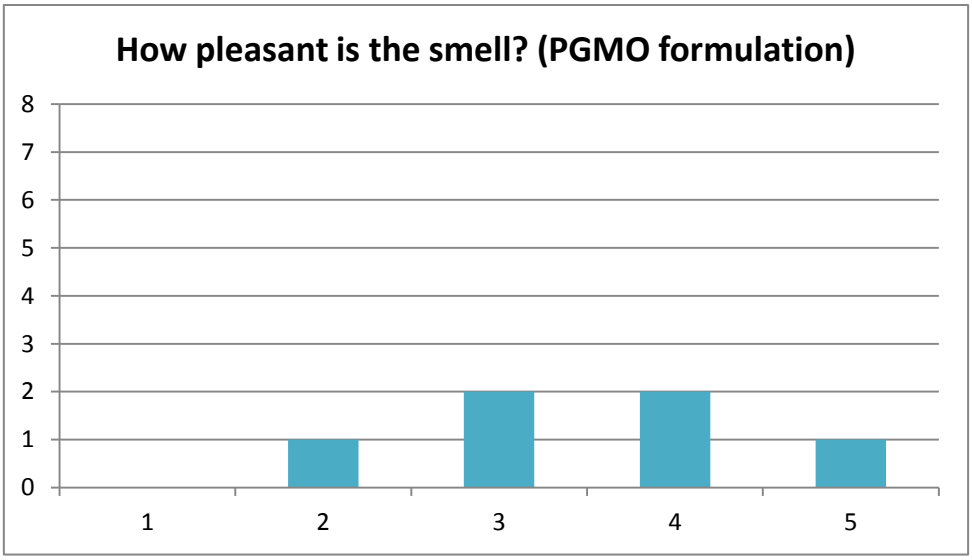

Figure 73. The diagram illustrates how pleasant the smell of the PGMO formulation was.

65 % of the participants could taste the GMO formulation. Only 10 % of the participants could taste the PGMO formulation. The taste of the GMO formulation was described as linseed oil, menthol, bubblegum or dry. The taste of the PGMO formulation was described as food oil.

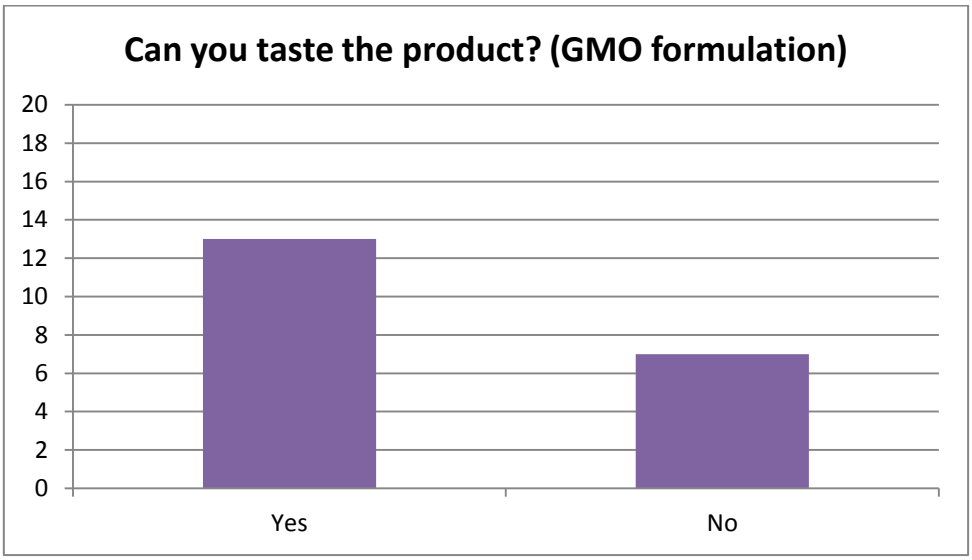

Figure 74. The diagram shows how many of the participants could taste the GMO formulation.

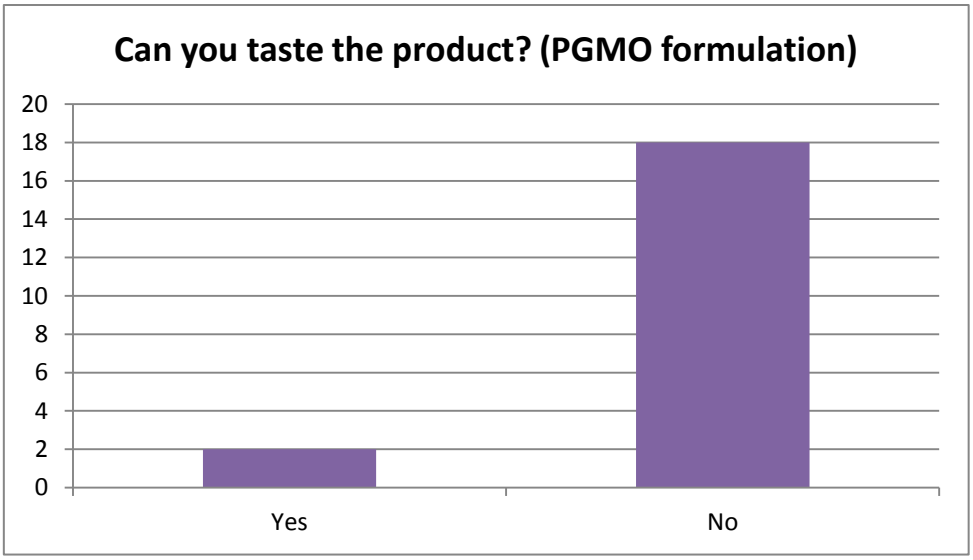

Figure 75. The diagram shows how many of the participants could taste the GMO formulation.

