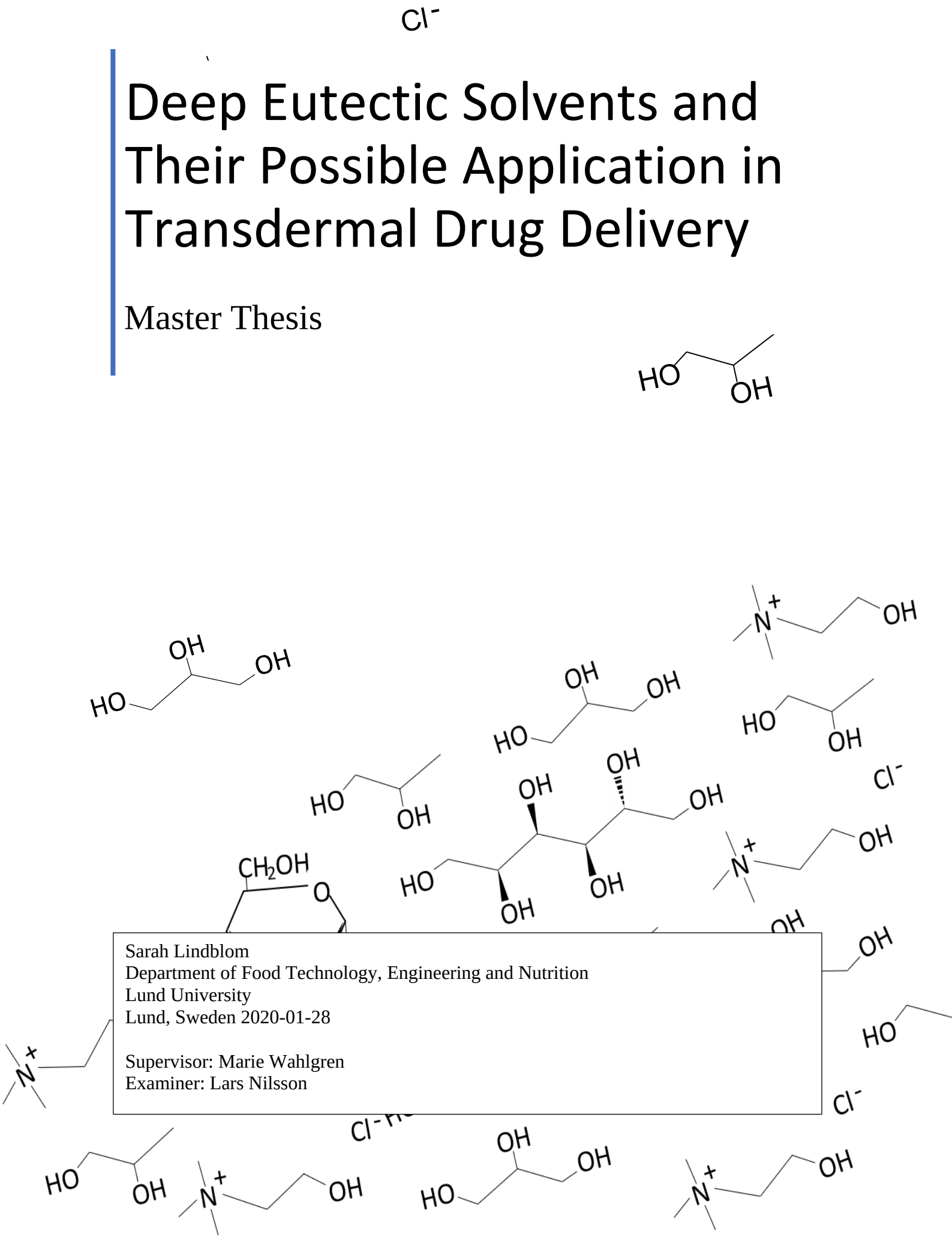
Deep Eutectic Solvents and Their Possible Application in Transdermal Drug Delivery

Master Thesis



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Abstracts

Deep eutectic solvents, DES, is a type of solvent made by mixing a quaternary ammonium salt with a hydrogen bond donor at the eutectic point of the system. Most common quaternary ammonium salt is Choline Chloride, ChCl. A specific DES, Choline:CAGE has shown promise in delivering insulin through the skin of mice. Four different systems containing Choline chloride were chosen: ChCl:glycerol, ChCl: propylene glycol, ChCl:glucose and ChCl:sorbitol. Initial viscosity measurements were done to see if the viscosity of the DES would be a problem and how water would affect them. They all behaved like Newtonian liquids, but glucose and sorbitol had to high viscosity to continue work with in their pure form. The protein BSA were added to ChCl:glycerol and ChCl:propylene and it was found that to solve the protein, water was necessary to be present at low concentration in the DES. ChCl:glycerol kept stable the BSA solved and stable for a longer time compared to ChCl:glycerol. Since ChCl:gly had quite low viscosity and behaved like a liquid, time was put in to trying to change their properties to become suitable for topical use. Carbopol, polyacrylic acid, in combination with water made this possible as it formed a shear thinning gel. Franz cell diffusion test were made on ChCl:glycerol with fluorescent BSA to see if had any effect on skin penetration. Fluorescent BSA in PBS were used as control. On undamaged skin either had a significant penetration, 1-2 %. On damaged skin both penetrated the skin well, DES about 20%, but the PBS were about double as effective. Telling that DES had limited penetration through the skin but might be interesting for drug delivery to the skin.

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Abbreviations

Name	Short
Deep eutectic solvents	DES
Hydrogen Bond Donor	HBD
Natural deep eutectic solvents	NADES
Therapeutic deep eutectic solvents	THEDES
Blood glucose level	BGL
Choline Chloride	ChCl
Choline chloride:glucose	ChCl:g
Choline chloride:glycerol	ChCl:gly
Choline chloride:propylene glycol	ChCl:pg
Choline chloride:sorbitol	ChCl:s
Geranate (geranic acid)	CAGE
Bovine Serum Albumin	BSA
Phosphate buffer saline	PBS
Active Pharmaceuticals Ingredient	API
Microcrystalline cellulose	cellulose
Carbopol 974P NJ	Carbopol
Pemulen™ TR-2 NF Polymer	Pemulen™

Introduction

Deep eutectic solvents, DES, is a relative new, green and cheap type of solvent that can be used from everything from synthesising organic molecules to biocatalysts. (Li and Lee 2016) DES can be made by mixing a quaternary ammonium salt with a hydrogen bond donor at the eutectic point. (Smith, Abbott et al. 2014) The eutectic point is a specific point in the phase diagram between two substances where a liquid with significant lower melting point than the individual substances is obtained. (Liu, Friesen et al. 2018) Solutions with properties like the ones at the eutectic point can also be achieved close to the eutectic point, these solutions can be of interest as well. (Li and Lee 2016) DES has gain interest in the green chemical community for being environmentally friendly, relative cheap and their negligent volatility, making them easy to handle compared to a lot of other solvents. The biggest drawback is their high viscosity. (Dai, Van Spronsen et al. 2013, Shekaari, Zafarani-Moattar et al. 2017) One way of combating this is adding in water to the systems. (Ma, Laaksonen et al. 2018)

Solvents with high viscosity can be of interest for topical formulations of drugs. One DES that has showed promise of topical use is choline Choline bicarbonate:geranate, Choline:CAGE, that changes the structure of the skins out most layer to help drug molecules penetrate the skin. Choline:CAGE was compared to traditional chemical skin enhancer and no enhancer at all when the protein insulin was mixed in an spread on mice skin and it showed great improvement in the blood glucose level of the mice. (Banerjee, Ibsen et al. 2017)

It is of interest in the pharmaceutical industry since topical delivery of drugs is good for localized treatments, dermal delivery, as well as systemic treatment, transdermal delivery. Transdermal drug delivery also bypasses the first pass effect in the liver. (Brown, Martin et al. 2006). Usually there is a limit to the size and hydrophilicity of the drug molecules that can be transported through the skin and as a rule of thumb only small lipophilic molecules can pass through the outer most layer of the skin unaided. Making most drug molecules too big or too hydrophilic for passing through the skin. It is possible to change the skin penetration by either physical or chemical means. (Banerjee, Ibsen et al. 2017)

To simulate skin penetration Franz cell diffusion were used instead with a fluorescent labelled protein Bovine Serum Albumin, BSA. Franz cell diffusion is a type off cell with a donator and acceptor chamber separated with a membrane. Together with a visual inspection of the membrane in a microscope under fluorescent light a good picture of skin penetration can be found. (Zsikó, Csányi et al. 2019)

For this work 4 different DES system what chosen to be investigated and see if they could help with skin penetration in the same way the ChCl:CAGE system had; ChCl:glycerol, ChCl:propylene glycol, ChCl:glucose and ChCl:sorbitol.

These systems were evaluated to see how well they would be suited to become solvents for topical formulations and then put to the test to see if they could help enhance skin penetration like the Choliine:CAGE system could.

Aim

They aim of this is to look closer into deep eutectic liquids and their application in transdermal delivery. Research into the use of deep eutectic solvents, to what extent have they been included in topical formulations. Find a protein to look at for penetration studies of proteins through the skin. See how soluble and stable proteins are in deep eutectic solvents. Find some eutectic systems and do skin penetration test with the help of Franz cells. For topical formulations the consistency and feel of the product is very important so looking into formulation of topical formulations and see if the deep eutectic solvents could be formulated into some pleasant consistency and nice skin feel, making them plausible to use as topical formulations. These indevers can be summarized in these research questions.

- Does the eutectic mixture give the system the best stability and rheology properties, or is it another molar ratio?
- Can any enhancement in skin penetration be proven from the different systems chosen?
- Can good cosmetic properties be obtained by formulating the DES to have wanted rheological properties?

Background

Ionic Liquids

Ionic liquids, IL, are room temperature liquids made from mixing organic salts together. The composition is that of an organic cation with an organic or inorganic anion. The cation is usually an asymmetric substituted one while the anion is usually halogen based. This results in a potential toxicity. A promising property of IL is that they are almost completely non-volatile which is a big step from a lot of common organic solvents. This together with promising physicochemical properties in thermal stability, non-flammability and solubility for organic compounds makes them of great interest. IL can be finetuned in polarity and viscosity depending on which ions that are used. (Dai, Van Spronsen et al. 2013) Since IL possible are toxicity, have high synthesis cost and high amount of impurities present they never got a wide industrial application. (Li and Lee 2016) This toxicity have prevented them from being implemented in the food industry and thus they cannot be used as excipients as they are not generally regarded as safe. (Faggian, Sut et al. 2016) This lead to another type of solvents being investigated called deep eutectic solvents as a greener alternative. (Liu, Friesen et al. 2018)

Deep Eutectic Solvents

Deep eutectic solvents, or DES, is a type of solvent that is formed from mixing two organic compounds together and achieving a liquid with lower melting point than the two starting compounds. (Lu, Cao et al. 2016) They are formed by mixing a quaternary ammonium salt with a hydrogen bond donor, HBD, for example a sugar or a carboxylic acid. (Shekaari, Zafarani-Moattar et al. 2017) The most commonly used quaternary ammonium salt is choline chloride, ChCl. (Lu, Cao et al. 2016)

For a true DES the molar ratio should be the one that gives the lowest melting point of the mixture. This is where the eutectic point is located and what has given the name to the solvents. See figure 1 for an example of a phase diagram and where the eutectic point is located. But for different reason other molar ratios than the one at the eutectic can be of interest to work with and these solvents are called deep eutectic solvents derivates, DESD. In this report the umbrella term DES will be used for all mixed solvents for simplicity. Molar ratio from 2:1 up to 1:5 of ChCl:HBD is the most commonly occurring. (Hayyan, Mjalli et al. 2013, Li and Lee 2016)

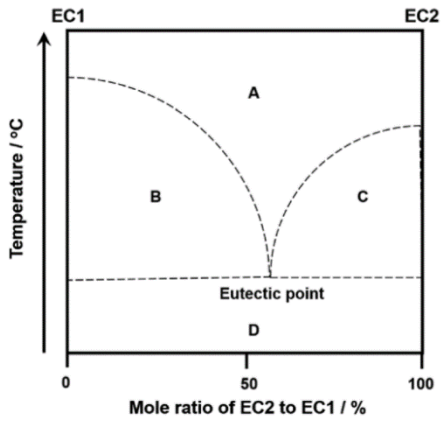


Figure 1. Simplified phase diagram of a eutectic system. EC1 stands for eutectic component 1 and EC2 for eutectic component 2. On the x- axis the molar ratio between the eutectic components are represented and on both the y- axis the temperature for each eutectic component are represented. (Liu, Friesen et al. 2018)

DES are easy and cheap to make, environmental friendly and can be prepared from biodegradable and natural components. (Shekaari, Zafarani-Moattar et al. 2017) It has been considered as a drawback of DES that they have high viscosity, although this do of course depend on the application of interest. They can also be unstable if not mixed in the correct molar ratio and have unknown corrosivity. (Juneidi, Hayyan et al. 2018) In some cases water is used to dilute the DES to lower the viscosity and making the DES easier and cheaper to work with. Water can be added to a certain degree without much change to the system but when the amount water becomes too high the DES dissociates and get hydrated. Ma et al. discuss this in detail in her review of the properties of IL and DES. See figure 2 for a visualization of water added to a DES. (Ma, Laaksonen et al. 2018) The components used in DES has a low toxicity which makes them good for application within different fields. (Shekaari, Zafarani-Moattar et al. 2017)

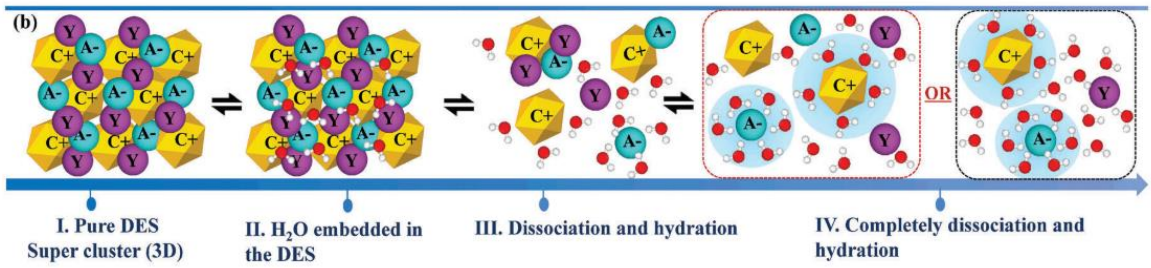


Figure 2. A visual representation of what happens to a DES system when water is added in different amounts. First how the water embeds itself in the system and when too much is added how the DES system dissociate and becomes a water solution instead. (Ma, Laaksonen et al. 2018)

DES has been divided into different subcategories. The most common one is Natural deep eutectic solvents, NADES. These DES are made from natural compounds and have become popular in both green chemistry area and in food and pharma industries. NADES are usually made form organic acids, amino acids and sugars. These type of solvents are good for solving organic compounds. (Faggian, Sut et al. 2016) NADES are both biodegradable and biocompatible.(Liu, Friesen et al. 2018)

Another popular subcategory of DES that is used exclusively in the pharma industry is therapeutic deep eutectic solvents, THEDES. The reason behind this is that instead of the usually quaternary ammonium salt the active substance from the drug is used as one of the two components in the DES. This is of interest if the Active Pharmaceutical Ingredient, API, is not water soluble or has very low water solubility. (Duarte, Ferreira et al. 2017)

Four different DES were chosen for this work and they were compared to ChCl:geranate that was investigated by Banerjee *et al.* and they are listed in table 1 with accomplishing information of molar ratio and melting points.

Table 1. Summary over the different DES systems and the most common occurring molar ration, for the ChCl:g the molar ratio with lowest melting point was chosen. The melting point reported in the table is for when melting the eutectic components together.

DES	Molar ratio	Melting point (°C)
ChCl:glucose	2:1	80
ChCl:sorbitol	1:1	99
ChCl:glycerol	1:2	20-23

ChCl:propylene glycol	1:2	75
ChCl:geranate	1:2	~60

Choline Chloride

Choline chloride is a quaternary ammonium salt used as a food additive. (Lu, Cao et al. 2016) The FDA has it listed as a dietary supplement and recognizes it as generally safe. (FDA 2019) Choline chloride has shown no genotoxicity, no reproductive or developmental toxicity when tried as a feed additive for animals. It has neither indicated to be an irritant in its undiluted form when tested on skin. It is also suspected that it will have no significant environmental impact since choline is widely found in nature and is biodegradable. (Li and Lee 2016) The Scientific Committee on Cosmetic products and non-food products writes back in 2003 how choline chloride should be allowed in cosmetic application, since it doesn't appear to cause any irritation when applied to skin. (SCCNFP 2003) Another benefited is that Choline chloride is the most common hydrogen bond acceptor in DES. It can be combined with a string of different hydrogen bond donors, for example sugars, alcohols or carboxylic acids. (Lu, Cao et al. 2016)

Choline chloride: glycerol

Choline chloride: glycerol is an environmentally friendly, nontoxic DES. Different molar ratio has been mentioned through articles but is seems that 1:2 and 1:3 are the most commonly used molar ratio for choline chloride: glycerol. (Dai, Van Spronsen et al. 2013, Troter, Todorovic et al. 2017)

Choline chloride: glucose

The molar ratio for choline chloride: glucose that gives the lowest melting point is 2:1. Other molar ratio that forms eutectic mixtures are 1:1 and 1:2,(Radošević, Cvjetko Bubalo et al. 2015, Aroso, Paiva et al. 2017) but these ratios have slightly higher melting points making them deep eutectic solvents derivatives. Mixtures where the ratio of glucose is higher than choline chloride is not stable and forms a semi solid once cooled down. Sugar based eutectic liquids are of interest as a new alternative to extract glucose from bio-materials. (Hayyan, Mjalli et al. 2013)

Choline bicarbonate: geranate

Choline bicarbonate:geranate has been investigated for its properties to enhance skin penetration for delivery of proteins. Banerjee et al. writes that the eutectic solvent choline bicarbonate:geranate, ChCl:CAGE, can be used to deliver insulin through the skin in mice. It is shown to have an antibacterial activity which could help transdermal delivery of antibacterial drugs. The study was carried out on both pig skin and on mice where a significant loss in blood glucose levels, BGL, could be seen in both experiments. With the help of confocal microscopy of the pig skin it could be seen that the CAGE-protein mixture had penetrated into the deep skin layers. This was compared to traditional chemical enhancer and in comparison, the CAGE-protein mixture penetrated deeper into the skin. The test done on mice showed a significant drop in BGL when the CAGE-insulin mixture was applied on the skin. This was compared to only CAGE, were a significant drop was not observed, insulin in a traditional chemical enhancer, which didn't show a significant drop in BGL either. Lastly

the CAGE-insulin mixture was compared to the traditional way of injecting insulin concluding that CAGE-insulin mixtures could be a new way of delivery insulin to diabetic patients without the need for needles. (Banerjee, Ibsen et al. 2017)

Choline Chloride: sorbitol

Sorbitol is a polyol and used as a sweetener in food and beverages. Sorbitol, like saccharides can form DES with choline chloride. The molar ratio for choline chloride:sorbitol DES is 1:1 and has a melting point at 99 °C. (Maugeri and Domínguez De María 2012) Sorbitol is listed in the excipient handbook for pharmaceuticals. (Rowe, Sheskey et al. 2009)

Choline Chloride: propylene glycol

Is a DES with a low melting point. Pure propylene glycol has a melting point around -59 °C, making the mixture with choline chloride have an even lower melting point, which makes it an interesting solvent since it is a liquid at so low temperature. (Kuehn, Massmann et al. 2017) Propylene glycol is a common solvent in pharmaceuticals and can be found in the excipient handbook for pharmaceuticals. (Rowe, Sheskey et al. 2009) Kuehn *et. al* reports that mixtures with molar ratio of 1:5, 1:6 or 1:7 forms stable DES, while anything lower will not be stable and become semi solids once cooled down. (Kuehn, Massmann et al. 2017) Contrary to Kuehns words, Troter *et al.* has done studies on ChCl:propylene glycol at a molar ratio of 1:2, not mentioning anything about it forming semi solids at this ratio. (Troter, Todorovic et al. 2017)

Adding protein to DES

Protein has been added to DES to see how the change of solvent effects the protein. A. Sanchez-Fernandes *et. al.* confirms that the protein Bovine Serum Albumin, BSA, folds itself differently in pure DES than in buffer and that solving the protein in DES has no protection against denature when heated to 80°C. The mixture of DES/water was tested as well, 50/50 or 25/75 wt% of DES and in these mixtures the protein had similar folding as in buffer. (Sanchez-Fernandez, Edler et al. 2017)

Bovine Serum Albumin

Bovine Serum Albumin, BSA, is a type of serum albumin protein that is isolated from cows. (Rockland antibodies & assays n.d.) BSA is structurally very similar to the human serum albumin, HSA, making it a good model for it. HSA is a globular protein that plays a important role in maintaining plasma pressure and the nutritional balance. (Jahanban-Esfahlan, Ostadrahimi et al. 2019) BSA is used in a lot of different application, one of them being a standard for protein quantification where a fluorescent tag can be attached to the BSA for easy fluorescent detection. (ThermoFisher Scientific n.d.) It is also cheap as it is readily purified out of cow blood, a by-product of the meat industry. (Rockland antibodies & assays n.d.)

Skin

The skin is the largest organ of the body. (Kolarsick, Kolarsick et al. 2009) It cover the entirety of the body's external surface and acts as the body's first line of defence. It protects against UV-light, pathogens and chemicals. It also works as a mechanical barrier. Besides that it helps control temperature and the amount of water released from the body. (Yousef and Sharma 2017) Kolarsick

et al. describes the skin being composed of three layers, the epidermis, the dermis and the subcutaneous tissue. (Kolarsick, Kolarsick et al. 2009) While Hunter et al. describes the skin being composed of two layers; the epidermis and the dermis, and saying that below there is loose connective tissue where an abundant of fat usually is collected. (Hunter, Savin et al. 2003) The thickness of the skin varies, the epidermis is the thinnest at the eye lids, measuring less than 0.1 mm but the thickest at the soles of the feet and palm where it measures 1.5 mm. For the dermis it is the thickest on the back, measuring in on 30-40 times thicker than the epidermis. (Kolarsick, Kolarsick et al. 2009)

The epidermis is structured from many layers of closely packed cells, the outer most layer of flattened cells filled with keratins making it a stratified squamous epithelium. The epidermal contains no blood vessels. The thickness of the epidermis is kept at a constant thickness from cell dividing in the deepest layer of the epidermal. The cells moves or is pushed through the epidermal before they die in the in the stratum corneum, SC. (Hunter, Savin et al. 2003) The epidermis is mostly made up of two cells: keratinocytes and melanocyte. Langerhans cells and Merkel cells can also be found in the epidermis. (Kolarsick, Kolarsick et al. 2009) The keratinocytes originate in the in the basal layer of the epidermal and produces keratin. Melanocyte are the only cells that synthesis melanin. Melanin a pigment responsible for the colour of hair and skin. (Hunter, Savin et al. 2003)

The epidermis is divided into four layers; the deepest layer closest to the dermis the basal layer (stratum germinativum), the squamous cell layer (stratum spinosum), the granular cell layer (stratum granulosum) and the outer most layer the horny layer (stratum corneum). (Kolarsick, Kolarsick et al. 2009) The SC works as a barrier, preventing the loss of interstitial fluid form within and prevents penetration of potentially harmful substances. The barrier layer of the SC can be affected by essential fatty acid deficiency, excessive hydration, detergents or injuries or skin diseases. The rate of penetration of a substance through the epidermis is directly proportional to the difference in concentration and indirectly proportional to the thickness of the stratum corneum layer.

The epidermis is connected to the dermis by interlocking it with downward projections, epidermal ridges or pegs, and the dermis connects with upward projecting dermal papillae to the epidermis to keep them together. (Hunter, Savin et al. 2003)

The dermis supports the epidermis structure. (Hunter, Savin et al. 2003) The dermis makes up the bulk of the skin. It is what provides the skin with its elasticity, pliability and tensile strength, as well as it binds water, and contains nerves and blood vessels. (Kolarsick, Kolarsick et al. 2009) For drug delivery into the systemic circulation the API needs to reach the dermis and the blood vessels it contains. (Brown, Martin et al. 2006)

Topical formulations

The skin is an interesting way of delivering drugs to the bloodstream. The reason is that delivering drugs through the skin bypasses the 1st pass of metabolism. Problem is that only lipophilic molecules smaller than around 500 Da normally can pass through the skin unaided. Since most drugs don't fit this description it poses a problem. There are different strategies to combat these limits and they are categorized into passive and active methods.

The passive method is based on trying to change the properties of the skin by how the drug is formulated. This includes ointments, creams and passive patch technology. This can be done by enchanting the diffusion through the skin and/or increase the permeability of the skin. Examples of this is supersaturated systems, prodrugs, metabolic approach or liposomes. Unfortunately, most

drugs still has problems passing through the skin with these methods since the skin is such a good barrier.

Active methods are based on physical and mechanic changes to the skin. This involves using external energy to enhance the driving forces and/or reduce the barrier of the outer most layer of the skin, the stratum corneum, SC. This method can be further divided into electrically assisted devices, mechanical devices, ultrasound, magnetophoresis, radio frequency and temperatures. Examples are iontophoresis, microneedle- based devices, skin puncture and perforation, needleless injection or ultrasound. (Brown, Martin et al. 2006)

Rheology

Rheology describes the deformation and flow behaviour of materials. Rheology includes different areas of how to describe how the material flows and deform. Viscosity is one of them and describes a fluid's internal resistance to flow, this relates to the internal friction of the material. (Anton Paar 2019) Larger components in a fluid gives rise to higher viscosity. (Anton Paar 2019) Viscosity measurements gives information on how thick a fluid is and how easily it flows. (Anton Paar 2019)

All materials are put on a scale between solid and liquid. Solid materials are described as being elastic and liquids as viscous. Most thing aside from pure liquids are somewhere in between and called viscoelastic materials. (Anton Paar 2019)

Fluids are divided into three categories depending on how they behave when external force is applied to them. If the fluids internal flow is independent of the external force applied to it, it is ideally viscous and are called Newtonian liquids. If the viscosity gets higher with higher amount of external force it is a shear-thickening fluid. If it gets thinner the more external force is applied, it's a shear-thinning fluid. (Anton Paar 2019) See figure 3 for how a graph of viscosity against shear strain can describe the three different behaviour of a liquid. Aside from external force, temperature influences the viscosity greatly. The higher the temperature the lower the viscosity – the easier it flows. Pressure affects the viscosity but to a lesser degree, with increased pressure the viscosity increases. (Anton Paar 2019)

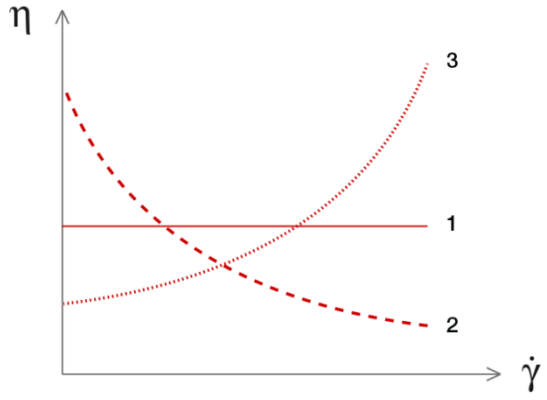


Figure 3. A viscosity graph with viscosity plotted against shear strain. The first line, 1, describes a Newtonian fluid, the second, 2, a shear thinning fluid and the third, a shear thickening fluid.

As mentioned above, a lot of material are either solid or liquid, instead they are viscoelastic materials. This means they behave as both a solid in some cases but as liquids in other cases. (Anton Paar 2019) To help determine if the material is a very viscous liquid, or if the elastic behaviour dominates oscillatory tests can be performed to help understand what type of material once are

dealing with. (Anton Paar 2019) To begin with an amplitude sweep with a cut of after the linear viscoelastic region, LVE region, is performed. This to prevent destroying the structure of the sample. (Anton Paar 2019) With a value from the LVE range a frequency sweep can be made to understand how the sample with behave over time in the non-destructive deformation range. (Anton Paar 2019) At last an amplitude sweep can be done that goes outside the LVE range to see what happens after deformation of the samples structure, will it start to flow or go brittle. After this test is done the sample is destroyed and no further test can be done on it. (Anton Paar 2019)

For both amplitude and frequency sweeps graphs containing G' and G'' is obtained. G' is the storage module and describes the elastic behaviour of the sample while the G'' is the loss module and describes the viscose behaviour of the sample. If G'>G'' the storage module is dominating, and the samples is a gel or viscoelastic. For the reverse, G''>G', the sample is a fluid. (Anton Paar 2019)See figure 4 for a visualisation on this for an amplitude sweep. As can be seen in figure 4 to the left the G'' crosses over the G' line the more strain is added to the sample, this is called the flow point, and beyond this point the viscous portion of the sample will dominate making it flow. (Anton Paar 2019)

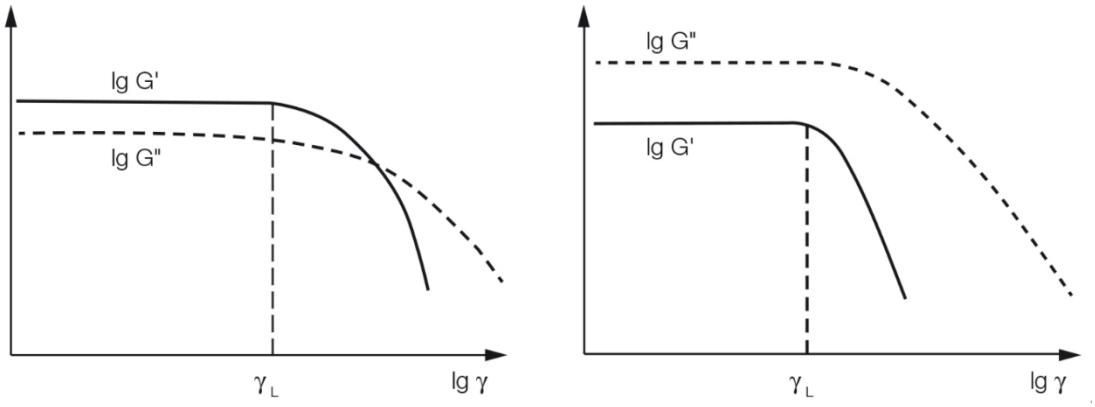


Figure 4.Left: A viscoelastic solid material in the LVE range, G'>G'' with a flow point. Right: A viscoelastic fluid, G''>G'. γ_L shows the end of the LVE range.

Skin penetration

Dermal and transdermal drug delivery has become more important with time because of their many benefits of being non-invasive and avoids first pass metabolism. Modelling the penetration into the skin and through the skin is complex. (Zsikó, Csányi et al. 2019) Since the SC is consists of dead cells the driving force through the skin is passive diffusion according to Fick's law. (Brown, Martin et al. 2006) Making it important having good methods for measuring skin penetration. Topical products are well accepted by patients therefore interesting in developing new drug formulations. But topical formulation is complex, many different factors need to be considered when making them, including particle size and distribution, surface tension between phases and rheological behaviour. The start, duration and therapeutic effect depends on three consecutive processes: amount of API in the formulation; penetration of said API through the SC and/or other skin layers; the effect at the targeted area. Human skin gives the most accurate information but of high costs so simpler methods are used, especially early in the drug formulating.

There are three different routes allowing substances to pass through the barrier the SC creates, see figure 5. The first being the intercellular penetration pathway, which has been the most important one for many years. The second it the follicular penetration pathway and is a second essential pathway. The last one, transcellular penetration pathway, where materials pass through both the

corneocytes, the dead cells of the SC, and the lipid layers if of no importance now. (Zsikó, Csányi et al. 2019)

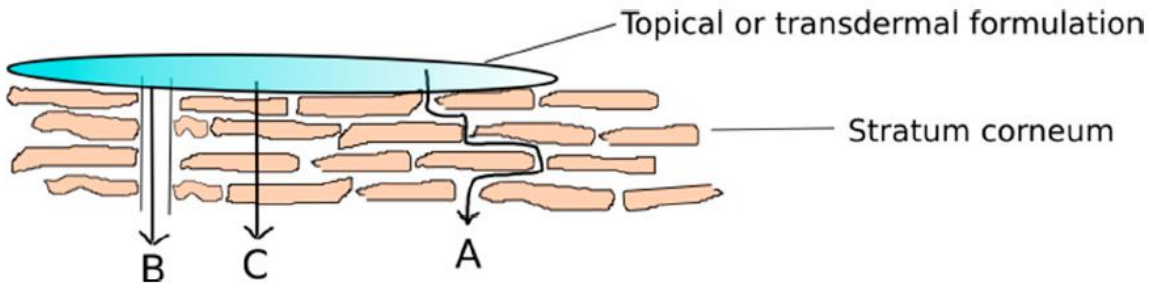


Figure 5. Stratum Corneum with a topical formulation on top with 3 different ways of penetration; A: intercellular penetration pathway, B: follicular penetration pathway and C: transcellular penetration pathway. (Zsikó, Csányi et al. 2019)

Getting the API from the formulation and into the systemic circulation is a multistep process and many factors affecting the process. Among them, molecular weight, low melting point, high but stable partition coefficient and solubility in water and oil to achieve a concentration gradient over the skin. The skins penetration is also affected by the individual such as age, hydration, gender, where and malformation due to damage or illness.

To keep skin test as uniform as possible, documents containing standard procedures have been developed by both World health organisation, WHO, and Economic Cooperation and Development, OECD.

There are two types of methods for modelling skin penetration; quantitative and qualitative or semiquantitative techniques. The quantitative method includes diffusion cells and Skin-PAMPA, these are usually used for measuring the amount of API permeated through the membrane over time. Where the qualitative or semiquantitative methods are different microscopic and spectroscopic methods or a combination of them and focus more on follow the API, see how it moves through the skin. (Zsikó, Csányi et al. 2019)

Diffusion Cells

The skin can be looked at as a selective permeable biological membrane.(Zsikó, Csányi et al. 2019) Since the SC is made of dead cells passive diffusion according to Fick's law is what drives the diffusion. (Brown, Martin et al. 2006) Diffusion is the random movements of molecular through a domain driven by a difference in concentration, going from high to low concentration. (Permegear 2015)

The standard was set after Dr. Thomas J. Franz developed the "Franz cell" in 1970. This helps terminate the relationship between API, skin and formulation. The diffusion cell consists of a chamber where the formulation is put on the membrane where the drug can diffuse through and into an acceptor chamber filled with receptor fluid. (Zsikó, Csányi et al. 2019) This fluid should be maintained at a constant temperature mimicking normal skin temperature of 32 ± 1 °C. (OECD 2004)

There are two main types of diffusion cells; static and flow-through. The cells are normally made of glass. The Franz diffusion cell is a static vertical cell, see figure 6 for an example of what a Franz diffusion cell can look like. There are also horizontal diffusion cells. New models of Franz diffusion cells have autosampler to take out the human error of sampling by hand. (Zsikó, Csányi et al. 2019)

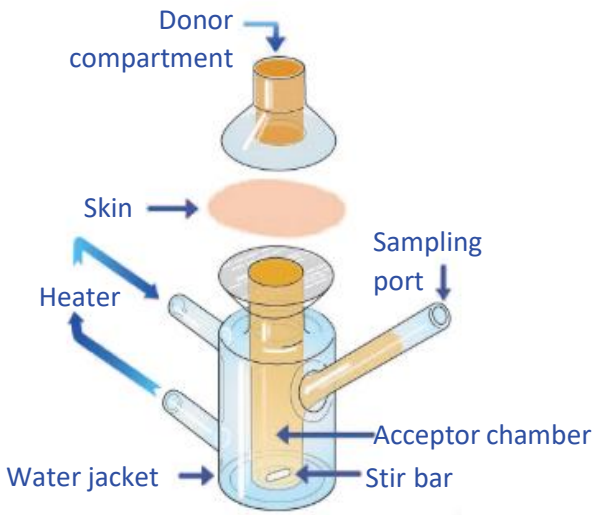


Figure 6. Schematic picture of how a static vertical Franz cell can look with its different parts named. Picture taken from PermeGear. (Permegear 2015)

Membrane

The skin of the pig's ear is a good alternative to human skin for in vitro testing of transdermal absorption. The reason for this is the similarities in physiology, chemistry and anatomy. (Pavlačková, Egner et al. 2019) Other option is human skin from cadaver, surgical biopsies or cosmetic surgeries or rodents as rats or mice. Drawbacks with this is they can be expensive, hard to get a hold of and can give a variability of results. An alternative to natural membrane is synthetic ones. Synthetic membrane has high reproducible results, are commercial availability employed for quality control to evaluate diffusion of topical formulation. A drawback is that the results vary with which type of synthetic membrane you choose. (Sguizzato, Valacchi et al. 2020)

Microscopic and Spectroscopic Methods

Microscopic methods give insight in distribution of the drug inside the different layers of the skin. It can also help explain the mechanism of penetration. A plus is that these are non-destructive in vitro methods. Fluorescence microscopy is often used. By labelling the API with a fluorescent tail it can easily been seen in a fluorescence microscope. This can give an idea if the fluorescent labelled API made it passed the SC and penetrated deeper into the skin or if it was stopped by the SC. (Zsikó, Csányi et al. 2019)

Materials and method

Materials

Chemicals

Choline chloride, >99%, Sigma Aldrich, D-(+)-Glucose, Sigma Aldrich, D-(-)-Sorbitol extra pure, Merck, Glycerol, 100%, VWR Chemicals, Propylene glycol, >99%, VWR, Choline chloride, >98%, Sigma Aldrich Bentonite, Merck, Cellulose Microcrystalline, Merck, Carbopol 947NF, LubrizolPemulen™ TR-2 NF Polymer, Lubrizol

Method

Sample preparation DES:

5ml DES was prepared by weighing up appropriate amount of ChCl and an HBD, see table 2, into a 10ml glass vial with crimp lid. A lid was crimped on. The glass vial was then place in a heating module (Pierce, Reacti- Therm, Heating/stirring module, no. 18971) and the temperature was set to 85°C. Once the mixture started to melt, the vial was gently shaken to help mix the two chemicals. When the liquid had turned clear and no trace of either chemical could be seen with the naked eye, the vial was removed from the heat and left to cool down to room temperature. The samples were stored in a desiccator.

Table 2. Molar ratio of the DES being prepared. The amount of ChCl and HBD is reported on how to mix together 5 ml of the specified DES.

DES	Molar ratio	Amount ChCl (g)	Amount HBD (g)
ChCl:glucose	1:1	2.979	3.844
ChCl:sorbitol	1:1	2.914	3.802
ChCl:glycol	1:2	2.651	3.496
ChCl:propylene glycol	1:2.4	2.387	3.084

Stability of BSA in DES

5 ml of ChCl:glycerol and ChCl:propylene glycol DES were made. Half of each DES was then poured into a new 10 ml glass vial and set aside. 10 mg BSA were added into each DES before they were left in the exicator.

20 mg BSA was mixed into 1 ml of water creating a concentration of 20 mg BSA/ml. 10 wt% of 20 mg BSA/ml were added to 2.5 ml DES before they were placed in the exicator.

2.5 ml ChCl:propylene glycol with molar ratio 1:3, 1:5 and 1:7 were made by mixing 1.030 g ChCl with 1.684 g propylene glycol for the 1:3 molar ratio, 0.717 g ChCl with 1.954 g propylene glycol for the 1:5 molar ratio and 0.550 g ChCl with 2.989 g propylene glycol for the 1:7 molar ratio. The DES were heated until a transparent liquid was formed and stored overnight in a desiccator. The next day 10 wt% 20 mg BSA/ml H_2O was added to each DES before they were put back into the desiccator.

Rheology measurements

Initial viscosity measurements

The Rheometer (Kinuxus, Malvern) was equipped with a C14 DIN standard bob and PC14 C0088 AL cup and initial routines and zero gapping was performed. The cup was loaded with 4-5ml DES. The temperature was set to 32°C. For ChCl:glycerol and ChCl:propylene glycol the start shear rate was set to 0.100 and end shear rate to 200 with 10 point for every decade. For ChCl:glucose and ChCl:sorbitol the end shear rate was changed to 1000. Every measurement was run three times with the same settings. An average was used. The measurements were repeated two times after the initial measurement.

5 wt% deionized water was added to each DES, put on heating at 85°C and gently shaken from time to time until the water was mixed in with the DES. It was then cooled down to room temperature before it was left in a desiccator for at least 2 days. The viscosity was measured again with the same settings as before. The same procedure was repeated with 10 wt%, 15 wt% and 20 wt% water with a waiting period of at least two days before each new addition of water.

Measuring low addition of water to ChCl:glycerol and ChCl:propylene glycol

New DES were made and 1 wt% 20 mg BSA/ml in water was added to each DES and stirred into the DES before the viscosity was measured. The same bob, cup and setting was used as for *Initial viscosity measurements*. This was then repeated with 2 wt%, 3 wt%, 4 wt% and 5 wt% 20 mg BSA/ml H_2O with no significant waiting in between measurements.

Measure viscosity on ChCl:glycerol with heating in between addition of water

A new 5 ml ChCl:glycerol DES were made. The viscosity on the DES were measured. The same bob, cup and settings were used as in *Initial viscosity measurements*. 1 wt% water was added to DES, it was then placed in the heating module for 5 minutes at 85°C. Once it cooled down to room temperature, the viscosity was measured with same bob, cup and settings as in *Initial viscosity measurements*. This was repeated for 2 wt%, 3 wt%, 4wt%, 5 wt%, 10 wt%, 15 wt% and 20 wt%. All samples were run within the period of a day.

Measure viscosity on ChCl:glycerol without heating in between addition of water

The same procedure were done as in *Measure viscosity on ChCl:glycerol with heating in between addition of water*, except that instead of heating the DES after each addition of water it was only stirred into the DES until a clear homogenic liquid was formed.

Formulating DES

Bentonite

5 ml ChCl:glycerol DES were made according and 5 wt% of bentonite was then added to the DES. The bentonite content was increase in 5 wt% increment until 20 wt% were reached.

Bentonite were mixed in with water to form a clay and was later added to a 2 ml ChCl:glycerol DES.

A new 5ml ChCl:glycerol DES were divided in two 10 ml glass vials. 10 wt% of a different brand of bentonite were mixed in.

Pemulen™ TR-2 NF Polymer

Big amount of Pemulen™ was added to ChCl:gly and set aside.

Cellulose

5 ml ChCl:glycerol DES and cellulose was added in 5 wt% increment until 20 wt% was reached. Another 5 ml ChCl:glycerol were created. The 5 ml DES were devided equally between 4 glass vials. Into the vials 5 wt%, 10 wt%, 15 wt% and 20 wt% were added respectively.

5 ml of ChCl:glycerol DES was made and dived into two different 10 ml glass vials. 10 wt% cellulose was added to both. One of them was heated for 20 minutes at 85°C, the other vial was left alone.

2.5 ml of ChCl:glycerol DES was made and to it 5 wt% cellulose and 10 wt% water were added and mixed together.

Carbopol 974P NJ

5 ml ChCl:glycerol DES was made and divided into two 10 ml glass vials. 10 wt% Carbopol was added to one of the vials and stirred through before left to thicken. In the other vial 5 wt% Carbopol was added, stirred through before left to thickening. Another 5 ml ChCl:glycerol DES was made and divided into two 10 ml glass vials. In one of the vials 10 wt% water was added together with 5 wt% Carbopol and stirred together before left to thickening. For the second vial 2 wt% Carbopol was added, stirred through and left to thickening.

Oscillation measurements on ChCl:glycerol with Carbopol 974P NJ

A series of 5 ml ChCl:gly DES were created and mixed with different amounts of Carbopol 974P NJ and water mixed into them, see table 3. The rheometer (Kinuxus, Malvern) was equipped with a plate din, PLC035 C0002 SS, with a diameter of 4 cm and a flat bob to match, PU40X SC0015 SS. Each sample were run a series of oscillation tests according to: LVE range measurement, frequency sweep measurements and viscosity measurements. The samples were then collected and left for the next day. Day 2 the samples were run through a series of oscillation test according to: LVE range measurements, frequency sweep measurements and amplitude sweep measurements. The parameters for each test can be found in table 4.

Table 3. Amount in wt% of Carbopol 974P NJ and water added to ChCl:gly DES

Sample	Amount Carbopol 974P NJ (wt%)	Amount water (wt%)
1	1	-
2	2	-
3	5	-
4	10	-
5	5	0.5

6	5	5
7	5	50

Table 4. Parameters used for rheology measurements in the Rheometer.

Sequence	Temp. (°C)	Start value sweep	End value sweep	Other information
LVE range	25	1 10^{-3} Pa	500 Pa	1 Hz
Frequency	25	10 Hz	0.01 Hz	-
Viscosity	25	0.1 s^{-1}	100 s^{-1}	-
LVE range	25	1 10^{-3} Pa	500 Pa	-
Frequency	25	0.1 Hz	100 Hz	-
Amplitude down	25	1 10^{-4} Pa	100 Pa	1 Hz
Amplitude up	25	100 Pa	1 10^{-2} Pa	1 Hz

Creating emulsion

5 ml ChCl:glycerol, Chcl:sorbitol, ChCl:glucose and ChCl:propylene glycol were made and 20 % H_2O were added to each DES. 3 g of each DES was then mixed with 2 g Miglyol 812 oil and 5 wt% TWEEN20 as surfactant. Each DES were run through the Ultra turax for 2 minutes. A plastic pipette was used to pipette up a drop of the emulsion, placed on an objective glass and looked at in a light microscope under 1x magnification. The rest of the emulsion was left standing.

Diffusion test with static Franz cells

Label BSA

Labelled BSA were gifted from RISE.

Calibration curve

Phosphate buffer with BSA

Phosphate buffer were prepared by mixing 405 ml 0.2 M Na_2HPO_4 was mixed with 0.2 M NaH_2PO_4 up to 500 ml. Then diluted with equal amount water, 500 ml. pH were checked and corrected. A calibration curve with concentrations 0.01, 0.03, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8 and 1 mg BSA/ml phosphate buffer, PB, with a pH of 7.4 was prepared in 5 ml glass vials. One stock solution was made containing 1 mg/ml and was then diluted into all following concentrations 160 µl of each concentration was pipetted into a well on a 96 welled microtiter plate (ThermoScinetific Nunclon™Delta Surface with 96 wells). To each well 20 µl 5 mg fluorescamine/ml acetone was added. The plate was covered with foil to hinder light to activate the fluorescamine. Buffer was added into

two wells before the fluorescence was measured in a plate reader (ThermoFisher Varioskan Lux) at an excitation of 260 nm and an emission of 465 with a gap of 12 mm. The four batches of Alexa labelled BSA was then measured with excitation of 480 nm and emission of 520 nm. Three of the batches had similar values and were combined into one stock solution, called batch 3.

DES with BSA

ChCl:glycerol was prepared and 1 ml DES was diluted 10 times with PB before a calibration curve was created. The concentrations for the calibration curve were 1 mg/ml, 0.8 mg/ml, 0.5 mg/ml, 0.3 mg/ml and 0.1 mg/ml. 160 µl of each concentration was added into a well on the microtiter plate. After 20 µl fluorescamine were added to each well before the fluorescence was measured at and excitation of 260 nm and an emission of 465 with a gap of 12 mm.

Intact skin2 ml of batch 3 was put in a centrifugation tub with a filter inside and was centrifuged for 20 minutes at 3632 rpm. The retentate was then rediluted to 2 ml with ChCl:glycerol in a 2 ml Eppendorf vial. The DES with retentate was then put on a tilting table for to mix the sample for 10 minutes. To calculate the concentration of BSA in the DES, 0.2 ml were diluted 10 times in a 0.5 ml Eppendorf vial. 160 µl of the diluted DES was then put in a well on a plate for the plate reader. Since the concentration didn't match up with the target concentration of 0.8 mg BSA/ml DES it was diluted with more DES.