When asked to evaluate how pleasant the taste was, where 5 is the most pleasant, the GMO formulation received the score 2.69 and the PGMO formulation 2.5, though this score comes from only two participants.

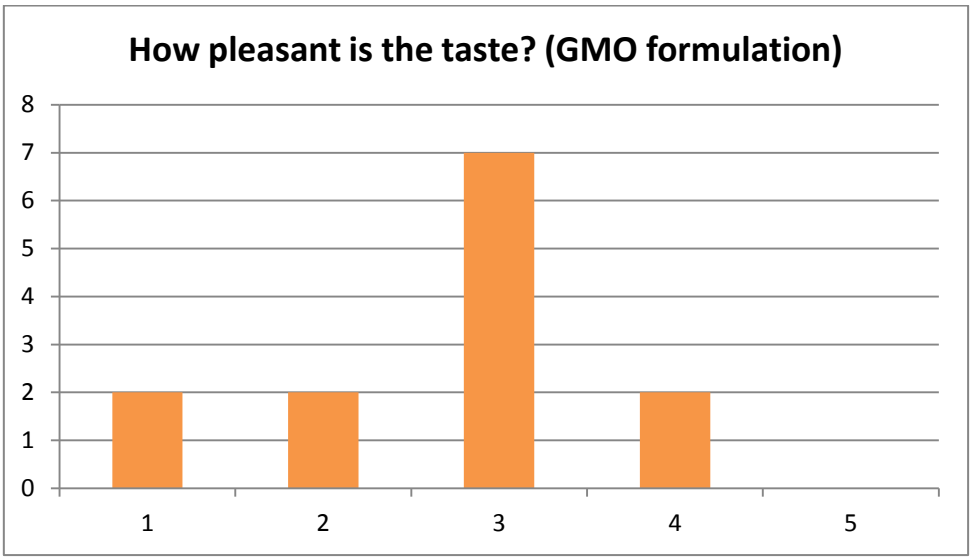

Figure 76. The diagram shows how pleasant the taste of the GMO formulation was, according to the participants.

The PGMO formulation taste was described as food oil. One participant felt that the taste was acrid.

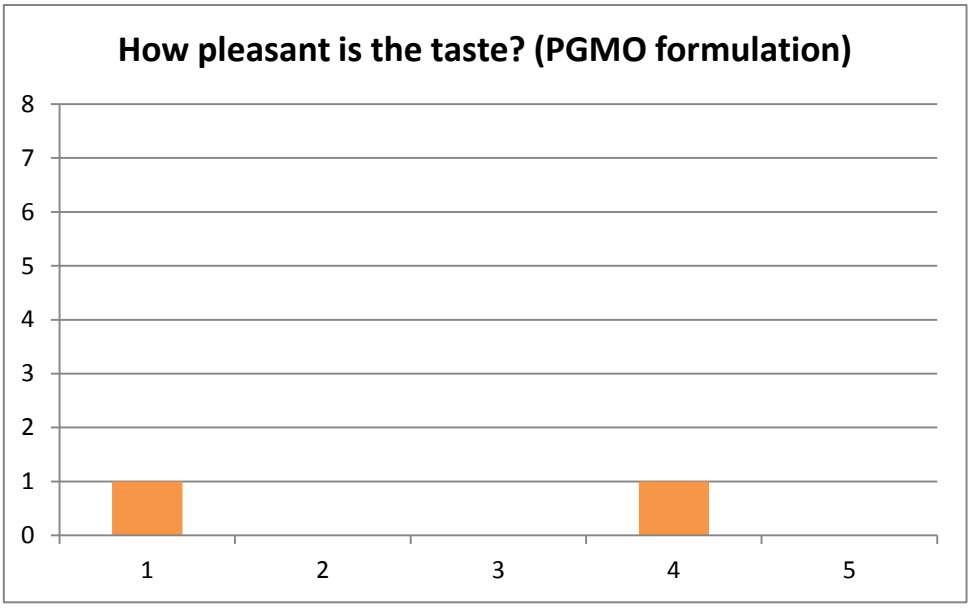

Figure 77. The diagram shows how pleasant the taste of the PGMO formulation was, according to the participants.

It seems that some people misinterpreted the question as irritating can mean many things. Most of the times it was made clear that a mild burning sensation was meant. When asked how irritating the product was inside the nose, where 5 is the most irritating the GMO formulation received the score 1.5 and the PGMO formulation received the score 1.25.