1 l sodium dodecyl sulphate cleaning solution with the concentration of 1 g/l was prepared. This was then used to fill the 6 5 ml Franz cells, the syringes and let lid, packings and magnetic stirrer steep in for 15 minutes. A 1 l of phosphate saline buffer, PBS, with pH 7.4 were made. The cleaning solution was poured out and everything was rinsed with deionized water. Once rinsed everything was filled/put in PBS buffer before it was left to sit overnight. The water jackets were connected to a water bath and checked it worked properly.

The second day was started by removing bubbles from the buffer by deairing it. The Franz cells had their buffer replaced 3 times, waiting 15 minutes between each change. During that time the syringes was rinsed three times with buffer before they were left for 15 minutes with buffer inside them. This was then repeated on more time. When everything was rinsed pieces of pig skin from 3 different pig's ears were defrosted and cut in half. The 2 pieces from the same ear was put in the same cup of PBS for 15 minutes, in order to keep track of which pieces belonged together. The Franz cells were then emptied of buffer and connected to water bath and placed in a plastic holder with a magnetic stirrer underneath. See figure 7.

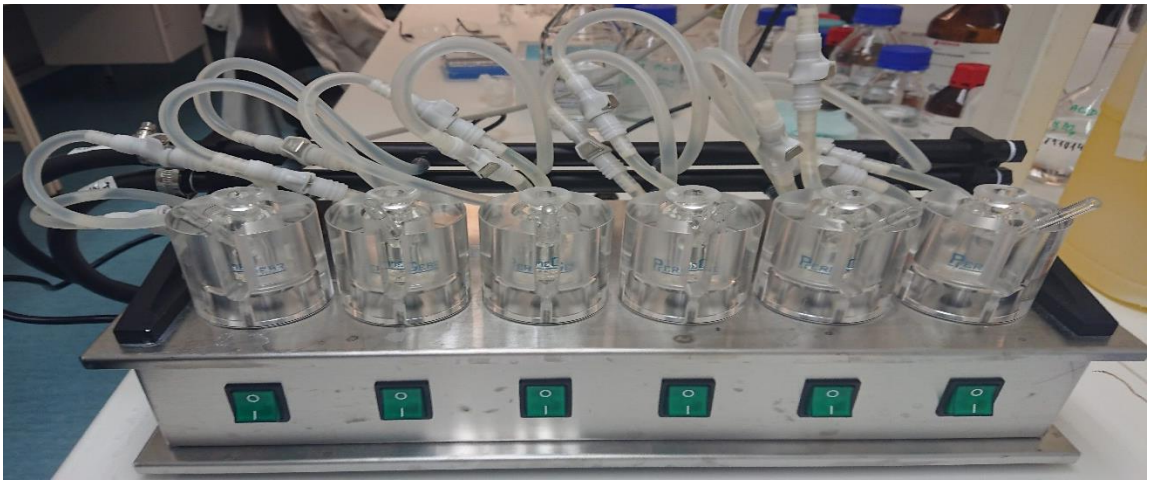


Figure 7. Picture taken of Franz cells placed in plastic holder with magnetic stirrer in the bottom and connected to water bath.

The water bath was then turned on and the temperature set to 32 °C. A magnetic stirrer was put into each of the Franz cells. Each Franz cell was then carefully filled and slightly overflowed with deaired PBS. A piece of pig skin was placed on top of the Franz cell, a packing on top making sure the pig skin was centered and covered the whole in the Franz cell. Above a lid was placed before it was clapped together. This were repeated for the other 5 Franz cells, while keeping track on which piece of pig skin went where. All Franz cells were then checked to make sure they contained 5 ml of PBS, if they did not PBS was either extracted or added to the cell. The stirrers were stared. The pigskin was carefully dried of before 200 µl of DES was put on top of three of them, all on different half of pig's skin. On the remaining three, 200 µl of batch 3 was used as a control. The lid of the 6 Franz cells were wrapped in parafilm to help avoid evaporation. The syringes were emptied out and a sample of 200 µl were taken from each Franz cell and put into 0.5 ml Eppendorf vials at time 0. These were wrapped in foil to avoid expose to light and put in the fridge. PBS were added to each Franz cell to keep the volume constant to 5 ml before the ends were wrapped in parafilm to avoid evaporation. Then the whole setup was wrapped up in foil to avoid exposure to light.

After an hour a new sample of 200 µl were taken from each Franz cell and put in a 0.5 ml Eppendorf vials, marked, wrapped in foil and stored in the fridge. New PBS was added to each Franz cell to keep the volume of 5 ml constant before everything was wrapped in parafilm and foil again.

This were repeated after 2 h, 3 h, 4 h, 5 h, 6 h and after 24 h. For the samples taken after 24 h triple samples were taken.

When the last samples were taken the pig skin were rinsed with PBS three times and dried of with tissue paper labelled and put in the freezer at -20 °C. All the labelled sample from the fridge were taken out and 160 µl of each was put into a well on a plate for the plate reader. After every sample were placed on the plate it was taken to the plate reader and measured at 480 nm to 520 nm with a gap of 12 mm.

Once the pig skin was frozen, the pieces were cut in half, diagonally and from each half a rectangle were cut and refrozen at -10 °C. The rectangle was mounted and cut into 50 µm thin slices by the crymicrotome (Leica RM22265 microtome equipped by Leica LN22 cryochamber). These slices were placed on slides and looked at in a fluorescent microscope (Zeiss Axiovert 100) under both visual light and fluorescence light with objective with 5x and 20x magnification. Additional zoom was provided by the camera used to take the pictures (Cannon PowerShot S80).

Damaged skin penetration test

The instructions in *Undamaged skin penetration test* were followed apart from how the pig skin were prepared. Instead the pig skin was placed in MilliQ water and heated in 60 °C for 90 seconds. Once heated the SC and epidermis was peeled off.

BSA treated vials

100 ml of 1 mg BSA/ml were made and filled 18 0.5 ml Eppendorf vial and left overnight. The liquid inside was poured out and replaced with 200 µl of the liquid from after 24 h of the *Undamaged skin penetration test*, 3 Eppendorf vials per Franz cell. 160 µl from each vial was put in a well on a plate and read in the plate reader at 480 nm to 520 nm.

Results and Discussion

DES

The most common molar ratio for each DES was chosen according to table 1. For ChCl:glucose Hayyan *et. al.* had written an article about where the eutectic point for the ChCl:glucose system was located and that molar ratio, 2:1, was chosen instead of the average between the articles. (Hayyan, Mjalli et al. 2013). As this molar ratio didn't form a DES at room temperature, the most common occurring molar ration was used instead, 1:1. (Radošević, Cvjetko Bubalo et al. 2015, Aroso, Paiva et al. 2017) The molar ratio for ChCl:propylene glycol did not form a homogenous liquid at room temperature, so 0.5 ml propylene glycol was added until it did.

ChCl:Glucose

ChCl:glucose with molar ratio 2:1 was heated for 2 days with mixing in between did not turn clear. Once it was taken of heating and cold it turned into a semisolid. It was then reheated but once cooled it turned semisolid again. See figure 8A.

ChCl:glucose with 1:1 molar ratio was made and heated for 1 day with stirring in between. Once cooled down it formed a lightly yellow-toned highly viscous liquid. See figure 8B. A summary of DES formulations can be found in table 5.

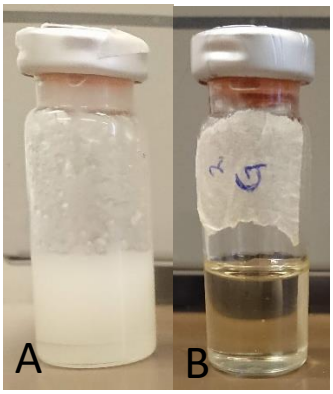


Figure 8. A: ChCl:glucose 2:1 when heated, cooled down the day after. B: Finished ChCl:glucose 1:1 once cooled down.

ChCl:Sorbitol

ChCl:sorbitol 1:1 molar ratio was slow to melt and stirring was needed to dissolve all sorbitol. It formed a clear highly viscous liquid, see figure 9. Final parameters can be found in table 3. It took time to form a DES out of sorbitol, it needed a lot of stirring and time to properly melt it together.

Figure 9. Finished ChCl:sorbitol DES with molar ratio 1:1.

ChCl:glycerol

ChCl:glycerol with molar ratio 1:2 was heated for about 30 minutes with slightly tilting during that time before a clear lightly viscous liquid was formed, see figure 10. See table 5 for parameters on creating DES. ChCl:glycerol melted together quickly.

Figure 10. ChCl:glycerol with a molar ratio of 1:2.

ChCl:Propylene glycol

ChCl:propyelen glycol with a molar ration of 1:2 crystalized as soon as it cooled down as seen in figure 11A. The crystals later fell out to a milky sediment. Reheating the sample did not improve the outcome for this ratio. The molar ratio was changed to 1:2.4 and this formed a clear viscous liquid, see figure 11B. For parameters of creating DES, see table 5.

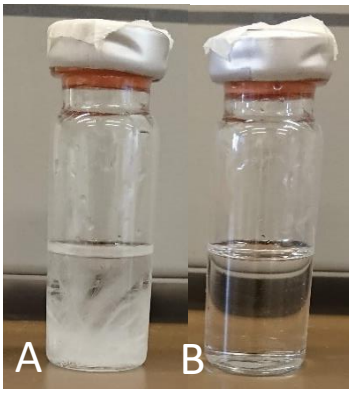


Figure 11. A: ChCl:propylene glycol with the molar ratio of 1:2 after it cooled down. B: ChCl:propylene glycol with a molar ratio of 1:2.4.

Final molar ratio for DES

In table 5 below a summary of how the stable DES from the different systems were created. Unfortunately, the stirring in the heating module was broken, meaning it had to be done manual by opening the lids and stirring it before replacing the lid. This could be a factor in why it took so long to form DES on the sugars as the sugar fell to the bottom of the vial. The liquid was then two viscous to shake it up from the bottom, creating the need open and stir them. This was not a problem for ChCl:gly and ChCl:pg. They were watery enough when hot that the vial itself could be lightly shaken to redisperse the unsolved salt to help it form a DES.

Table 5. Molar ratio for the different DES and how long time it took to form them and if any extra forces was needed in terms of stirring or tilting the vial up and down.

DES	Molar ratio	Time in ~85°C	Condition
ChCl:glucose	1:1	At least overnight*	Stirring
ChCl:sorbitol	1:1	3 h	Stirring
ChCl:glycol	1:2	0.5 h	Tilting
ChCl:propylene glycol	1:2.4	0.5 h	Tilting

*The sugar sinks to the bottom of the vial, so it had to be continuously stirred to form the DES.

BSA in DES

BSA were added to ChCl:gly and ChCl:pg DES with a concentration of 10 mg BSA/ml DES. See figure 11A-B to see that the BSA did not dissolve in the CHCl:gly DES, instead it got suspended in it while slowly sinking to the bottom of the vial. For ChCl:gly it looked the same even 2 months later. The ChCl:pg crystalized overnight and these did then dissolve into a milky sediment. After 2 months it looks the same. See figure 11C-E.

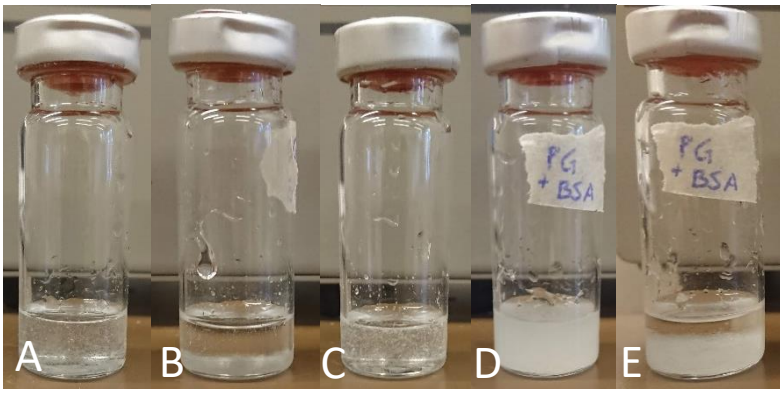


Figure 12. A: ChCl:gly with 10 mg BSA/ml DES. B: ChCl:gly with 10 mg BSA/ml DES after 24 h. C: ChCl:pg with 10 mg BSA/ml DES. D: ChCl:pg with 10 mg BSA/ml DES after 1h. E: ChCl:pg with 10 mg BSA/ml DES after 24 h.

It thus seems that in these systems a low amount of water could be needed to help solve the proteins in the DES. BSA was then solved in water with a concentration of 20 mg BSA/ml. 10 wt% of this solution was added to a new ChCl:gly and ChCl:pg.
The ChCl:gly this time stayed a clear liquid, successful keeping the BSA dissolved in the DES for 2 months before turned slightly yellow and milky. See figure 13. It could be interesting to look closer into the amount of solved BSA added to the DES and the BSA concentration in this solution to see if even longer time of stability could be achieved.

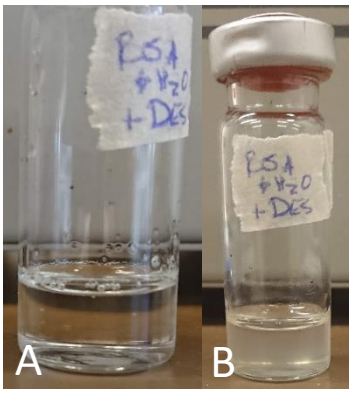


Figure 13. A: ChCl:gly with 10 wt% of 20 mg BSA/ml water after 1h. B: Same solution after 2 months.

The ChCl:pg also dissolved the BSA-water solution initially but overnight it had turned milky. See figure 14A-B. Since the ChCl:pg kept the BSA dissolved overnight different molar ratio was tried. Kuehn et al. wrote that with molar ratio up of 1:7 was possible to achieve DES. (Kuehn, Massmann et al. 2017) So DES with molar ration of 1:3 was prepared and into it 10 wt% 20 mg BSA/ml water was added. This as well turned milky overnight. See figure 14C-D. Another batch of ChCl:pg DES were made with molar ratio of 1:5 and 1:7. In these 10 wt% 20 mg BSA/ml were dissolved. This time the ChCl:pg 1:5 stayed clear for about a week before they turned milky, signifying the system not being stable any longer. The 1:7 one held out about another week before turning milky as well. See figure 14E-F. Compared to the ChCl:gly system this is a fourth of the time it kept the BSA solved. Between the ChCl:gly and ChCl:pg the ChCl:gly system seemed to be the better choice to use for further investigation.

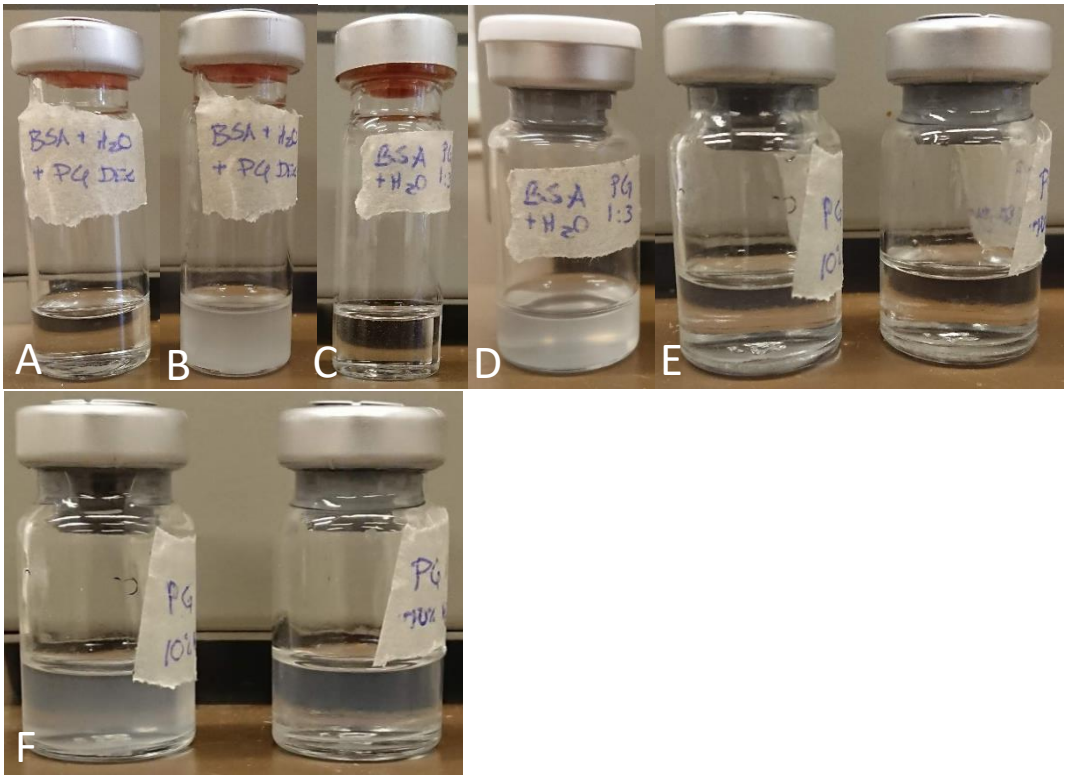


Figure 14. A: ChCl:pg with 10 wt% 20 mg BSA/ml water after 1h. B: ChCl:pg with 10 wt% 20 mg BSA/ml water after 24h. C: ChCl:pg molar ratio 1:3 with 10 wt% 20 mg BSA/ml water after 1h. D: ChCl:pg molar ratio 1:3 with 10 wt% 20 mg BSA/ml water after 24h. E: ChCl:pg molar ratio 1:5 (left) and 1:7 (right) with 10 wt% 20 mg BSA/ml water after 1h. F: ChCl:pg molar ratio 1:5 (left) and 1:7 (right) with 10 wt% 20 mg BSA/ml water after 24h.

Viscosity measurements

Viscosity was measured for ChCl:glycerol DES and ChCl:glycerol with 5 wt%, 10 wt%, 15 wt% and 20 wt% water mixed into it with the help of heating. An average of the three runs was calculated for each sample and plotted as viscosity against shear strain. The same was done for ChCl:sorbitol, ChCl:glucose and ChCl:propylene glycol. All plots showed that the DES behaved liked Newtonian liquids. An example of ChCl:gly with 10 wt% water can be seen in figure 15.

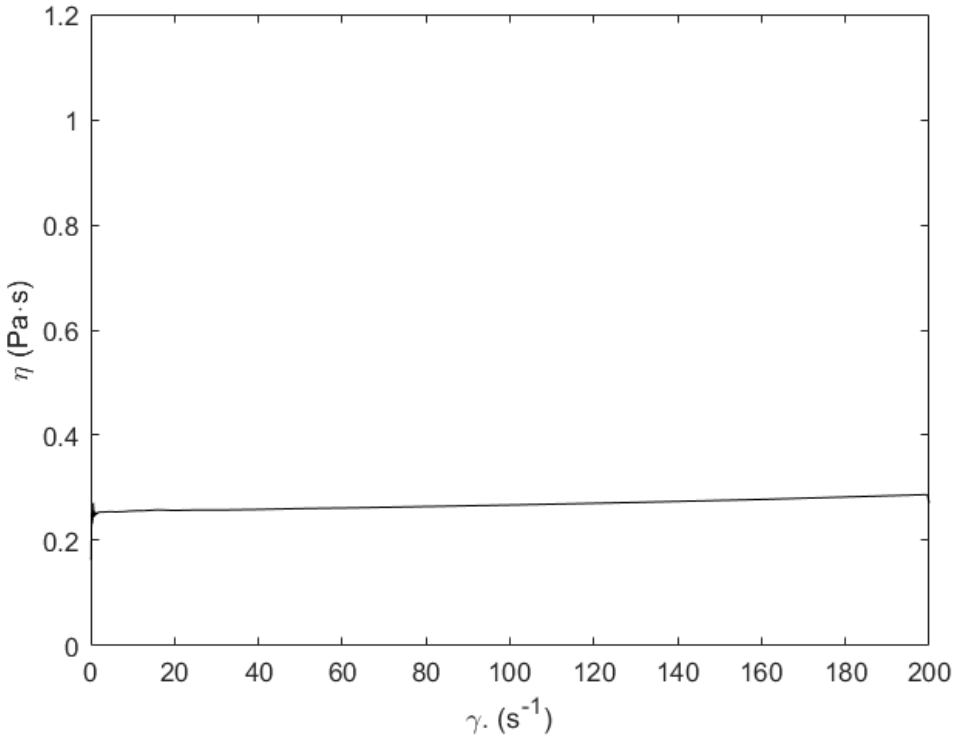


Figure 15. The plot shows an average of three measurements made in succession as to how the viscosity changes with the shear strain for ChCl:gly with 10 wt% water added with heat. The samples show a Newtonian behaviour.

From this an average viscosity was calculated from the Newtonian part of the measurements. This value was then plotted against the amount of water added to the sample. The same calculations were done for ChCl:sorbitol DES, ChCl:glucose DES and ChCl:propylene glycol DES and plotted against the amount of water added to the sample. Figure 16 shows how the different DES viscosity changes with the added amount of water. For more detailed graphs, see appendix A.

The different DES have quite different viscosity, which correspond to their behaviour in the vials. It can also be seen from figure 16 that the viscosity drops drastically for sorbitol and glycerol DES when 5 wt% water was added, but after that the viscosity seems to change less with the addition of water. The viscosity for ChCl:glucose DES seems to decline drastically for the first 10 wt% of added water before levelling out. ChCl:propylene glycol DES seem to get gradually thinner with each addition of water. The different DES have very different viscosity to begin with, from 0.2 to 1200 Pa·s. But with each addition of water they get thinner and seems to get closer to a common value. If more water was added they probably would have end up having a similar viscosity to water as the DES-system would have been dissociated with all the water. This point was not reached by adding 20 wt% water.

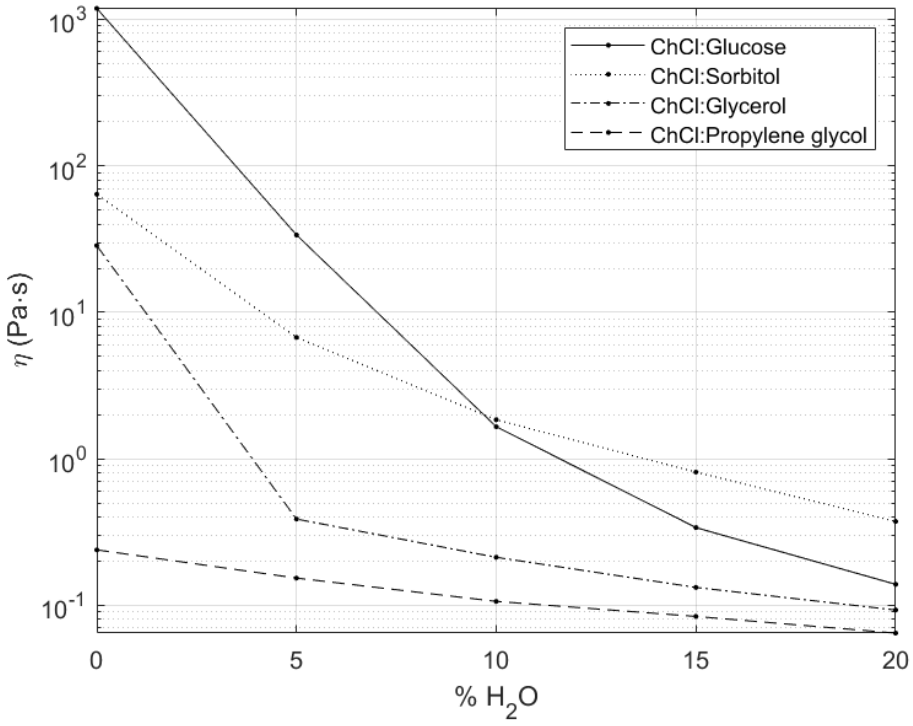


Figure 16. Viscosity measurements for ChCl:glucose, ChCl:sorbitol, ChCl:glycerol and ChCl:propylene glycol with 0, 5, 10, 15 and 20 wt% water added to them. Plotted on a logarithmic scale of viscosity against percent of water added.

To note here is that the measurement was not the best, the rheometer only measured a small interval of shear strain for some of the DES instead of the one asked for, this could be because the DES were too viscous for the bob to rotate or a mistake was made with the measurements. Since the largest different seems to be between no water and 5 wt% water added to the DES; 1 wt%, 2 wt%, 3 wt%, 4 wt% and 5 wt% water was added to a new DES of ChCl:glycerol and ChCl:propylene glycol and had their viscosity measured. They were then plotted into the same graph with the viscosity measured with higher amount of water as can be seen in figure 17.

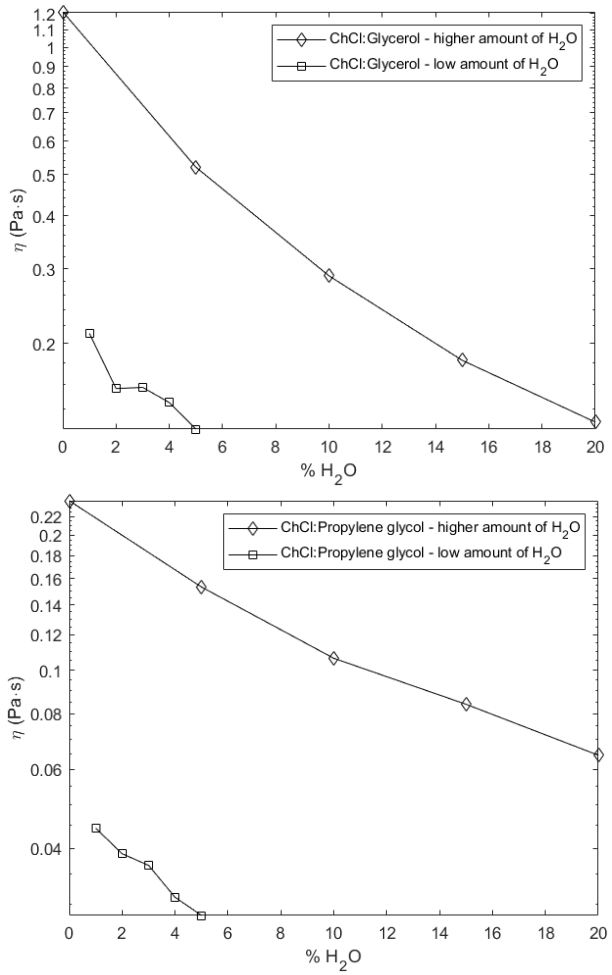


Figure 17. Left: ChCl:gly with small and large amount of water added to it with their viscosity measured and plotted as viscosity against shear strain. Right: ChCl:pg with small and large amount of water added to it with their viscosity measured and plotted as viscosity against shear strain.

The viscosity from the two runs that is seen in figure 17 does not match at all. One reason for this could be that the run with lower water amount added was not heated between each addition since the water contained BSA as well. The DES without water was forgotten to be measured so the zero point is unknown. The measurements were also made from different batches of Choline Chloride. So another set of ChCl:glycerol DES were made; one where the samples were heated between each addition of water, where the other was not heated between water addition instead the water was just stirred through – like the second batch with low addition of water. These were measured for 0, 1, 2, 3, 4, 5, 10, 15 and 20 wt% water. All four runs with ChCl:gly with addition of water is plotted in figure 18.

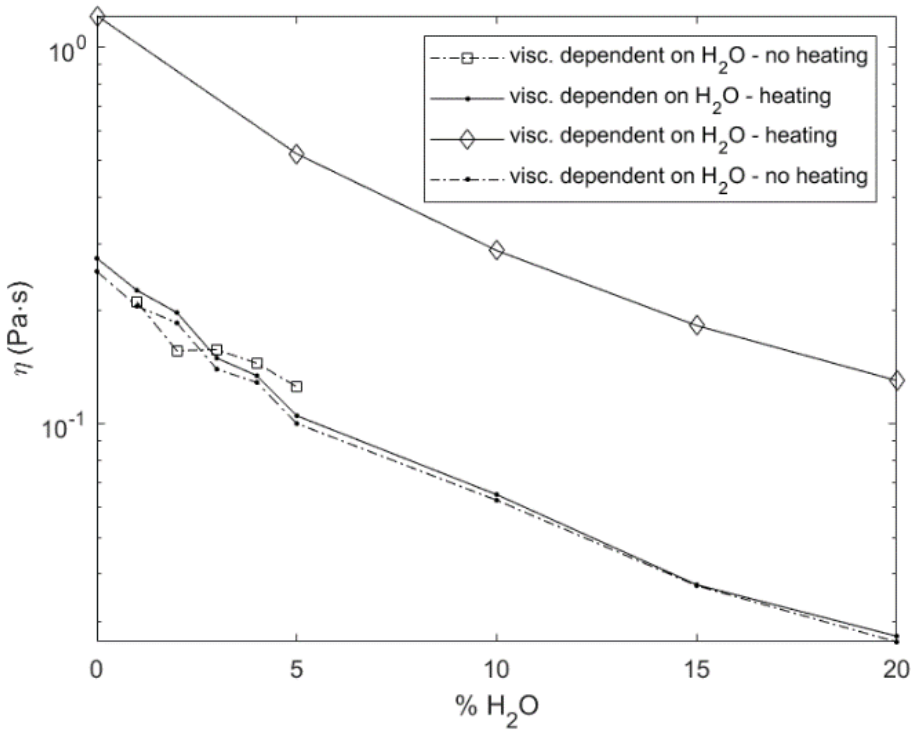


Figure 18. All runs of viscosity measurements on different batches of ChCl:gly. First: Large amounts of water added with heating. Second: Low amount of water added, no heating. Third: Wide range of water added with heating. Fourth: Wide range of water added without heating.

From figure 18 it is clear that mistakes could have been done while running the first set of viscosity measurements. If that is seen as an outlier, a new graph with the three last runs can be seen in figure 19. The second run with addition of water with heating was the same batch of ChCl as the measurements with low addition of water. For the last run with addition of water without heating another batch of ChCl were used, but this one still matched well with the runs with the second batch of ChCl. This lets on that the difference is not related to changes in the ChCl. From figure 19 it can also be seen that heating the DES is not necessary to solve the water in the DES, it can be done by stirring it into the DES at room temperature.

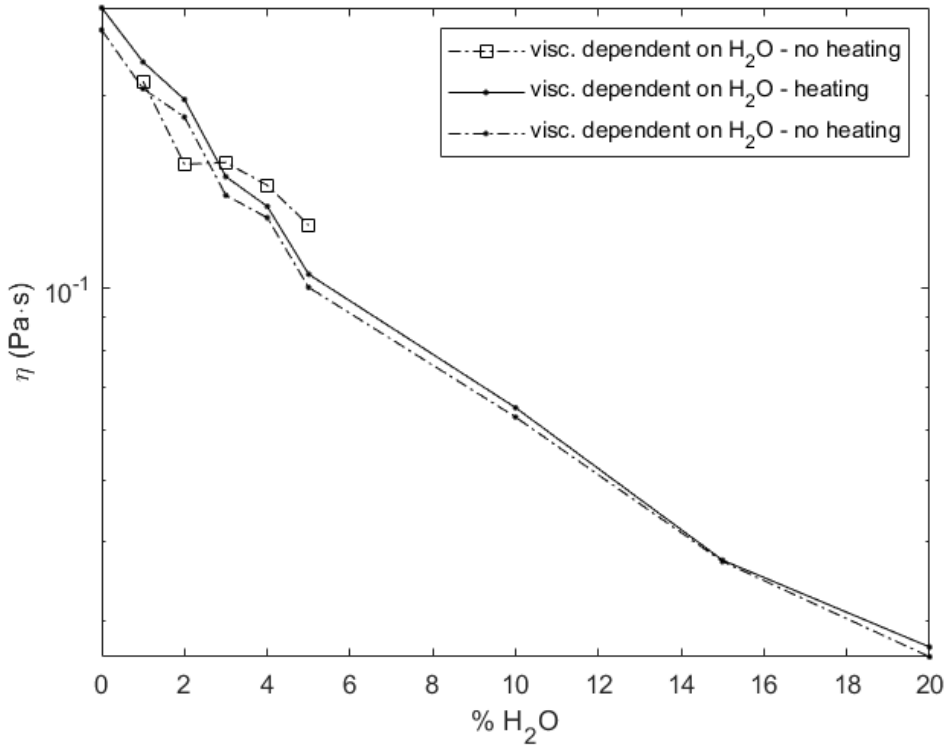


Figure 19. Second, third and fourth run of viscosity measurments of ChCl:gly plotted as viscosity against shear strain.

Formulation

Bentonite

Bentonite was mixed in different weight percent into ChCl:gly DES. In figure 20 a time laps can be seen of what happened if bentonite is mixed into ChCl:gly DES. It sinks to the bottom in about 30 minutes. The picture is of 20 wt%, but the same happened with 2 wt%, 5 wt%, 10 wt% and 15 wt%. Bentonite was also mixed with water, forming a loose clay and then mixed in with the ChCl:gly DES. This did not make any different. The bentonite still did not stay in the DES or thicken the DES to any noticeable degree.

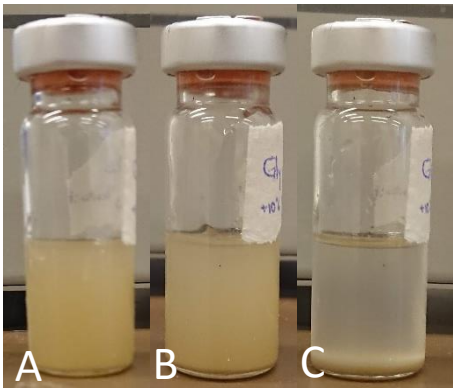


Figure 20. A: 20 wt% bentonite added to ChCl:gly. B: 20 wt% bentonite in ChCl:gly after 1 h. C: 20 wt% bentonite in ChCl:gly after 24h.

Bentonite usually works well when forming gels, but it had no effect on the DES, this might be because of the way the DES is made of a network of hydrogen bonds.

Pemulen™ TR-2 NF Polymer

Pemulen™ was added in big amount to ChCl:gly and after an hour it had formed a stiff gel, see figure 21.

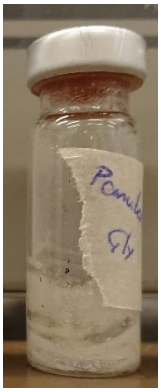


Figure 21. Pemulen™ added to ChCl:gly, 1 h after addition.

Microcrystalline Cellulose

Cellulose was added to ChCl:gly in 5 wt%, 10 wt%; 15 wt% and 20 wt% as seen in figure 22A. The consistency became softer, smoother and a little less sticky with increasing amount of cellulose added. But as can be seen in figure 22C the cellulose sedimented in the time frame of a few hours. The higher weight percent, the longer it took to sediment. The vials were then heated on 85 °C for about 30 minutes. The results were the same, after a few hours the cellulose had sedimented. A comparison was made between two ChCl:gly DES with 5 wt% cellulose added to both and 10 wt% water was added to one of them, see figure 22B. The cellulose in both sedimented overnight. They were heated for 20 minutes at 85 °C but the same results were achieved.

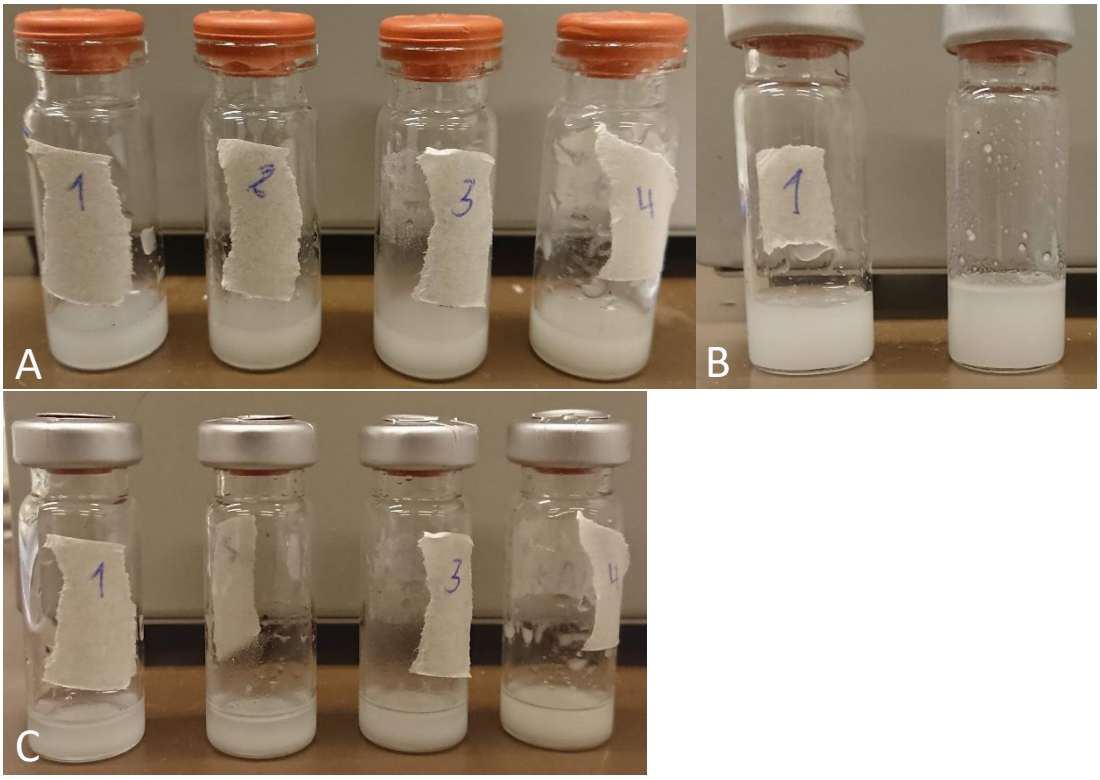


Figure 22. A: left to right: 5, 10, 15 and 20 wt% microcrystalline cellulose added to ChCl:gly. B: left: 5 wt% cellulose added to ChCl:gly, right: 5 wt% cellulose and 10 wt% water added to ChCl:gly. Picture taken after heating for 20 minutes at 85 °C. C: left to right: 5, 10, 15 and 20 wt% microcrystalline cellulose added to ChCl:gly, 24h.

The cellulose added a nice feel to the DES, making it more pleasant when spreading it on the skin. It seemed like neither adding small amounts of water or heating on the DES could help the cellulose incorporate into the DES.

Carbopol 974P NJ

Carbopol 974P NJ was added to ChCl:gly DES in 5 wt% and 10 wt%. It took about an hour until it was a thick, slightly stretchy gel. The one containing 5 wt% felt like Vaseline. The one containing 10 wt% had similar feel to the 5 wt%, but it was thicker and harder to spread, it felt a little like dried out slime. Both had a pleasant feeling when applied to the skin.

2 wt% Carbopol 974P NJ was added to ChCl:gly DES and after about an hour it had turned into a gel. This time it was a lot looser and wetter compared to the 5 wt% and 10 wt%. It felt more like a water-based gel, see figure 23. In another ChCl:gly DES 5 wt% Carbopol 974P NJ was added together with 10 wt% water. This formed a gel as well, but with lumps in it making it a bit of an interesting experience when applying it and spreading it on skin, see figure 23.

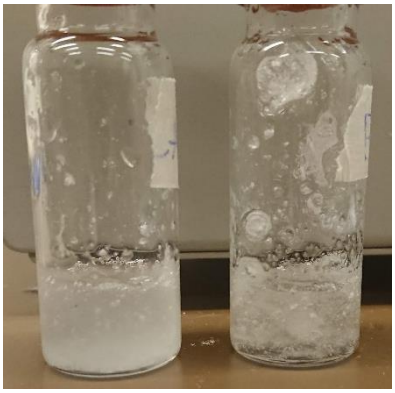


Figure 23. Left: 2 wt% Carbopol 974P NJ added to ChCl:gly, 2h. Right: 5 wt% Carbopol 974P NJ and 10 wt% water added to ChCl:gly, 2 h.

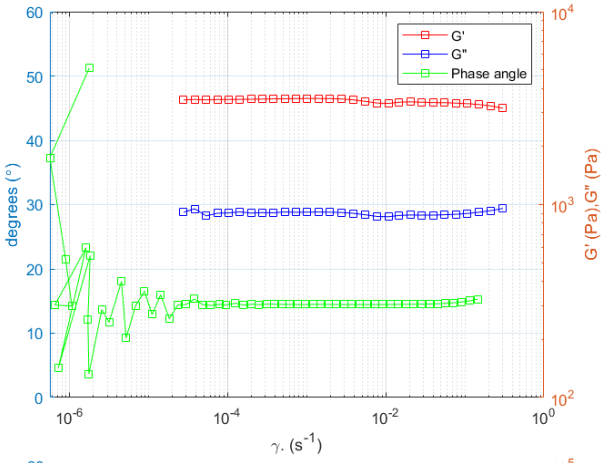
Oscillation

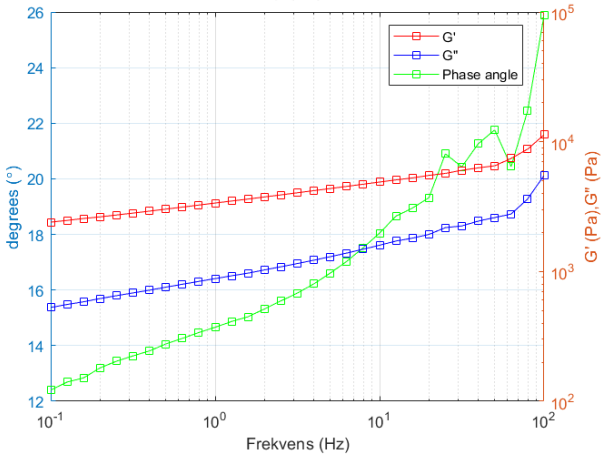
The samples that were prepared according to table 3 were run through oscillation measurements and a summary of their LVE range values from day one and two together with the shear strain value at the end of the LVE range can be found in table 6.

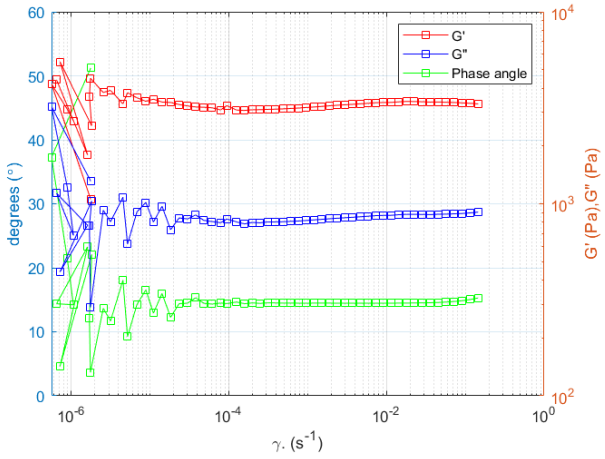
Table 6. Measured data of G', G'' and value of shear strain at then of LVE range for samples done day 1 and day 2.

Sample	G’ (Pa)		G’’ (Pa)		Υ_L (s^{-1})	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
1% Carbopol	19.92	7.836	18.07	1.889	0.04469	0.02425
2% Carbopol	58.19	264.3	57.08	118.1	$9.083 \cdot 10^{-5}$	0.007382
5% Carbopol	1359	1433	419.3	428.5	0.07295	0.003913
10% Carbopol	3056	3553	783.4	936.1	0.005728	0.1458
5% C + 0.5% H2O	1679	1528	487.7	431.4	0.0754	0.06185
5% C + 5% H2O	1321	1267	357.1	330.6	0.005301	0.007151
5% C + 50% H2O	428.8	435.9	44.84	44.64	$4.412 \cdot 10^{-5}$	0.006092

Below in figure 24 is a set of how the graphs looked for the samples. The sample presented in figure 24 is the one with 10 wt% Carbopol. For all graphs related to each sample, see appendix B.


G'
G"
Phase angle
degrees (°)
G' (Pa),G" (Pa)
γ. (s-1)


G'
G"
Phase angle
degrees (°)
G' (Pa),G" (Pa)
Frekvens (Hz)


G'
G"
Phase angle
degrees (°)
G' (Pa),G" (Pa)
γ. (s-1)

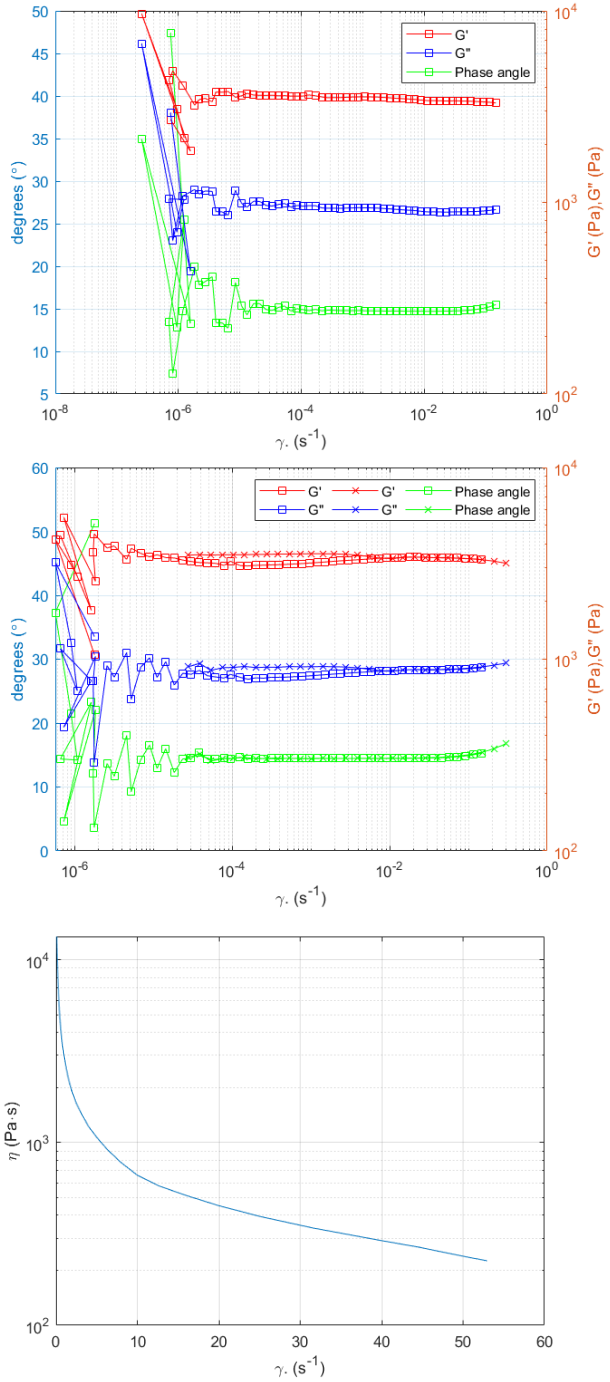


Figure 24. Graphs from oscillation measurments for 10 wt% Carbopol, day 1 and 2 combined.

From the graphs in figure 24 and appendix B then type of material can be figured out. All samples tested were shear thinning. Except for 1 wt% Carbopol, all of them had G'>G'' making them gels or viscoelastic solids. For an overview of what the samples were, see table 7.

Table 7. Summarization of how samples behaved from data collected in the oscillation measurements and all graphs used can be found in appendix B.