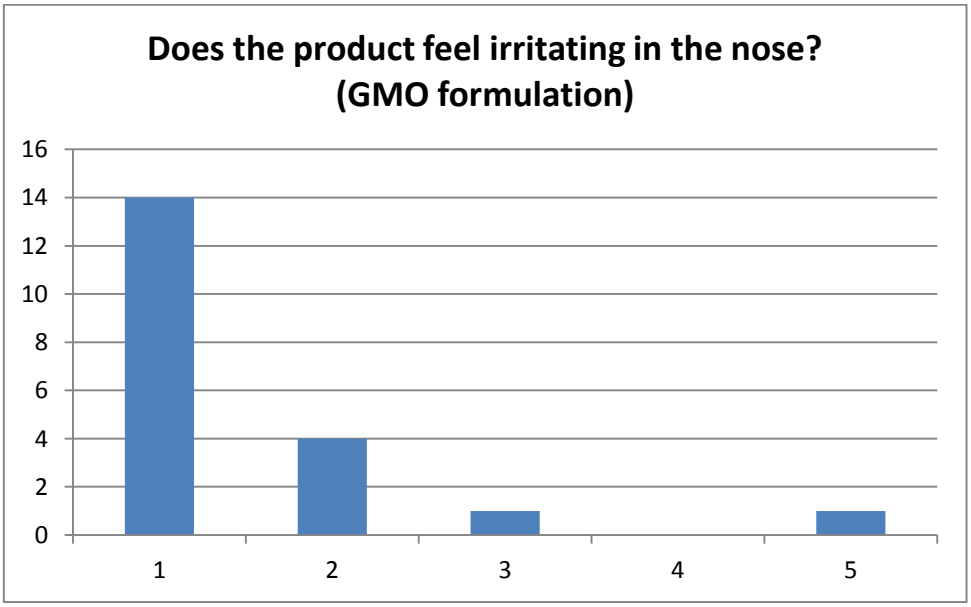

Figure 78. The diagram shows how irritating the GMO formulation was inside the nose, according to the participants.

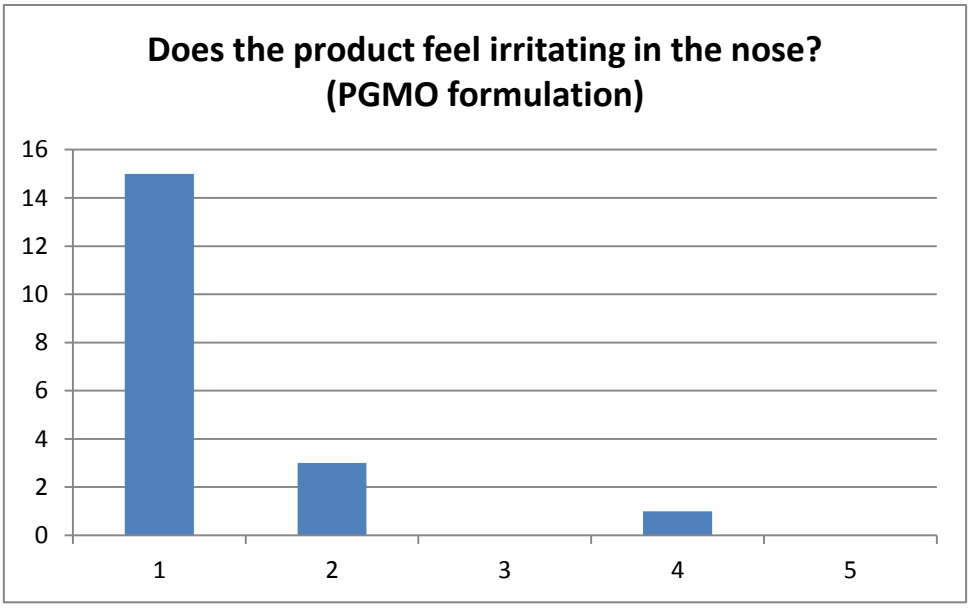

Figure 79. The diagram shows how irritating the PGMO formulation was inside the nose, according to the participants.

When asked how irritating the formulations were, where 5 is the most irritating, the GMO formulation received the score 2.0 and the PGMO formulation received the score 1.1.