Sample	From LVER/amplitude sweep	Frequency	Viscosity
1% Carbopol	G''>G' -> fluid	Over time liquid	Shear thinning

2% Carbopol	G'>G'' -> gel/solid	G'>G'', but not stable	Shear thinning
5% Carbopol	G'>G'' -> gel/solid	Gelled slight freq. dependant	Shear thinning
10% Carbopol	G'>G'' -> gel/solid	Gelled slight freq. dependant	Shear thinning
5% C + 0.5% H2O	G'>G'' -> gel/solid	Gelled slight freq. dependant	Shear thinning
5% C + 5% H2O	G'>G'' -> gel/solid	Gelled slight freq. dependant	Shear thinning
5% C + 50% H2O	G'>G'' -> gel/solid	Gelled slight freq. dependant	Shear thinning

From table 7 it can be seen that the Carbopol could gel the DES without problem. Even when adding water, they held together. 1 wt% Carbopol were more fluid like in structure than the other, telling that 1 wt% Carbol is too little to make a gel. When spread on the skin, 5 wt% Carbopol has the best feel. 10 wt% is too stiff, it needs to much force to spread it easily. When adding water to 5 wt% it gives them an even better feel, loosen up the gel and making it spread easier. The one containing 50 wt% water feels like it has lost its gel structure. This can be backed up by looking at the amplitude and frequency sweeps graph in appendix B, since these graphs were a bit all over the place. To get a feel of how they felt, see figure 25 for a view of how they looked in real life. If the viscosity of the different samples is compared, see figure 26, the feeling is backed up by the data. We see that 10 wt% Carbopol has outstanding viscosity, so high the rheometer couldn't measure the whole interval. The 5 wt% Carbopol is grouped together with the other 5 wt% Carbopol with 0.5 and 5 wt% water. It is also easy to see that 1 wt% Carbopol has very low viscosity compared to all other samples, which is backed up by it looking and behaving like a liquid.

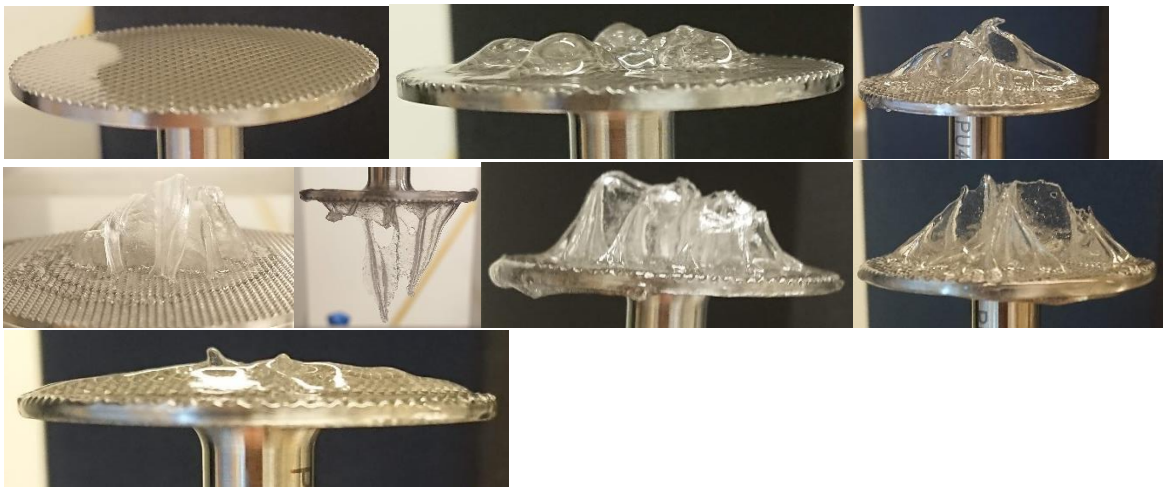


Figure 25. A look into how the different samples looked when running them through the rheometer. Left to right, top to bottom: 1 wt% Carbopol, 2 wt% Carbopol, 5 wt% Carbopol, 10 wt% Carbopol (2 pics), 5 + 0.5 wt% Carbopol + water, 5 + 5 wt % Carbopol + water and 5 + 50 wt% Carbopol + water.

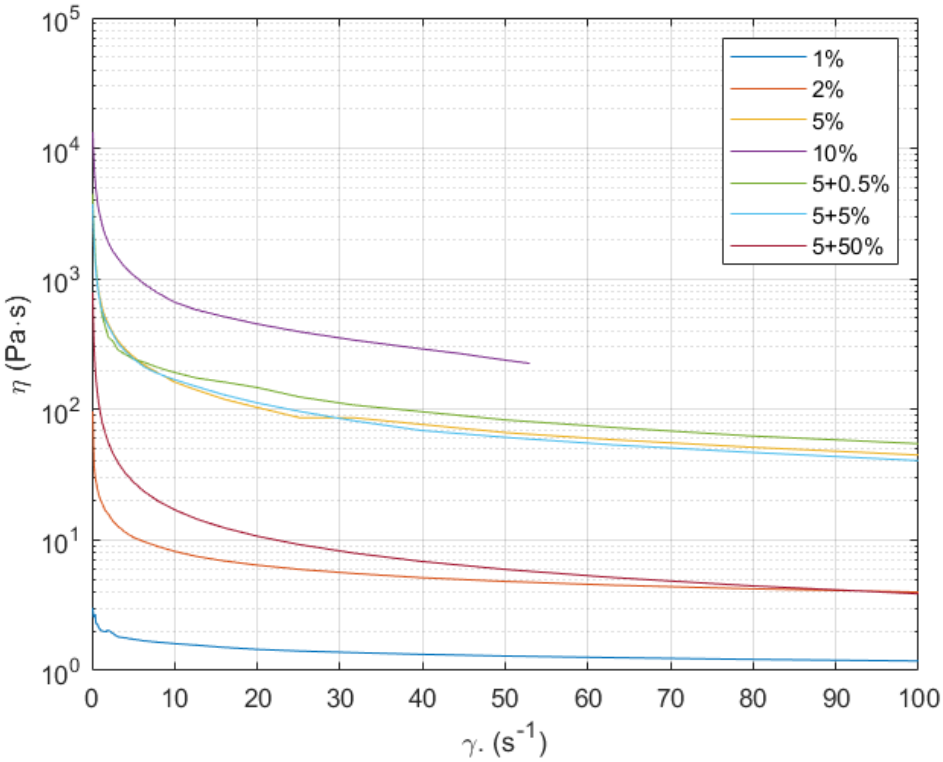
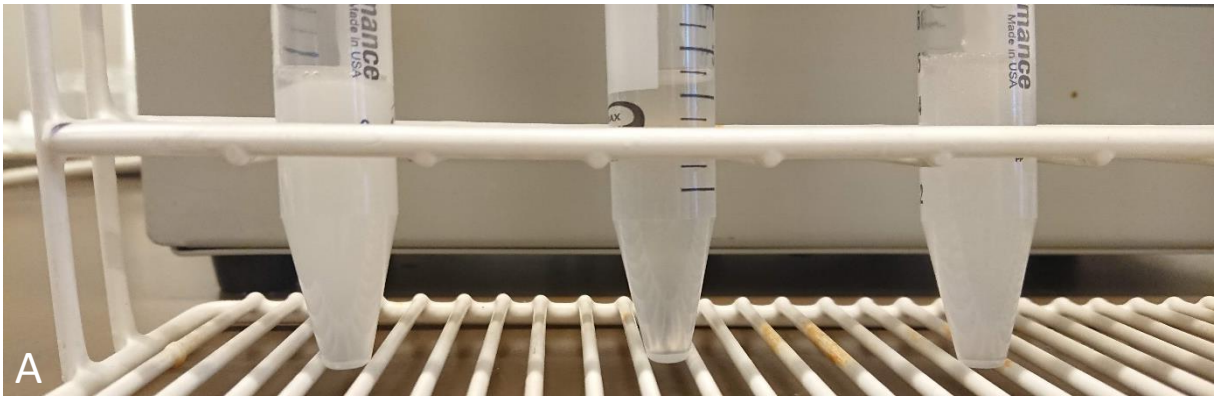


Figure 26. Viscosity of all different Carbopol samples in the same graph to see how the viscosity changes with shear strain.

Emulsions

Emulsion was created of the different DES with 20 wt% water, Mygliol 812 N and TWEEN20. The results can be seen in figure 27. The ChCl:g were not stable enough to get any microscopy pictures of it separated faster than the time it took to pipette it up and place it on a slide and as a result it could not be looked at under the microscope.



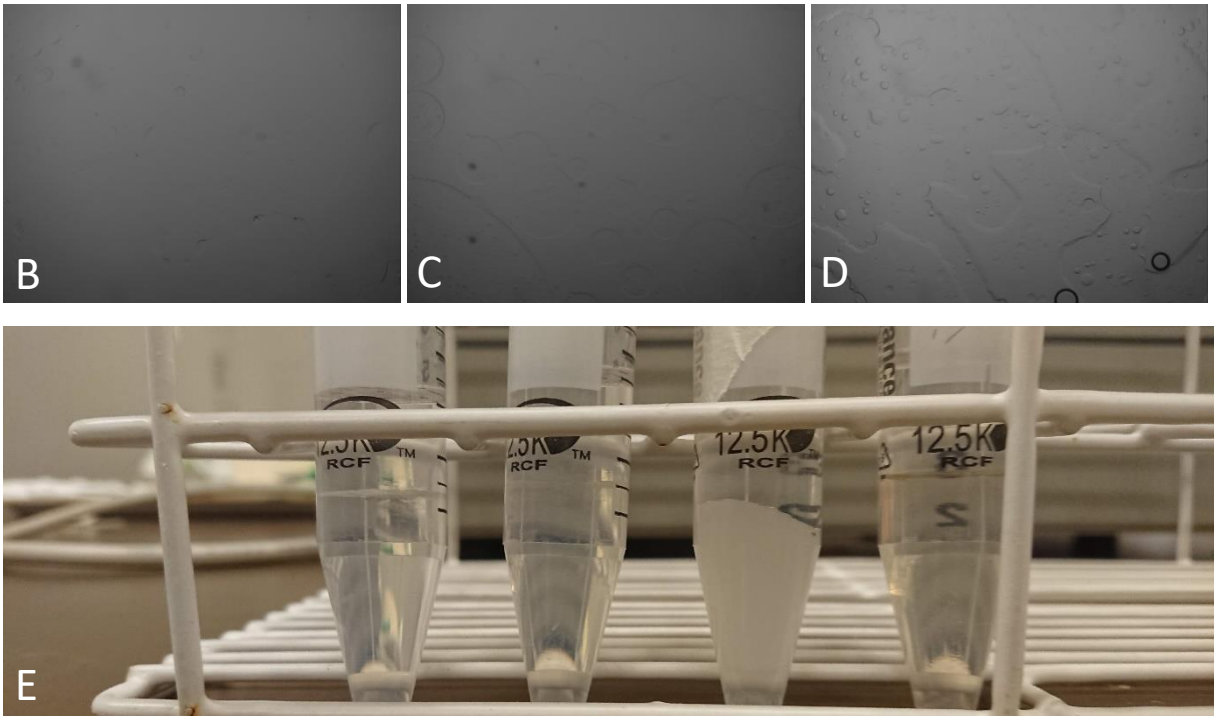


Figure 27. A: from left to right: ChCl:gly, ChCl:pg and ChCl:s emulsions, picture taken directly after all being mixed. B: microscopy picture of ChCl:gly emulsion, 1x zoom. C: microscopy picture of ChCl:pg emulsion, 1x zoom. D: microscopy picture of ChCl:s emulsion, 1x zoom. E: left to right: ChCl:gly, ChCl:pg, ChCl:s and ChCl:g emulsions after 1 month. The sorbitol has fallen out of the DES.

The emulsions were left by themselves and as seen in figure 27 the ChCl:s DES one seems to have had the sorbitol precipitated out of the DES and formed something like solid sorbitol in the solution. This might be because of the sorbitol is close to its stability as a solution and when adding something, it precipitates. Do not know why this would happen or why it only happened to the sorbitol. Otherwise as can be seen in figure 27 the emulsion did not keep stable for any period of time making this type of formulation a bad idea. It can be that other ways of making emulsions can fit with the eutectic liquids better, but this attempt was not successful.

Franz cell diffusion test

Undamaged skin

Absorbance from the Franz cell diffusion test with undamaged skin were run in a plate reader. From the measured absorbance concentration can be calculated with the help of measuring the full penetration and knowing the start value. Full penetration was calculated to 0.032 mg/ml. The table 8 shows how concentration changed over time for each Franz cell, the first three being the control one with BSA in PBS and the last three of the ChCl:glycerol DES with BSA in it. At the bottom of the table the percentage of penetration after 24 h was calculated. For a visualization of the results, see figure 28 for the plotted data of concentration against time for the different Franz cells.

Table 8. Concentration of Franz cell diffusion test on undamaged skin for 6 Franz cells, three as control with BSA solved in PBS and three with BSA solved in ChCl:gly DES.

Time	PBS 1	PBS 2	PBS 3	DES 1	DES 2	DES 3
0	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000

1	0,00001	0,00002	0,00001	0,00001	0,00001	0,00001
2	0,00003	0,00002	0,00002	0,00001	0,00001	0,00001
3	0,00005	0,00004	0,00002	0,00002	0,00002	0,00001
4	0,00009	0,00004	0,00003	0,00002	0,00002	0,00002
5	0,00014	0,00005	0,00003	0,00003	0,00002	0,00002
6	0,00019	0,00006	0,00003	0,00003	0,00003	0,00002
24	0,00163	0,00043	0,00016	0,00008	0,00013	0,00010
% penetration	5,09%	1,35%	0,50%	0,25%	0,39%	0,33%

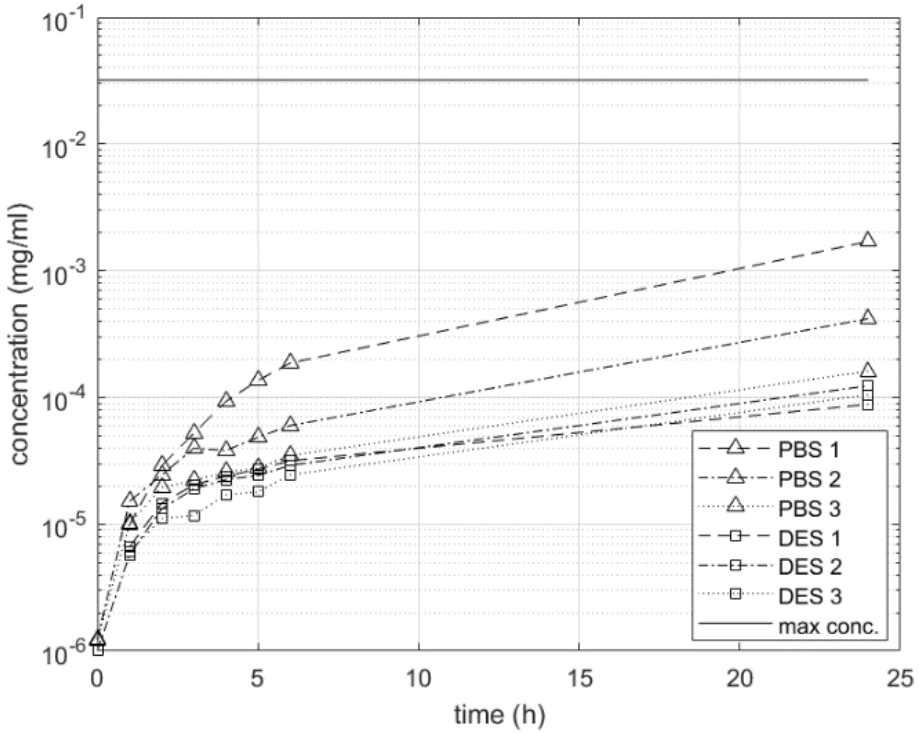


Figure 28. Concentration from Franz cell diffusion test on undamaged skin plotted against time. The straight line represents full penetration of BSA.

As can be read from table 8 and seen in figure 28 penetration was low. For the DES it got an average below 1 % after 24 h which is a very close to zero it doesn't make a difference it if was applied to the skin or not. The values for PBS were slightly higher with an average of 2 % penetration after 24 h. But to look at it as a whole it had no noticeable penetration for either sample. Then it should be noted that the buffer solution performed better than the DES which is not the desirable results. Protein are not stable in a buffer solution for long period of times which is why they are not turned into pharmaceuticals. So, having a formulation that is as bad is not a good sign. But it should be

mentioned skin penetration through undamaged skin without mechanic or chemical is enhancement is hard and the hope was at the ChCl:gly DES would work as a chemical enhancer.

Damaged skin

From the absorbance measured for damaged skin together with the value of full penetration the concentration can be calculated. The results can be seen in table 9. Included in the table is also percentage of total penetrated mg BSA after 24 h. The same data is plotted in figure 29 on logarithmic scale.

Table 9. Concentration of Franz cell diffusion test on damaged skin for 6 Franz cells, three as control with BSA solved in PBS and three with BSA solved in ChCl:gly DES.

Time	PBS-1	PBS-2	PBS-3	DES -1	DES-2	DES-3
0	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000
1	0,00006	0,00001	0,00004	0,00003	0,00003	0,00004
2	0,00024	0,00010	0,00008	0,00003	0,00004	0,00006
3	0,00058	0,00038	0,00030	0,00005	0,00005	0,00009
4	0,00104	0,00071	0,00061	0,00004	0,00007	0,00003
5	0,00153	0,00112	0,00098	0,00017	0,00008	0,00030
6	0,00193	0,00150	0,00132	0,00027	0,00011	0,00043
24	0,01257	0,01172	0,01105	0,00525	0,00284	0,00646
% penetration	39,28%	36,63%	34,52%	16,41%	8,89%	20,18%

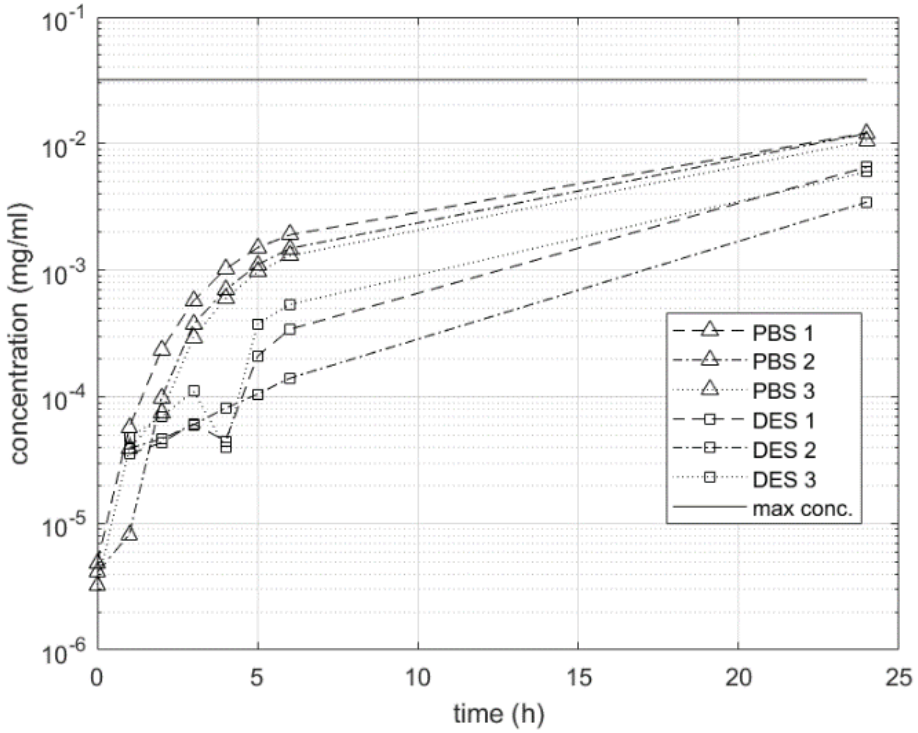


Figure 29. Concentration from Franz cell diffusion test on damaged skin plotted against time. The straight line represents full penetration of BSA.

The damaged skin follows the same trend as the undamaged skin; the PBS gives better penetration than the ChCl:gly DES formulation. This time PBS had an average penetration of 37 % penetration after 24 h which is more respectable. The DES formulation had an average penetration of 15 % after 24 h. This is 60 % less penetration than the PBS, which is unfortunate as buffer is not an option for a formulation. It could still be that another DES gives better results than the buffer, but for the ChCl:gly DES it does not seems to be a viable option. One thing to notice is that the liquid in the donor compartment of the DES Franz cell held the double amount of liquid, 400 µl, after 24 h compared to 200 µl when the experiment was started. This would result in that the concentration if BSA got lower over time and made penetration slower. The reason behind this unexpected change in volume could be because of the high osmolality of the ChChl:gly DES since it contains a lot of salt and has a high glycerol content this could have driven liquid from the acceptor chamber through the skin and into the donor compartments liquid. The choline chloride is also very hydroscopic, meaning it could have taken up water from the air and diluted itself that way as well. This resulting in the ChCl:gly DES not being a good option for a topical formulation.

BSA treated vials

While running the diffusion test on undamaged skin vials were filled with BSA and left overnight. The samples taken after 24 h of diffusion in the Franz cells were collected in both BSA treated vials and vials without BSA treatment. The results are shown in figure 30, where the specific Franz cell is plotted against the measured absorbance value for BSA treated vials and vials without BSA treatment.

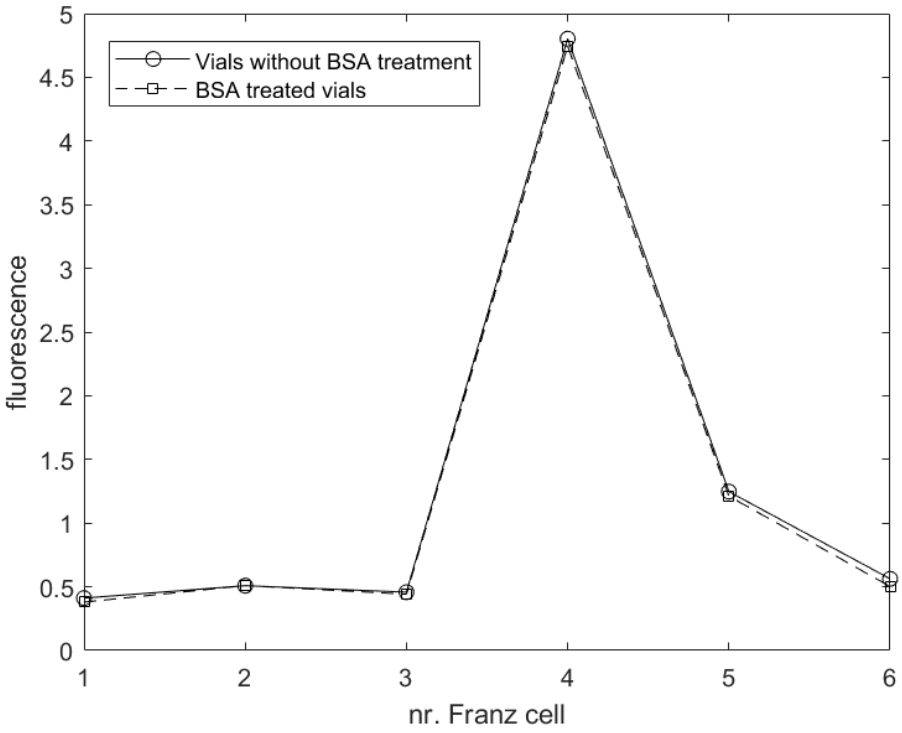


Figure 30. Comparison between BSA treated vials and vials without pre-treatment with BSA. Each square/circle represents one of the different Franz cells and how their absorbance compared between pre-treating the vials with BSA or not. The line is there to help visualize the difference between vials treated with BSA or without.

As can be seen from looking at figure 30, the absorbance is not affected by the vials. The fear was that the BSA would adsorb to the surface of the vials and give lower readings than the actual values. But as can be seen in figure 30, it didn't adsorb to the walls to such extent that it affected the measurements and thus precoating the Eppendorf vials and other equipment is not necessary to get a correct reading.

Fluorescence and microscopic pictures

Undamaged skin

Microscope pictures both under normal light and with a fluorescent light were taken of the pig skin sample from Franz cell 4 containing ChCl:gly DES after the diffusion experiment was finished, see figure 31. From figure 31 it can be seen that the fluorescence is weak, indicating that only a minor amount of fluorescent labelled BSA has penetrated the skin.