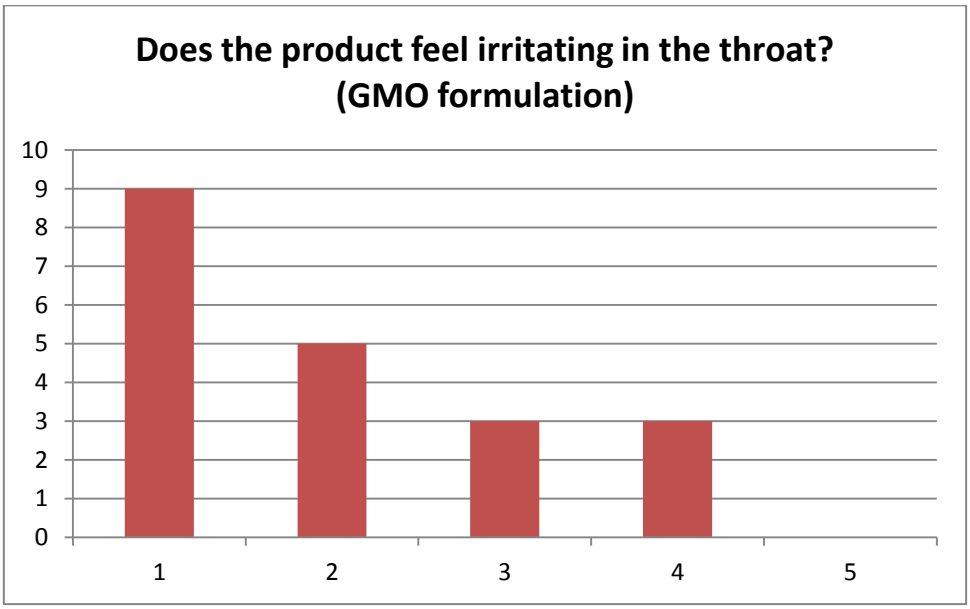

Figure 80. The diagram shows how irritating the GMO formulation is in the throat, according to the parcicipants.

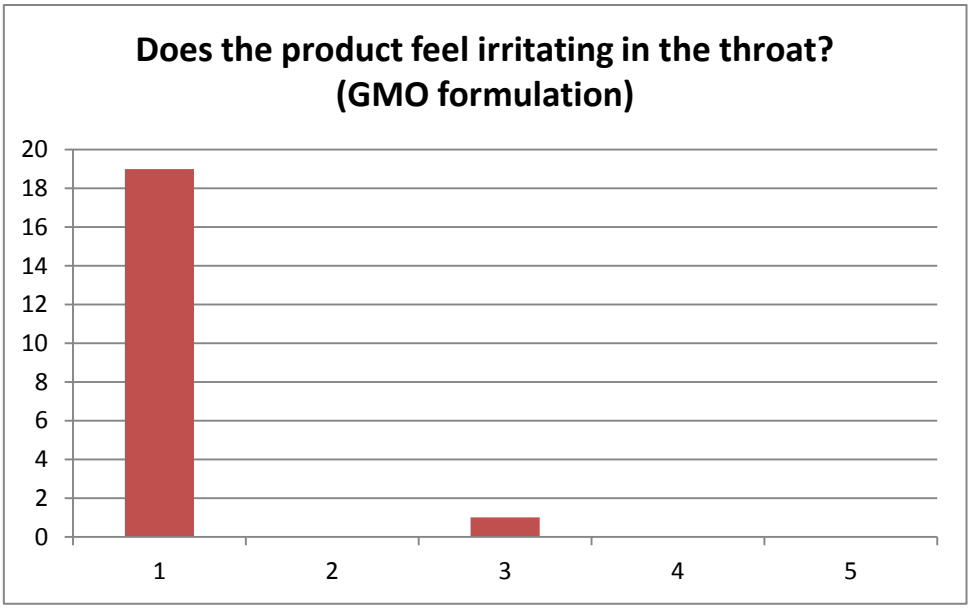

Figure 81. The diagram shows how irritating the PGMO formulation is in the throat, according to the parcicipants.

When asked how caring the product felt inside the nose, where 5 is the most caring the participants rated the GMO formulation with a score of 3.65 and the PGMO formulation with a score of 3.50.

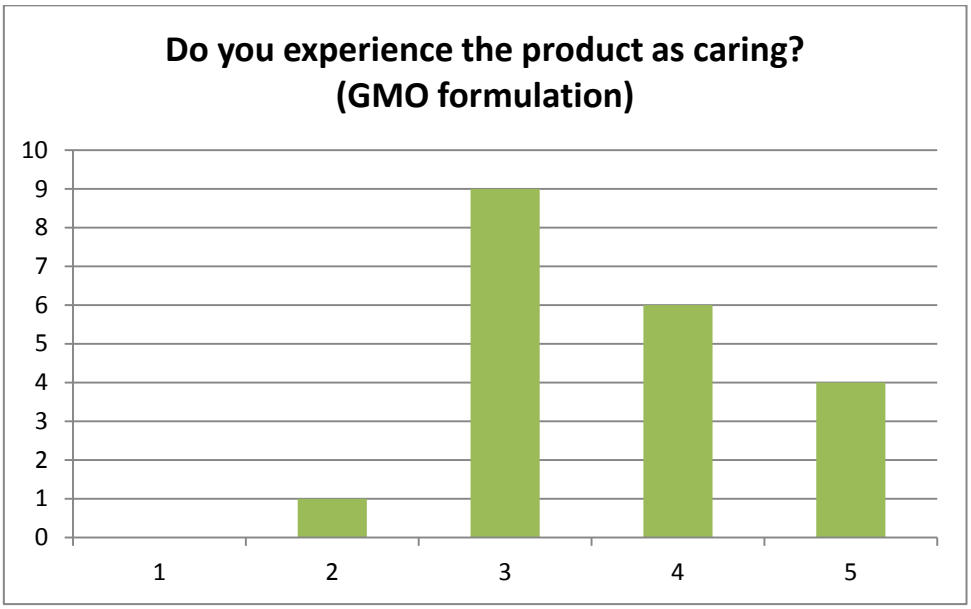

Figure 82. The diagram shows how caring the GMO formulation is, according to the participants.