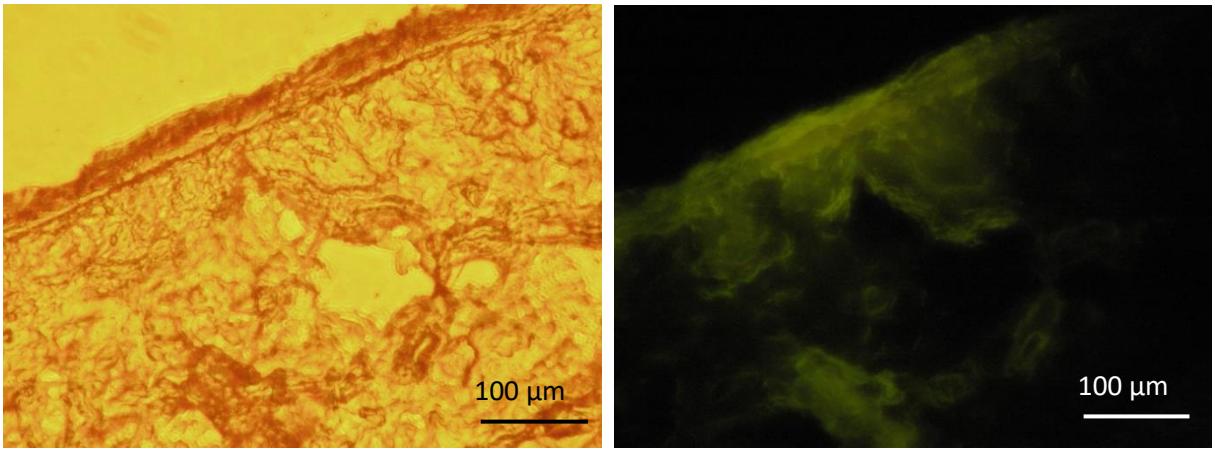


Figure 31. Left: Microscopy picture of undamaged pig skin, frozen, after 24 h of diffusion of BSA in ChCl:gly DES under normal light. Right: Microscopy picture of undamaged pig skin, frozen, after 24 h of diffusion of BSA in ChCl:gly DES under fluorescent light.

The same procedure was done for Franz cell 1 where the other half of the same pig skin were used. Making the conditions for the diffusion as similar as possible. The results of the phosphate buffer control can be found in figure 32, pictures were taken with both normal light and fluorescent light. Compared to the pictures taken of DES on undamaged skin, the pictures of PBS on undamaged skin in figure 32 has a brighter colour, meaning more fluorescent BSA has penetrated through, which matches with the results from the data from the diffusion measurements, seen in figure 28.

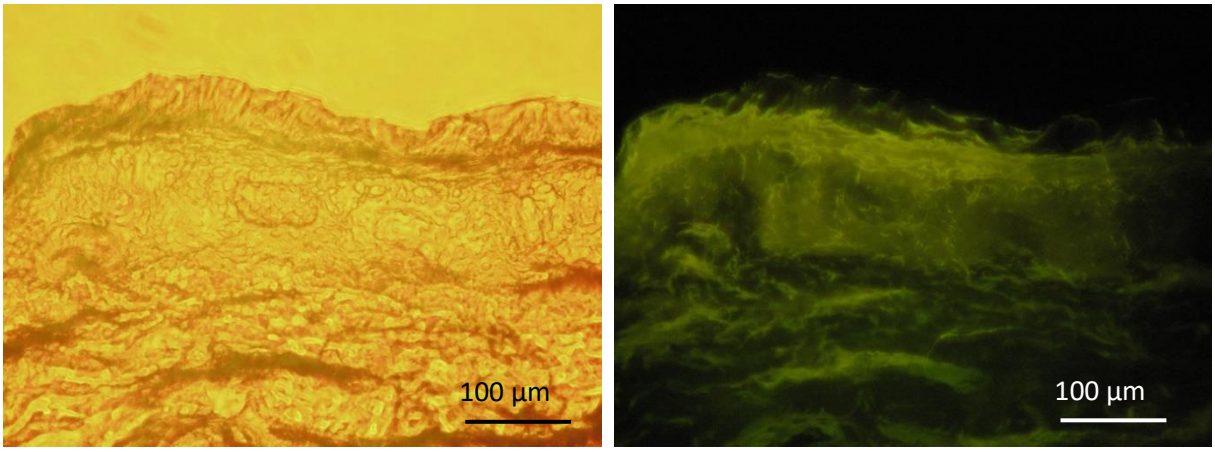


Figure 32. Left: Microscopy picture of undamaged pig skin, frozen, after 24 h of diffusion of BSA in PBS under normal light. Right: Microscopy picture of undamaged pig skin, frozen, after 24 h of diffusion of BSA in PBS under fluorescent light.

The whole of the skin pieces were looked at in the microscope under fluorescent light to determine the thickness of the skin. Zoom was set to 5 times and with that the thickness could be calculated. The thickness from the Franz cell 4 with DES formulation was calculated to 0.7 mm and for Franz cell 1 with PBS the thickness was calculated to 0.8 mm. For a visualization, see figure 33. Optimum the skin would have the same thickness, making the conditions of penetration as close as possible between the DES and PBS. As the PBS had better penetration, despite it the piece of pig skin being thicker it just enhances the fact that the PBS performed better than the DES.

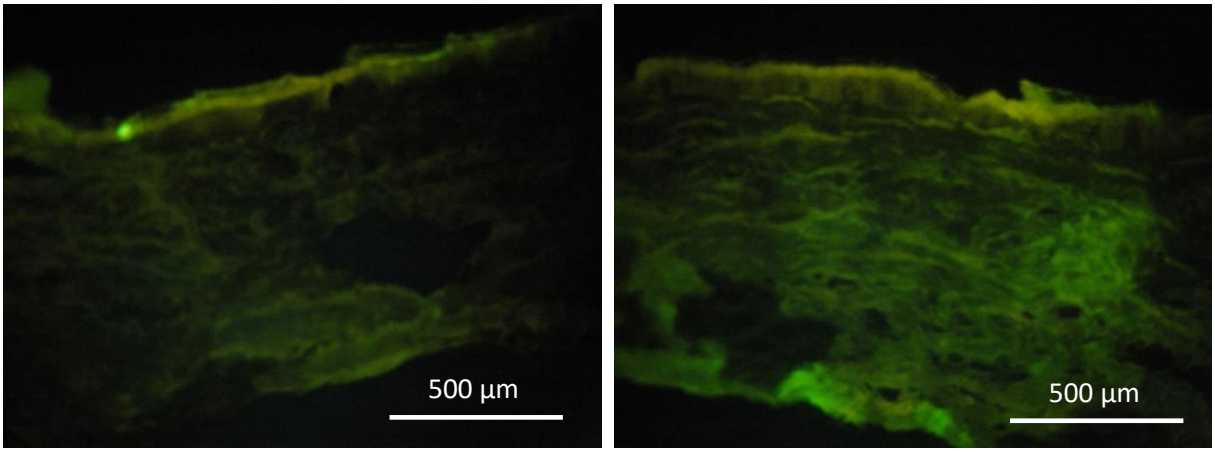


Figure 33. Left: Piece of undamaged pig skin, frozen, after 24 h of BSA in ChCl:gly DES diffusing through. Right: Piece of undamaged pig skin, frozen, after 24 h of BSA in PBS diffusing through.

Damaged skin

Figure 34 shows damaged skin after ChCl:gly for DES formulation after the diffusion experiment. If the picture in figure 34 is compared to the picture in figure 31 it can be seen that the fluorescence is considerable higher indicating that a higher amount of BSA has penetrated through the damaged skin.

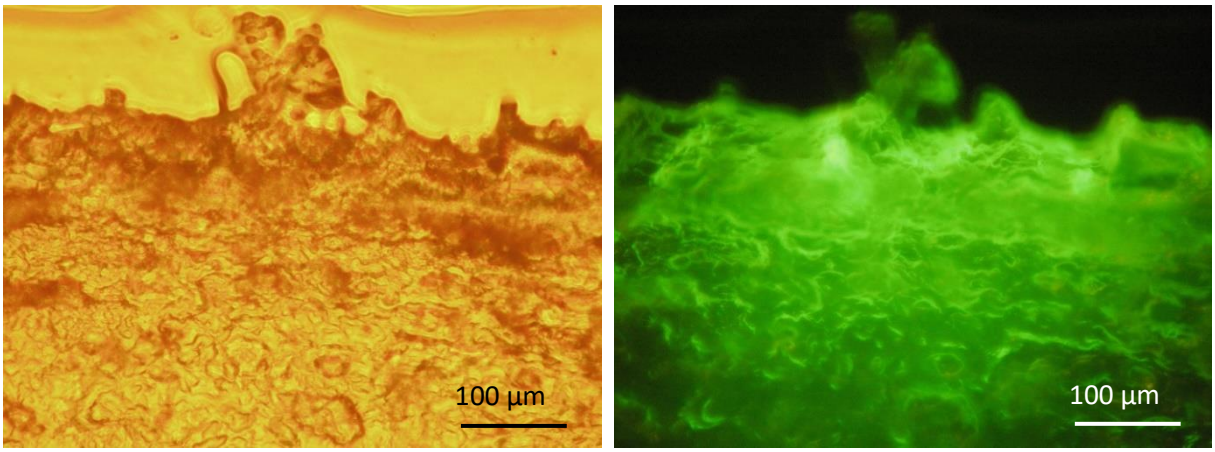


Figure 34. Left: Microscopy picture of damaged pig skin, frozen, after 24 h of diffusion of BSA in PBS under normal light. Right: Microscopy picture of damaged pig skin, frozen, after 24 h of diffusion of BSA in PBS under fluorescent light.

In figure 35 a microscopy pictures can be seen of damaged skin from Franz cell 1 PBS in normal light and fluorescent light can be seen. This is as well is the other half of the same piece of skin that was used for the damaged skin in Franz cell 4 with DES formulation. From figure 35 it can be seen that a lot more fluorescent BSA has penetrated compared to figure 32, PBS through undamaged skin. This follows nicely along with the diffusion experiment. Comparing the picture in figure 34 and 35 it is harder to visually spot any different in amount of penetrated fluorescent BSA from the brightness of the picture.

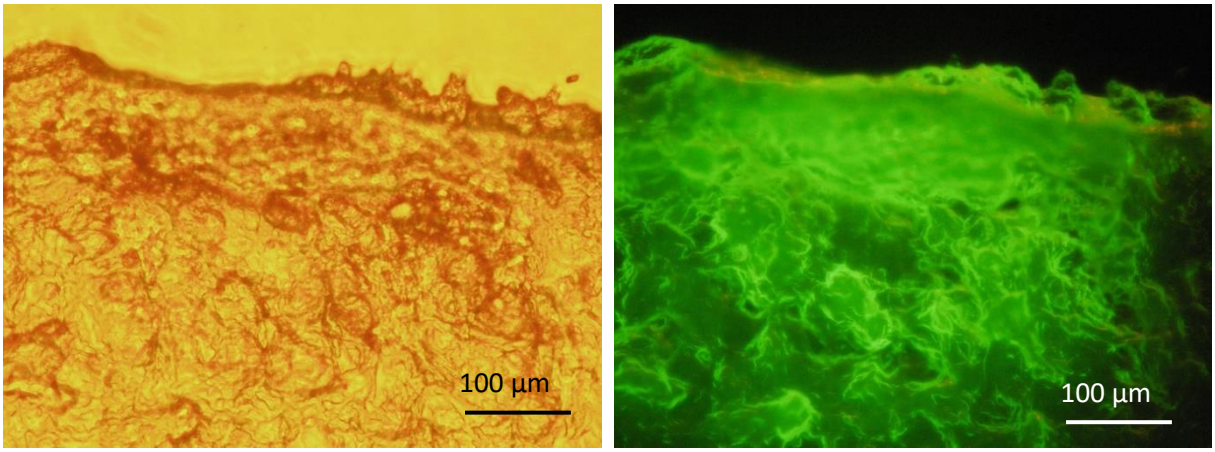


Figure 35. Left: Microscopy picture of damaged pig skin, frozen, after 24 h of diffusion of BSA in ChCl:gly DES under normal light. Right: Microscopy picture of damaged pig skin, frozen, after 24 h of diffusion of BSA in ChCl:gly DES under fluorescent light.

The thickness of the skin piece from the damaged skin were calculated to be 1.6 mm for the piece that PBS diffused through and 1.0 mm for the other half that DES formulation diffused through. In figure 36 the thickness of the pieces can be seen, for the one for where PBS diffused through it had to been puzzled together from three different pictures. Here the different in thickness is more significant than for the undamaged skin and becomes a bit more of a problem when comparing the penetration of BSA in PBS or DES through damaged skin. The numbers in table 9 shows that the PBS had higher concentration penetrated BSA than the DES. This together with the knowledge of that the skin piece for PBS was a half time thicker than the piece for DES shows that even if it had longer to wander into the acceptor liquid, it still had double the penetration of DES, showing that the PBS might have had even higher concentration in the acceptor fluid if the skin pieces would have been the same thickness and might be something to try and improve in the future when doing Franz cell diffusion test.

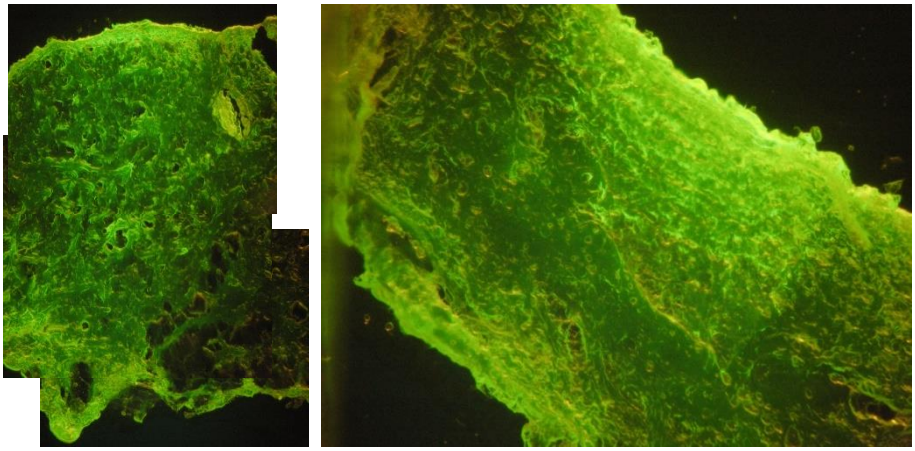


Figure 36. Left: Piece of damaged pig skin, frozen, after 24 h of BSA in PBS diffusing through. Right: Piece of damaged pig skin, frozen, after 24 h of BSA in ChCl:gly DES diffusing through.

Conclusion

Following the molar ratio that was most commonly mentioned in articles to prepare DES did not always create stable DES, some of them needed to be adjusted to make stable DES at room temperature. This could be because of the condition of the lab or inaccuracy in preparing the DES. If anything, the literature values was a good starting point.

The viscosity of the DES changes with added amount of water, more drastically in the initial addition of water. The more water added the closer the viscosity gets to the one of pure water. This matches up with the theory that if enough water is added the system breaks and becomes ions solved in water. It was also observed that DES with too high viscosity is hard to work with and it is easier to work with the once that have a lower viscosity and then try to thicken them later on.

Making typical emulsion of water, oil and surfactant with DES didn't work. This could be because of all the ions in the DES or that the DES is held together of hydrogen bonds and that is more favourable than breaking up and forming and emulsion with the oil and surfactant.

Thickening DES is possible with Carbopol 974P NJ, which is a crosslinked polyacrylic acid. Small amounts of carpool is enough to get a gel. The more Carbopol added, the thicker they get, and the more force is needed to spread them out. Addition of water lowers the viscosity, giving looser that are easier to spread. This also worked for the addition of Premulen™, which is as well a crosslinked polyacrylic acid in this case containing hydrophobic groups.

The protein BSA didn't manage to be solved in pure DES, but with the addition of small amounts of water it held stable between 2 weeks to 2 months, depending on the DES system. This is promising since it is an improvement from solving BSA in water and could be interesting to look further into and adjust the amount of water and BSA in relation to the DES.

Skin penetration of BSA performed better in PBS than in DES. For undamaged skin both of them performed poorly and had 1-2 % of penetration. For damaged skin penetration the PBS buffer had about double amount of penetration compared to the DES. Then the DES had about 20 % penetrated. For transdermal delivery the PBS outperformed the DES. But looking at the microscopic pictures, DES glowed as much as the PBS, making it interesting for dermal delivery.

From the penetration test it was also clear that the surfaces of vials and alike does not need to be precoated with BSA solution since it didn't make any different in the results.

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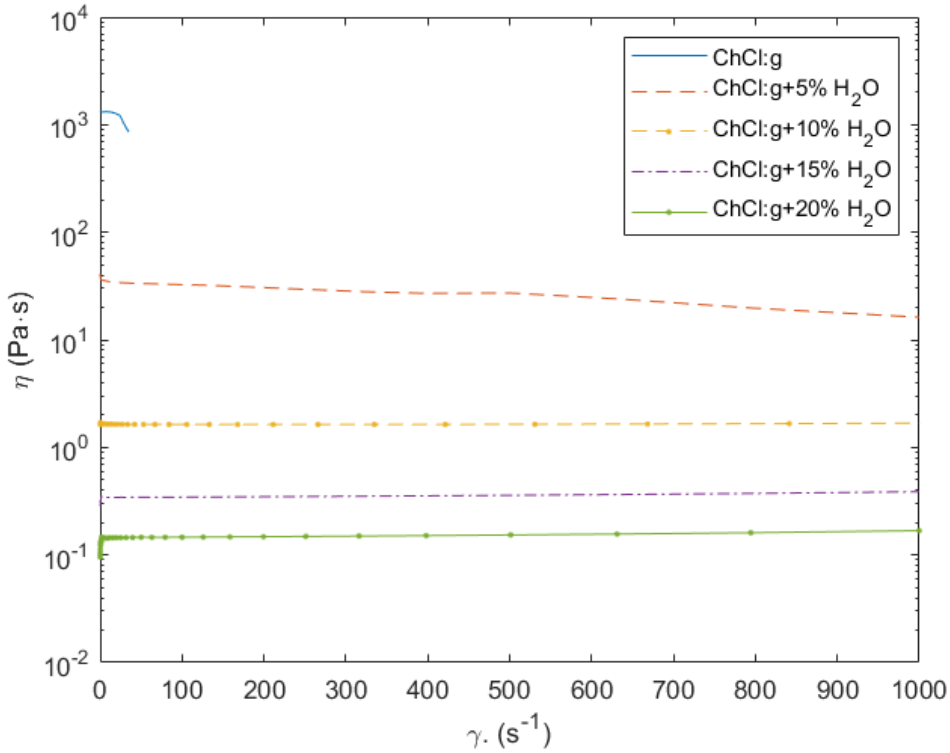
Appendix A

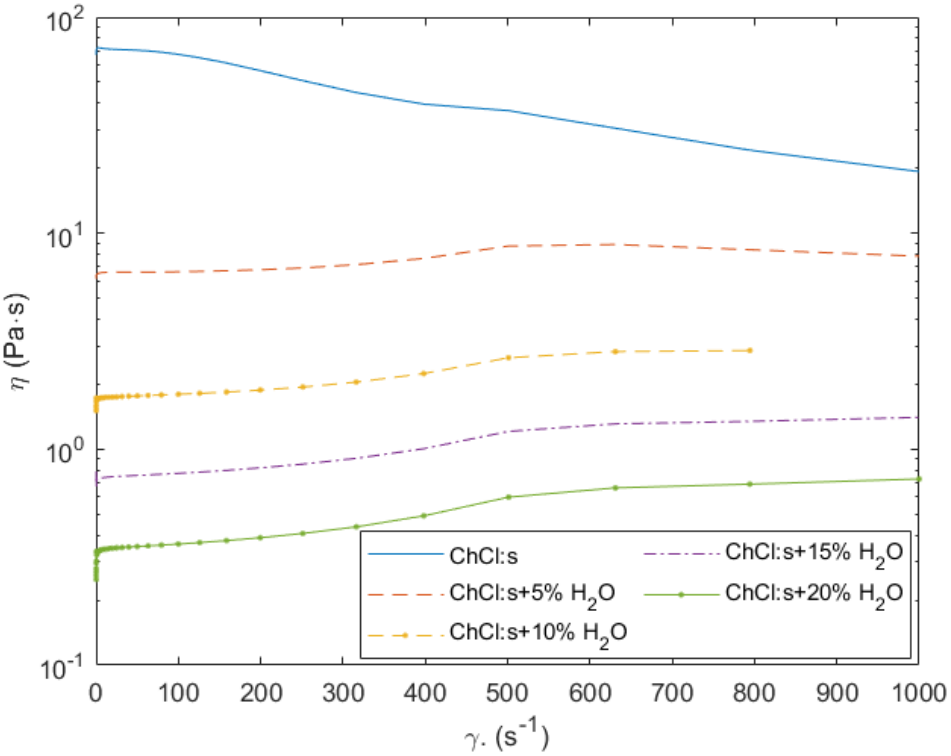
Viscosity measurements

ChCl:glucose



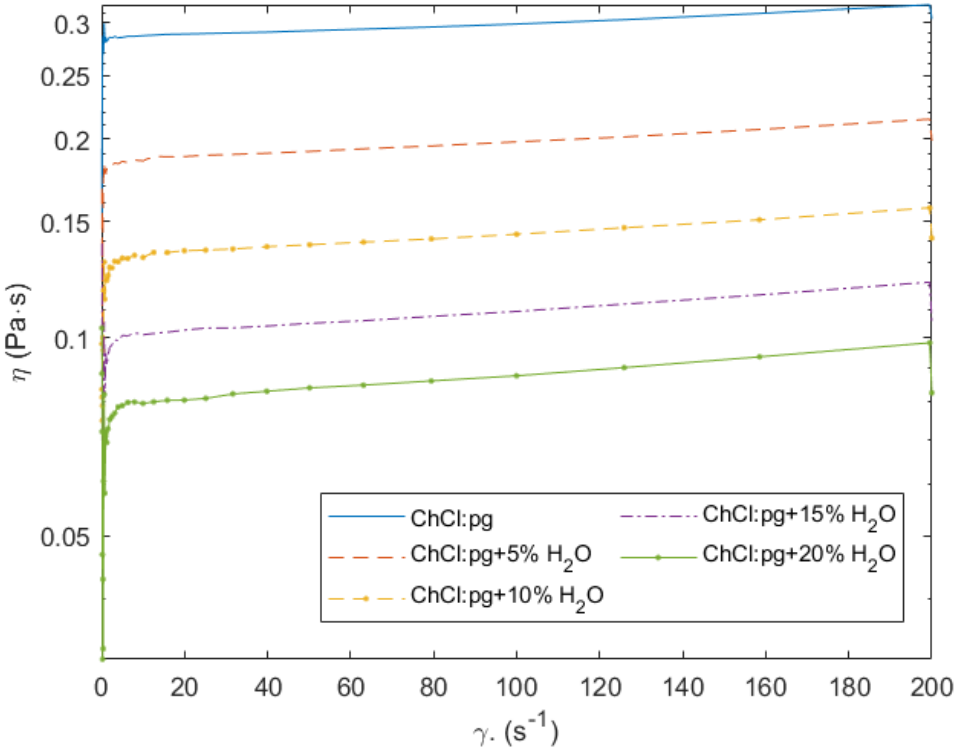
Average shear viscosity of ChCl:glucose with increasing amount of water.

ChCl:sorbitol

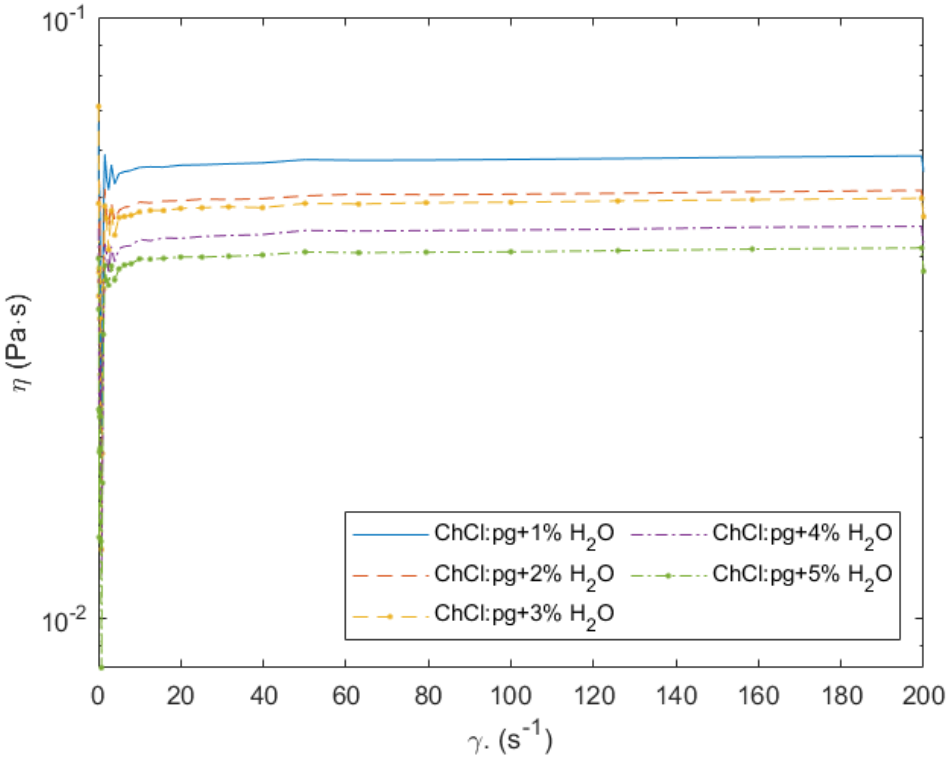


Average shear viscosity of ChCl:sorbitol with increasing amount of water.

ChCl:propylene glycol

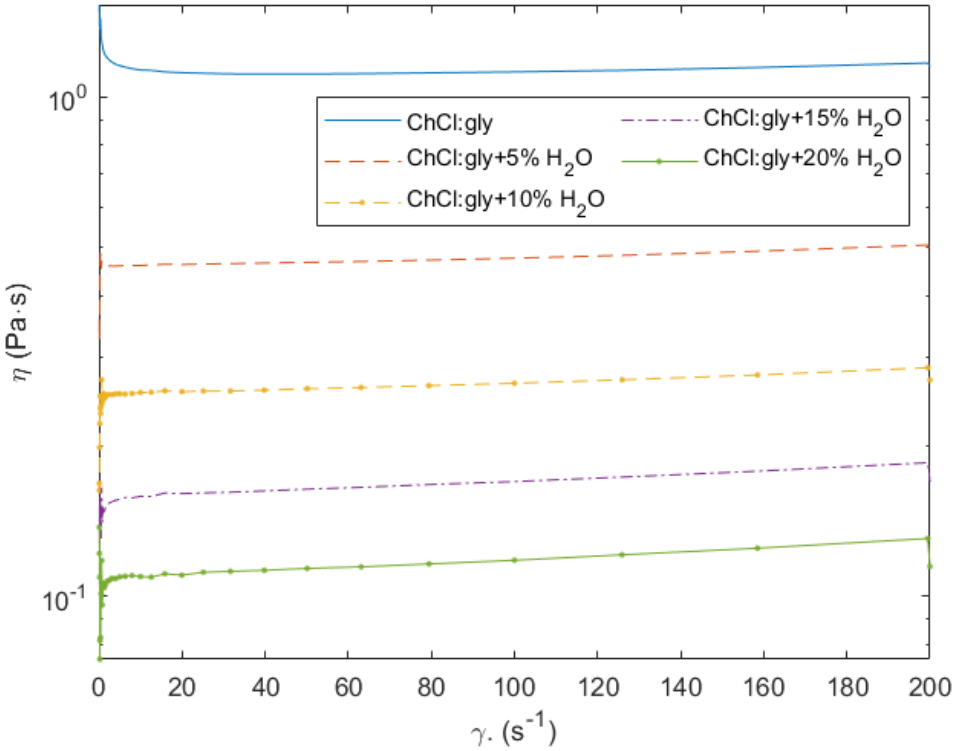


Average shear viscosity of ChCl:propylene glycol with increasing amount of water.

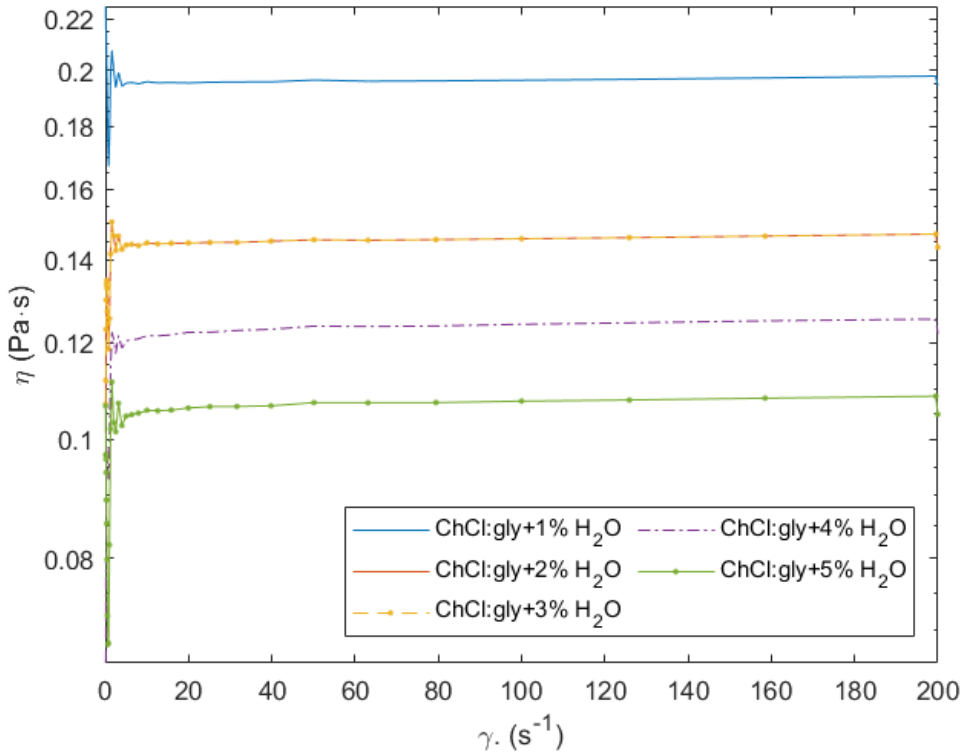


Average shear viscosity of second ChCl:propylene glycol with increasing amount of water.

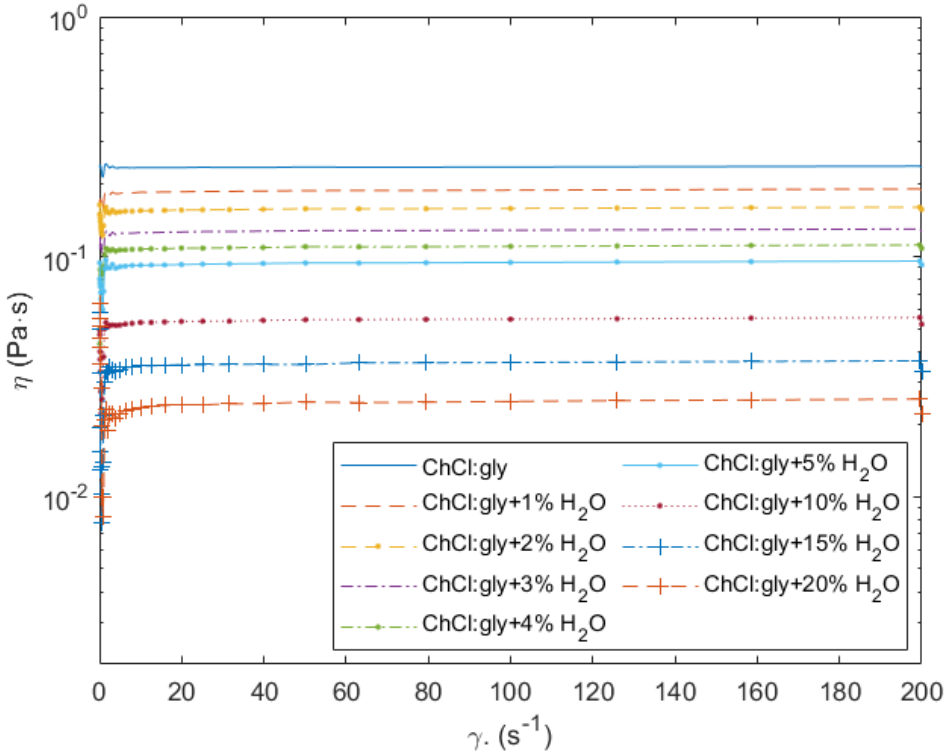
ChCl:glycerol



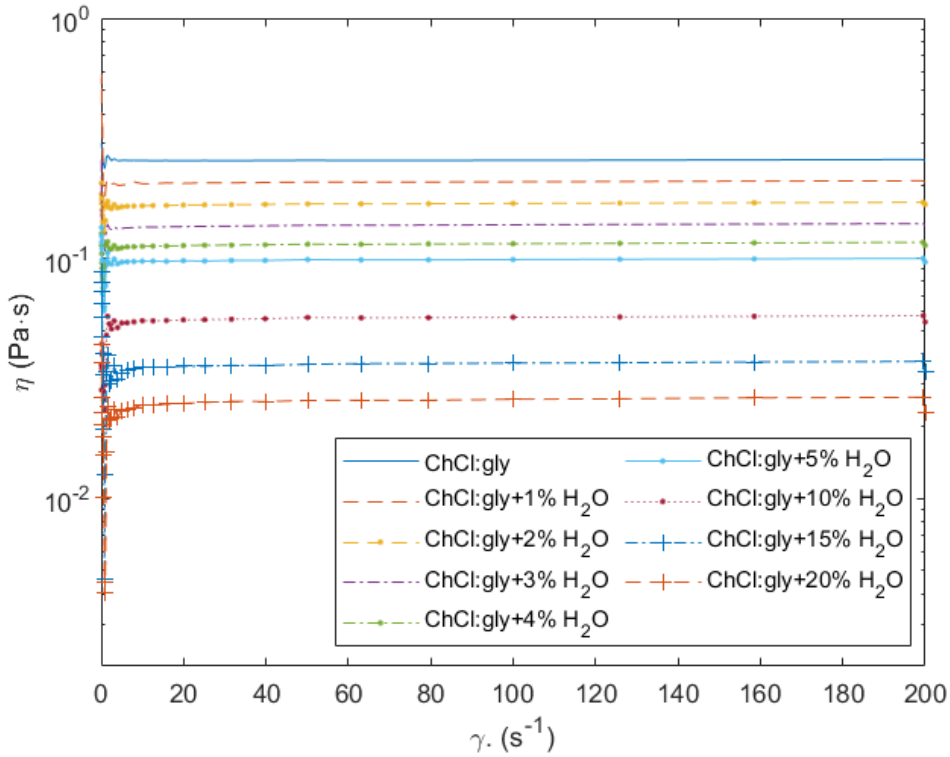
Average shear viscosity of ChCl:glycerol with increasing amount of water.



Average shear viscosity of second ChCl:glyerol with increasing amount of water.



Average shear viscosity of third ChCl:glycerol with heating with increasing amount of water.

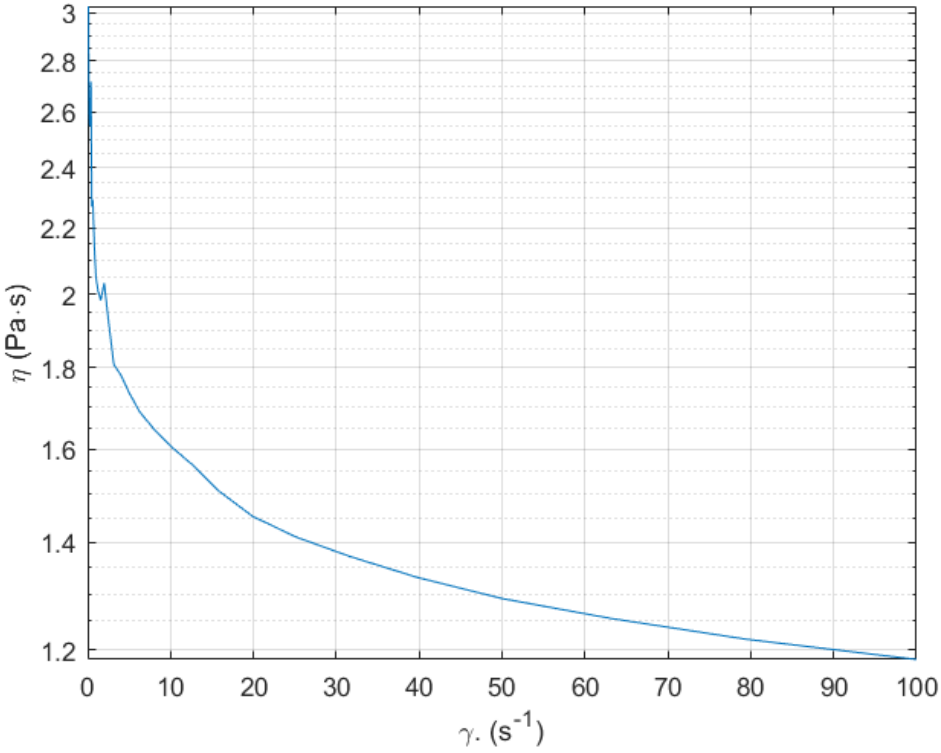


Average shear viscosity of third ChCl:glycerol without heating with increasing amount of water.