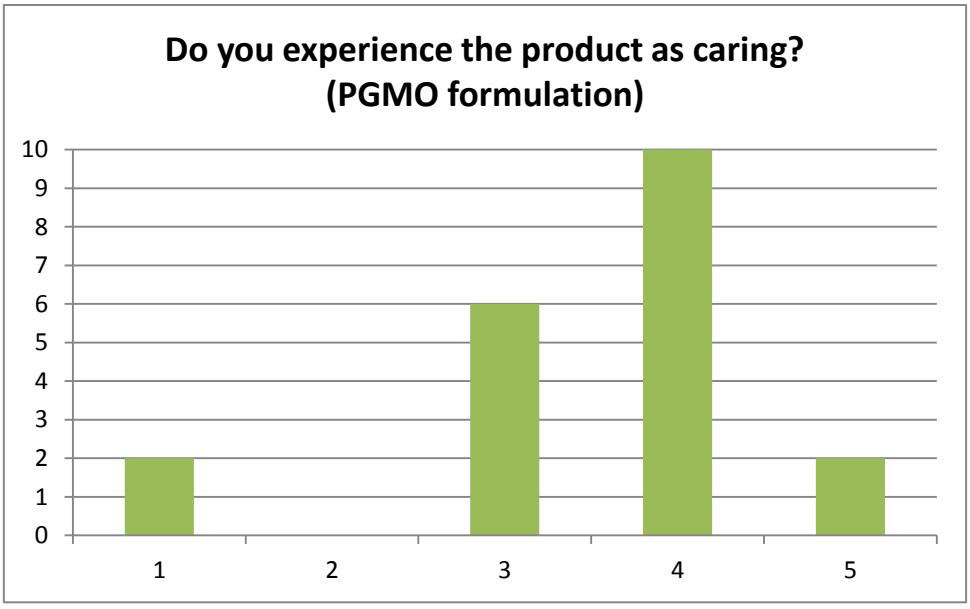

Figure 83. The diagram shows how caring the GMO formulation is, according to the participants.

Judging if the product was caring or barrier forming seemed like a difficult task. Here a little longer time than 10 minutes might have given more accurate answers.

A score of 5 means that the product gives the strongest barrier feeling. The GMO formulation received a score of 2.80 and the PGMO formulation received a score of 2.65.

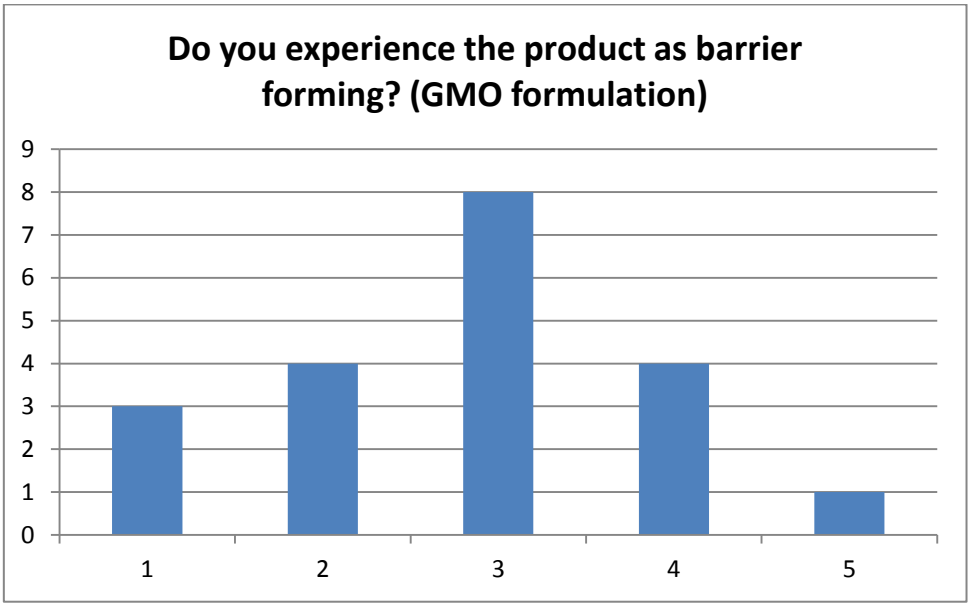

Figure 84. The diagram shows how strong the barrier feeling of the GMO formulation was, according to the participants.

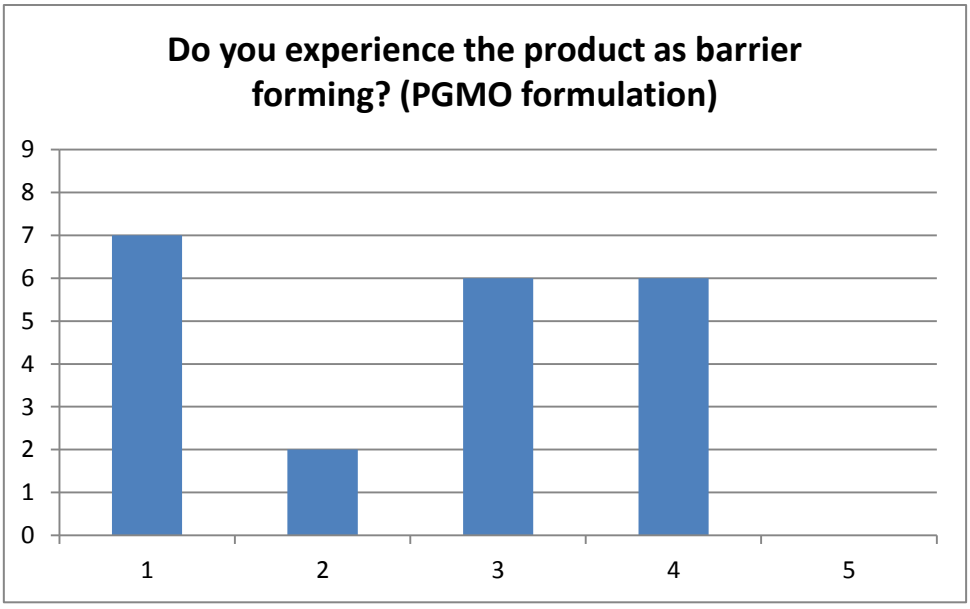

Figure 85. The diagram shows how strong the barrier feeling of the PGMO formulation was, according to the participants.

The PGMO formulation had a lower score, meaning less dripping, that the GMO formulation probably due to having a higher viscosity. When asked how much of the product dripped out of the nose where 5 is a lot dripping out and 1 is no product dripping out at all the GMO formulation received a score of 2.90 and the PGMO formulation received a score of 2.55.

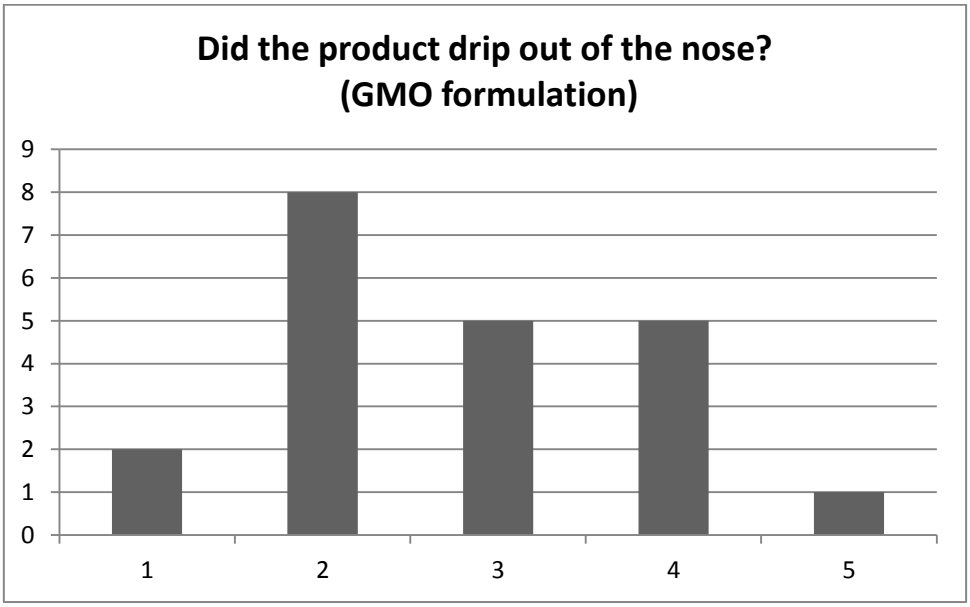

Figure 86. The diagram shows how much of the GMO formulation was dripping out of the nose, according to the participants.

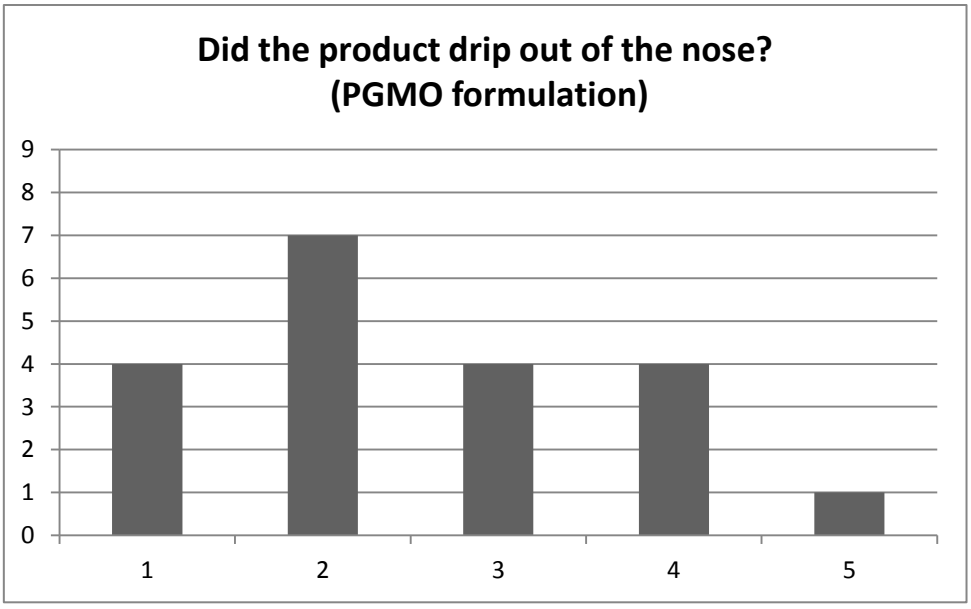

Figure 87. The diagram shows how much of the PGMO formulation was dripping out of the nose, according to the participants.

Some sensations felt in the nose from the GMO formulation were: blocking, oily, smoother, rehydrated, not as blocked running moisture, tingling.

Some sensations felt in the nose from the PGMO formulation were: blocked, itchy, oily, wet, runny nose, sticky.

Many users felt that there was something in their nose and they wanted to get it out to have clear nostrils again.

The PGMO formulation had the comments: “Took too much because I could not feel it.” “No effect, nose feels soft afterwards.” “It was annoying with oil in the nose.” “A nice smell would be better.” “I wanted to blow my nose.” “It is good to feel something inside your nose, like you have accomplished something when you take it.”

The GMO formulation had the comments: “It was irritating to the nose and throat.” “Nice smell.” “The nose was running a little.” “After 10 minutes I felt a burning sensation in throat.” “It seems to be useless.” “It drips out of the nose.”

When asked if they would consider using the product where 5 is most likely, the participants rated the GMO formulation with the score 2.3 and the PGMO formulation with the score 2.5.

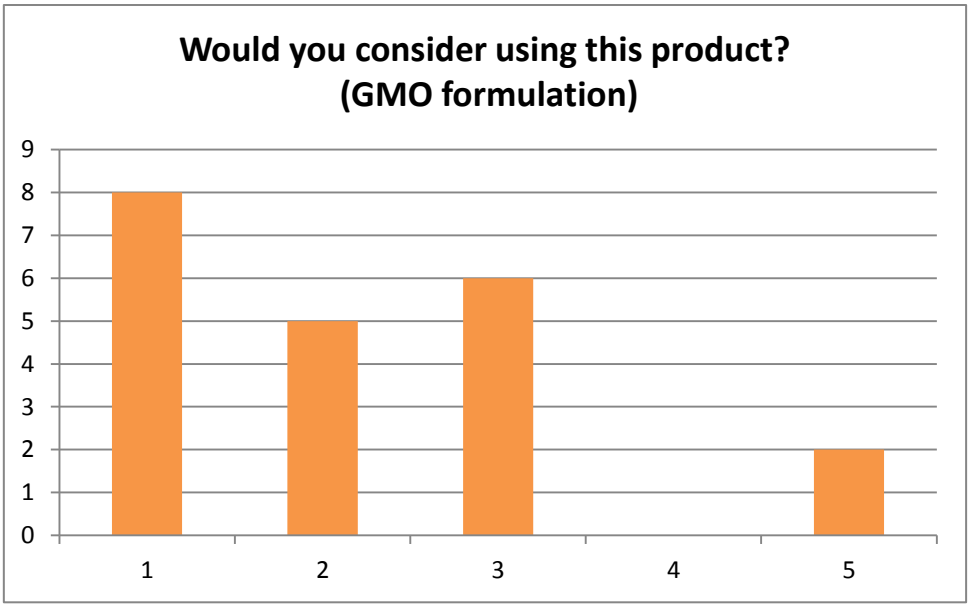

Figure 88. The diagram shows how likely it is that the participants start using the GMO formulation.

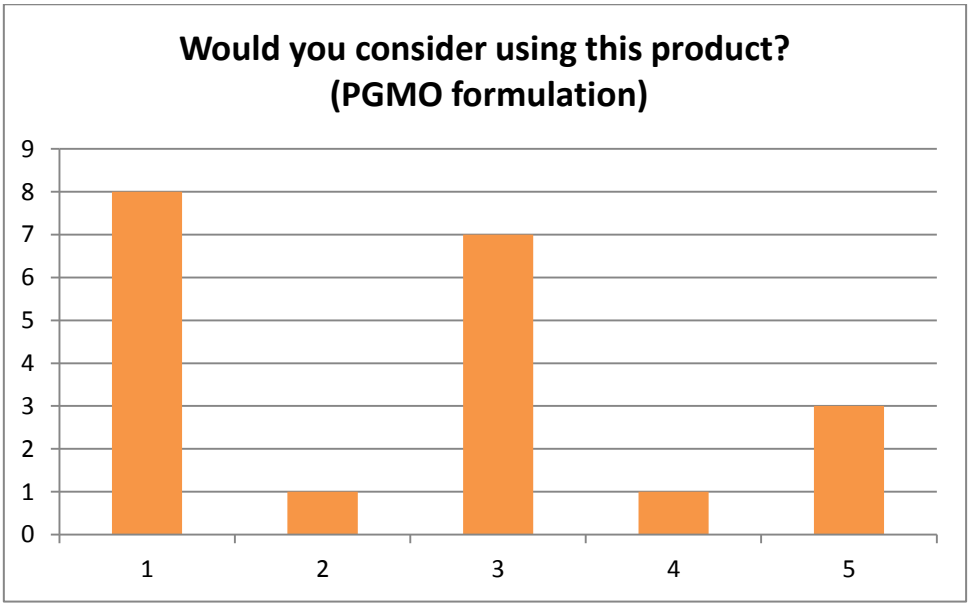

Figure 89. The diagram shows how likely it is that the participants start using the PGMO formulation.

When asked how likely it would be for the participants recommending the product to someone else, where 5 is most likely, the participants rated the GMO formulation with the score 3.1and the PGMO formulation with the score 2.85.

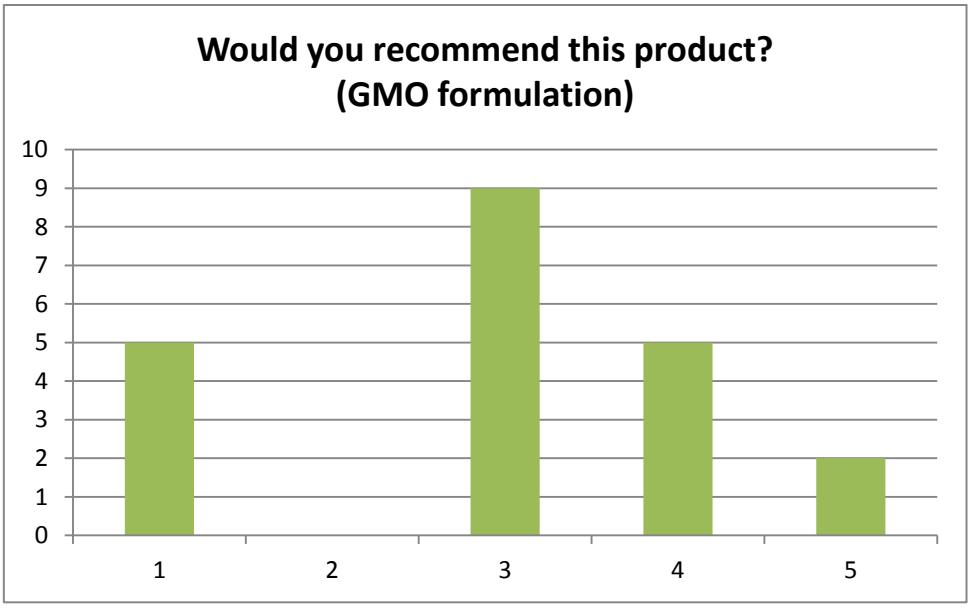

Figure 90. The diagram shows how likely it is that the participants recommend the GMO formulation.

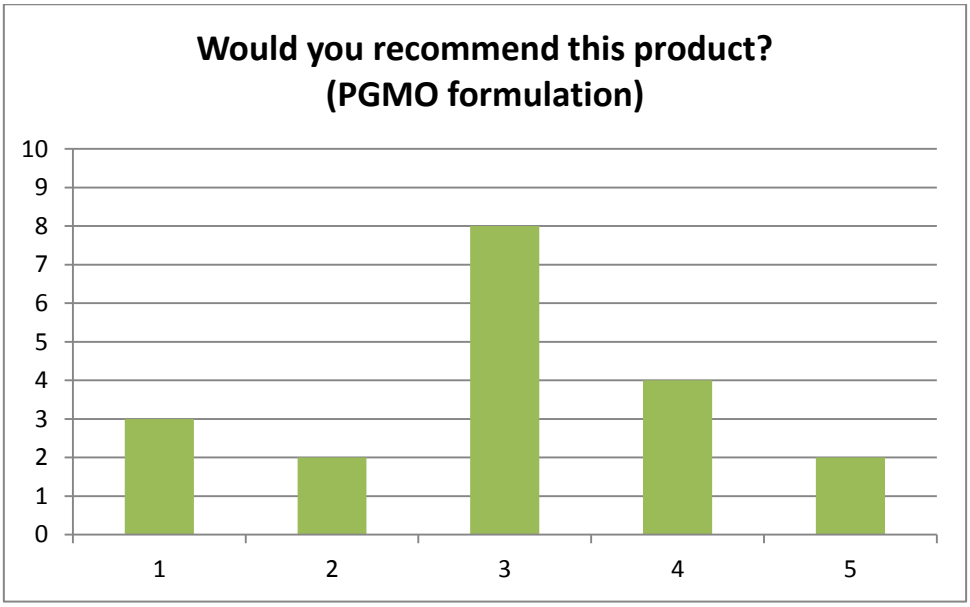

Figure 91. The diagram shows how likely it is that the participants recommend the PGMO formulation.