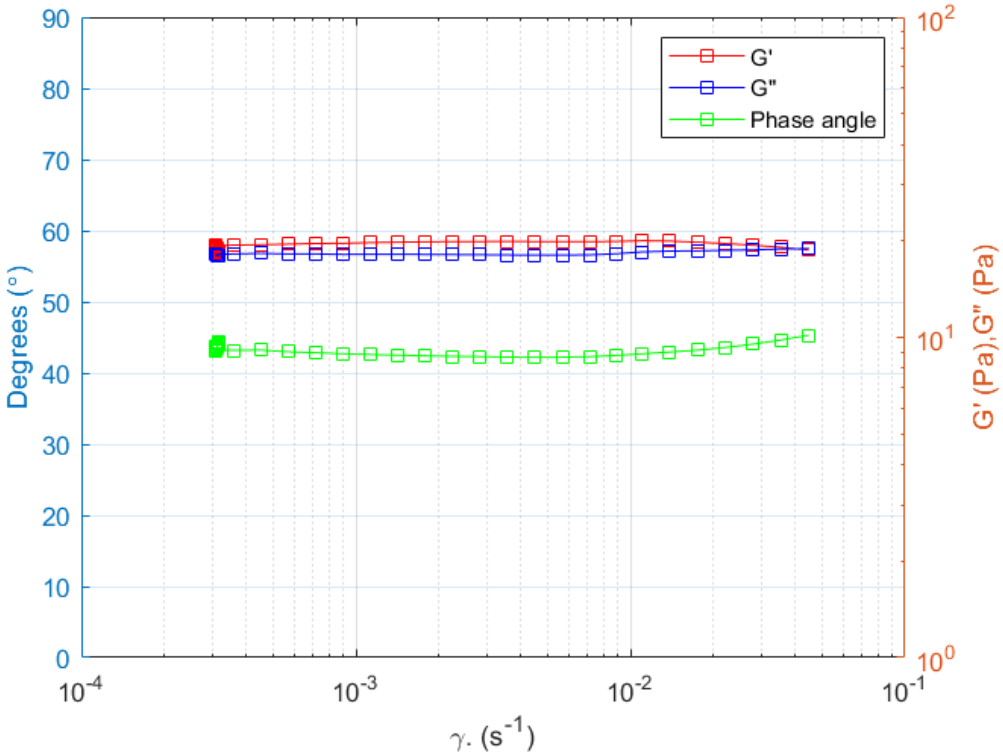
Appendix B

ChCl:glycerol + 1 wt% Carbopol 974P NJ

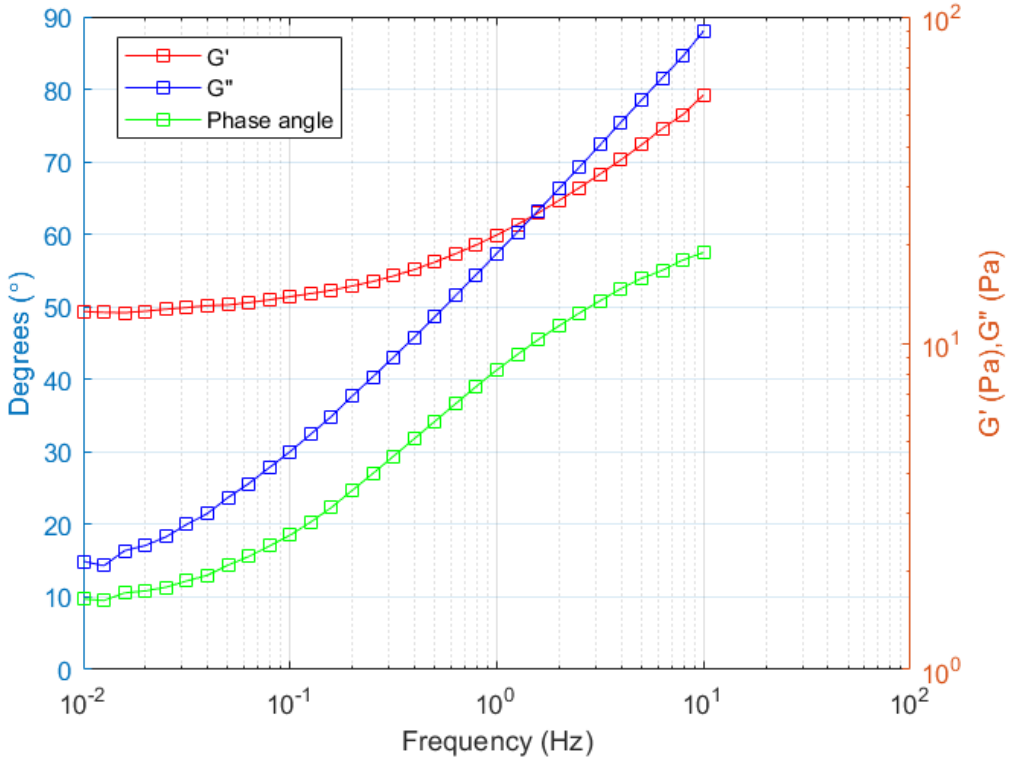
Day 1



Viscosity measurement

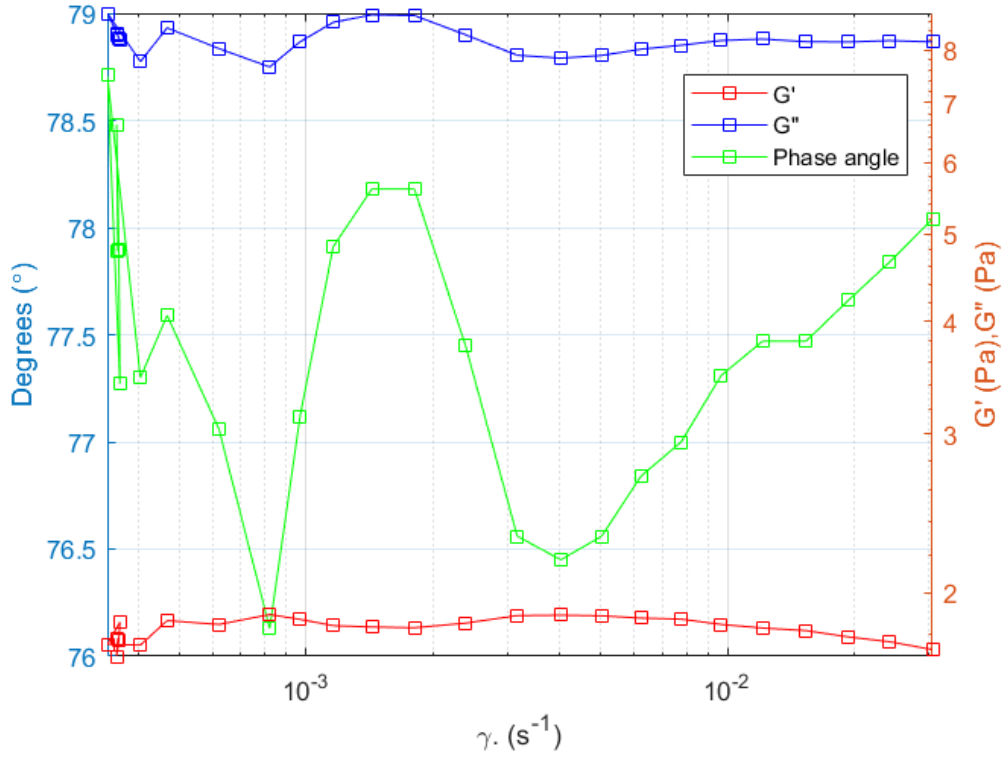


LVE range measurement

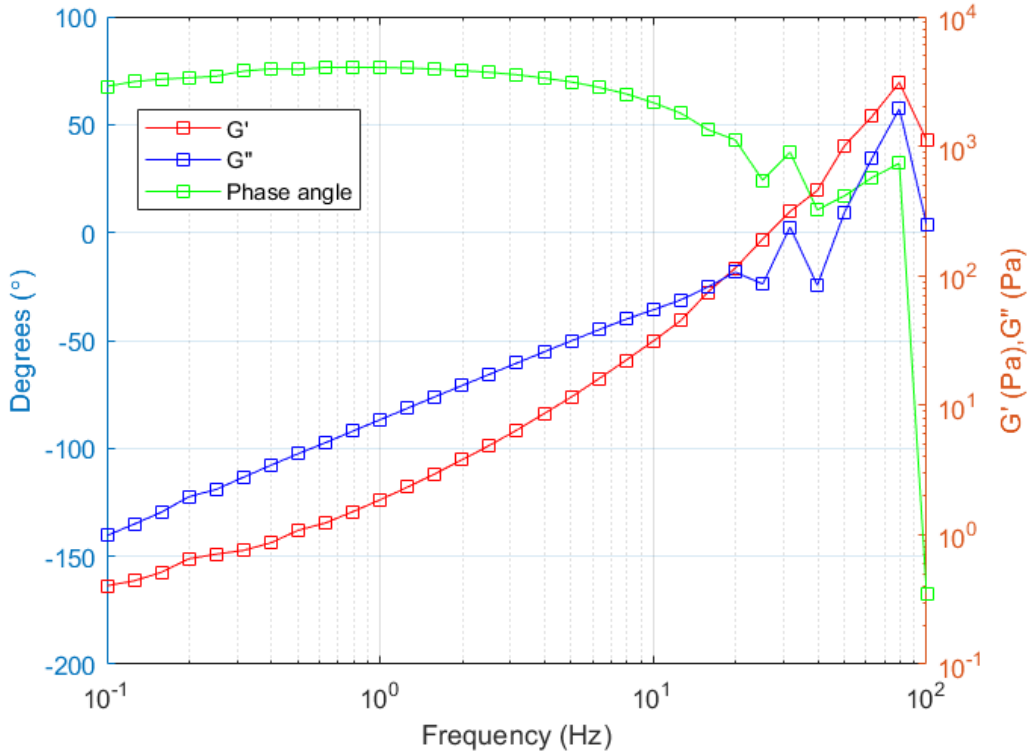


Frequency sweep measurement

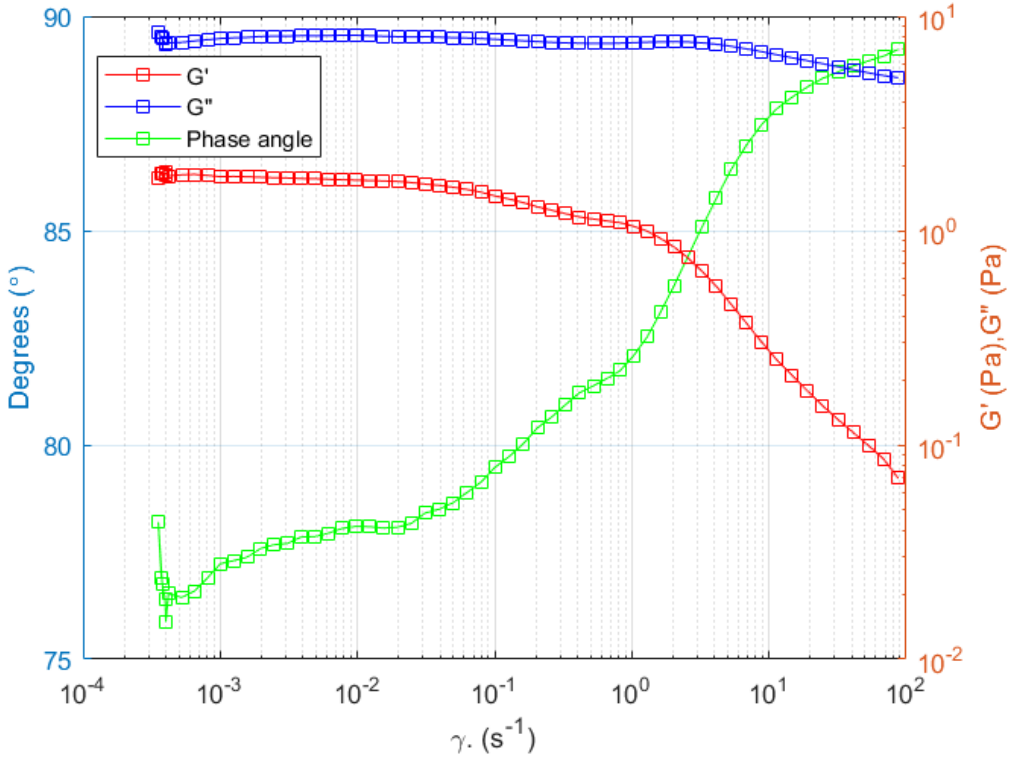
Day 2



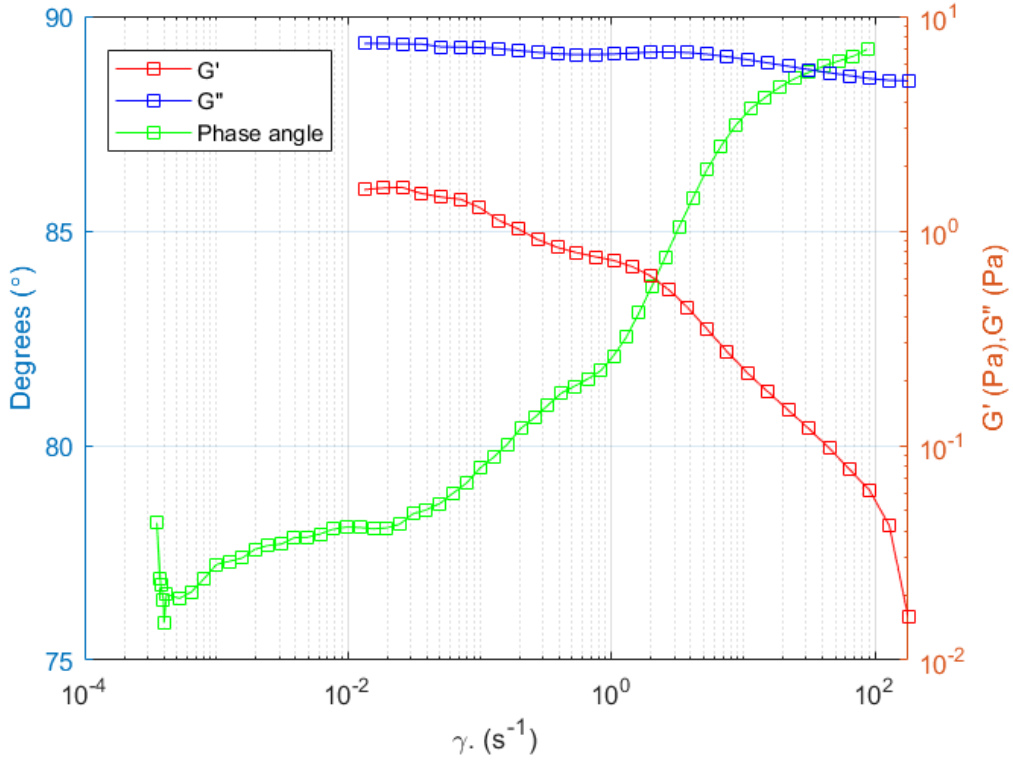
LVE range measurement



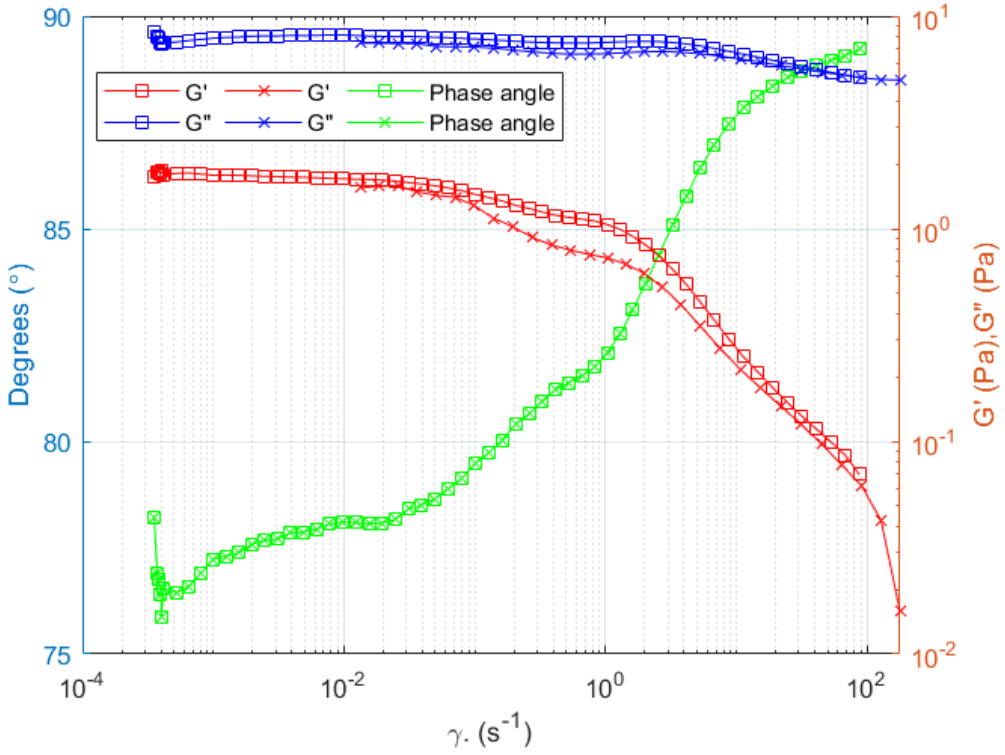
Frequency sweep measurement



Amplitude sweep down measurement



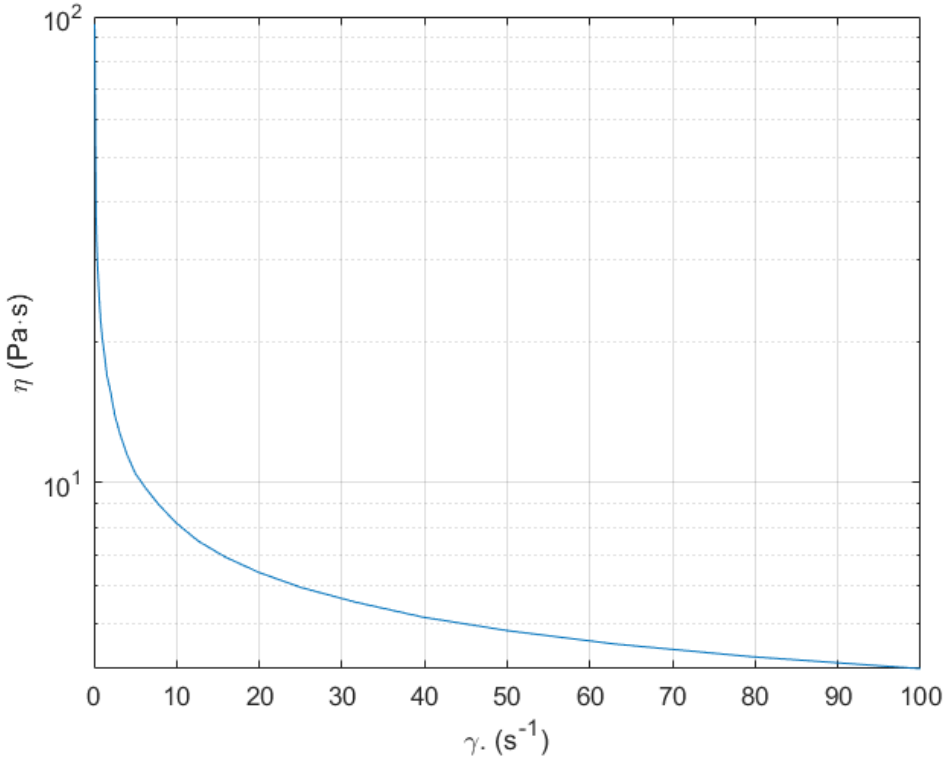
Amplitude sweep up measurement



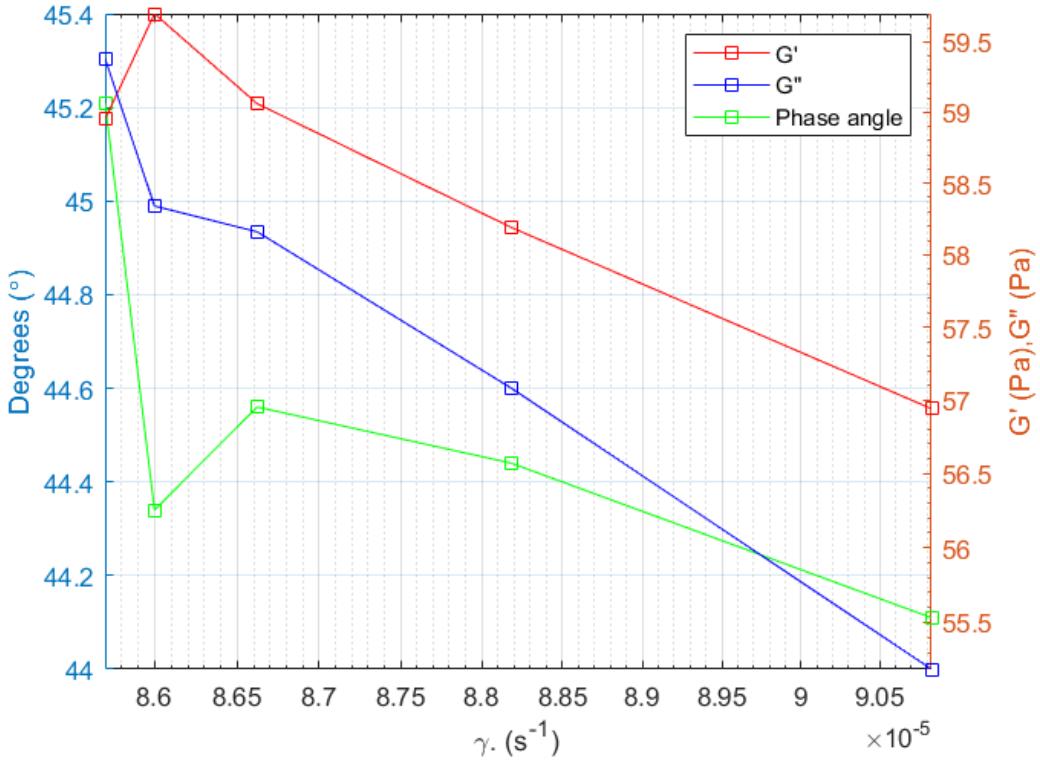
Amplitude sweep up and down combined

ChCl:glycerol + 2 wt% Carbopol 974P NJ

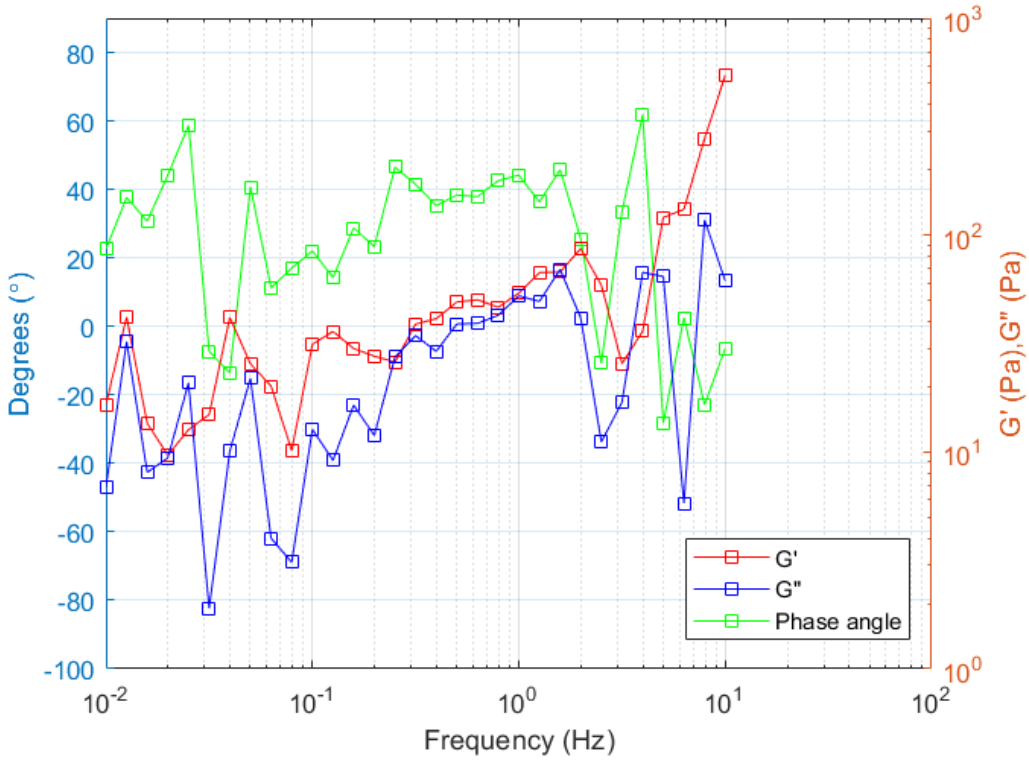
Day 1



Viscosity measurement

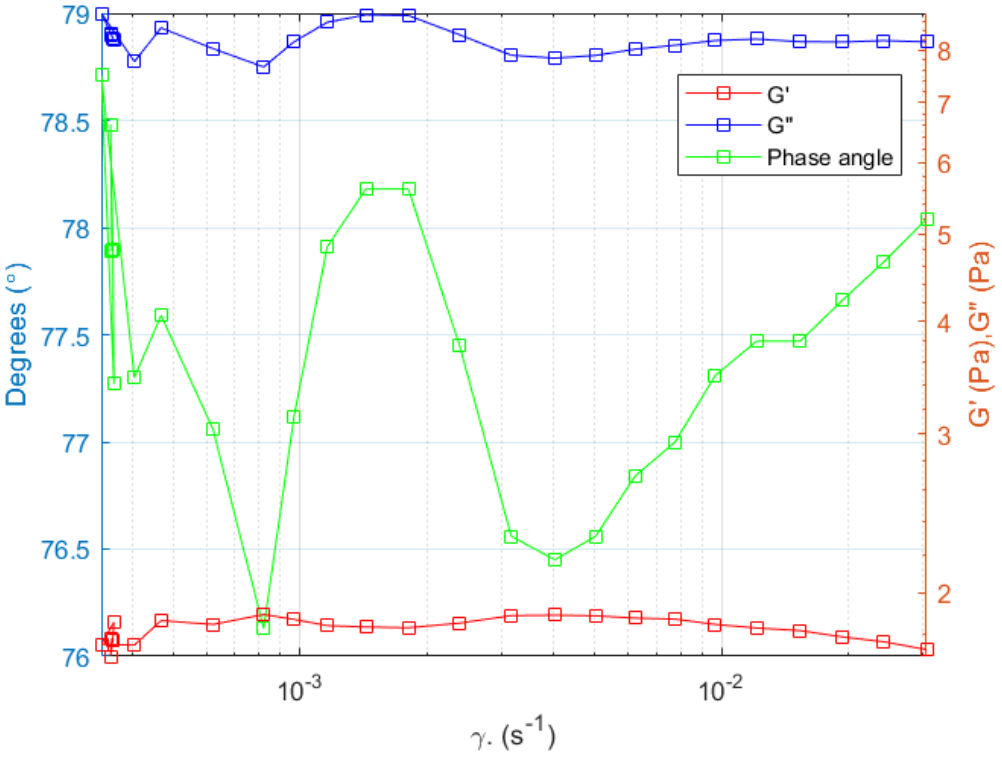


LVE range measurement

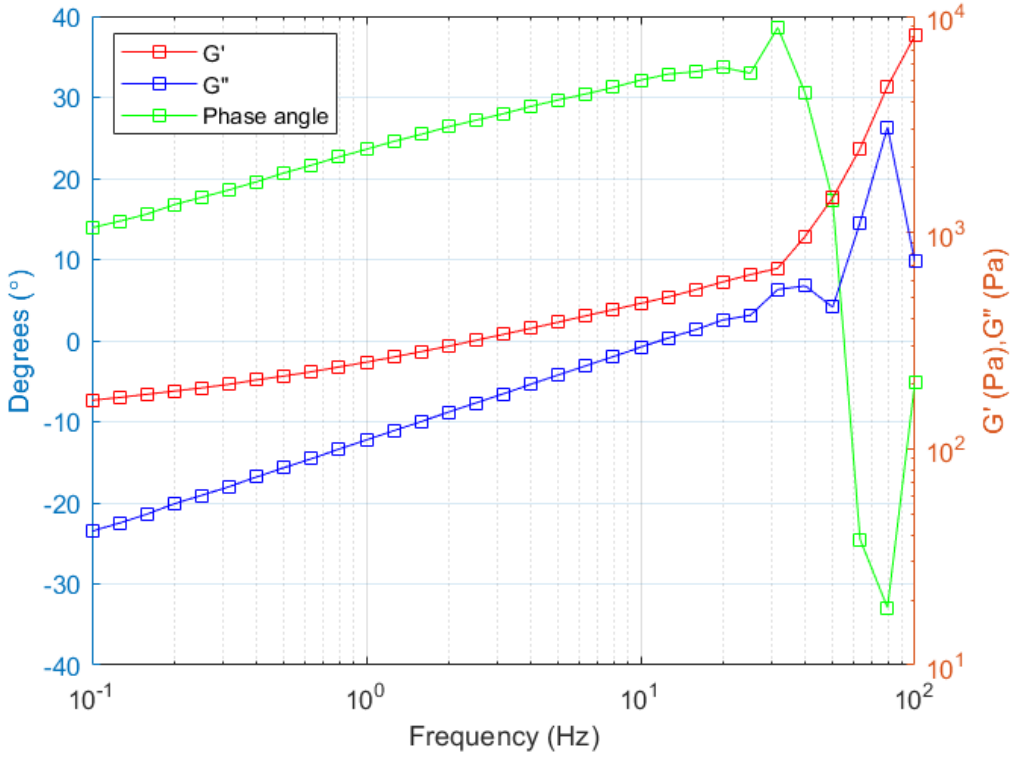


Frequency sweep measurement

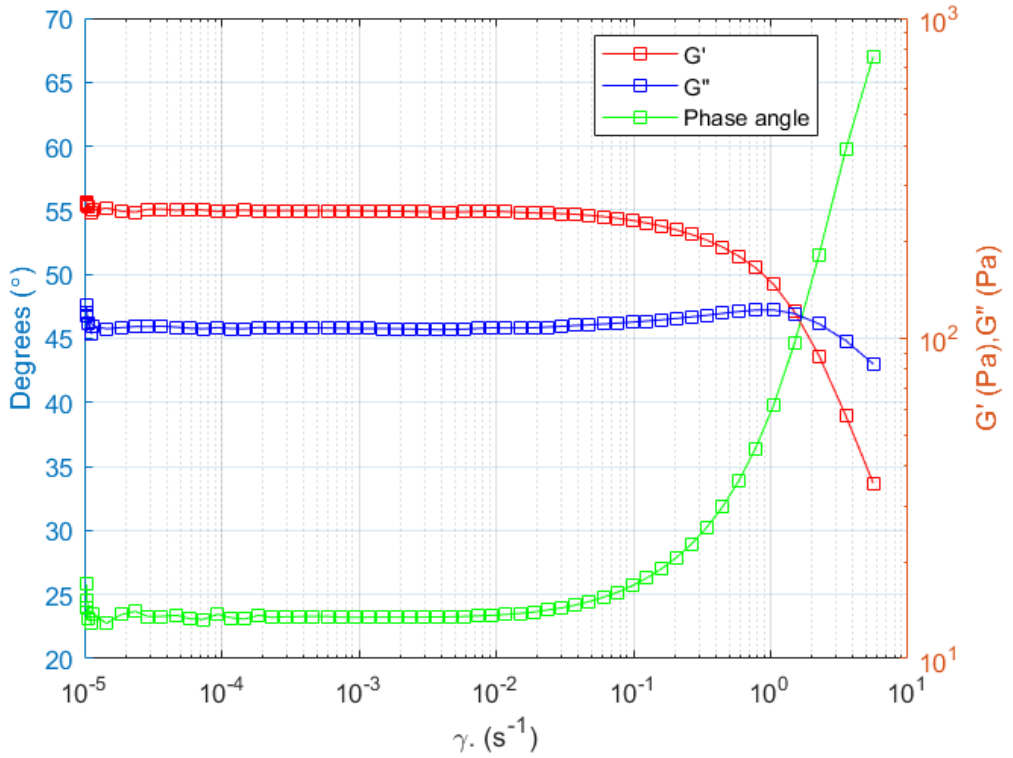
Day 2



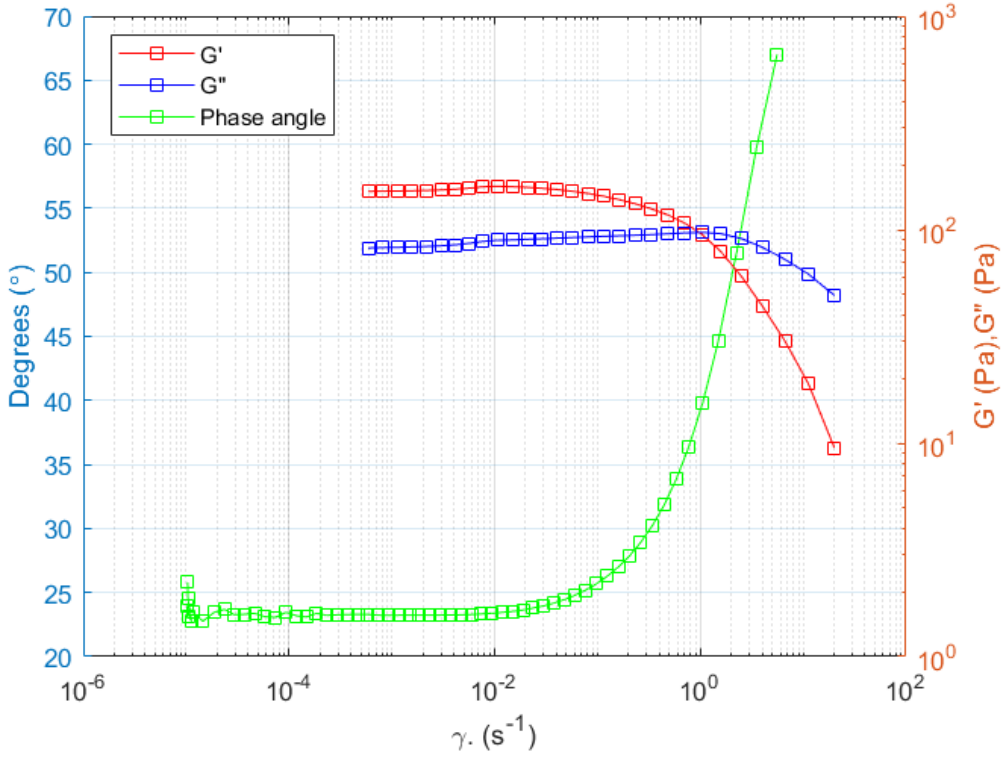
LVE range measurements



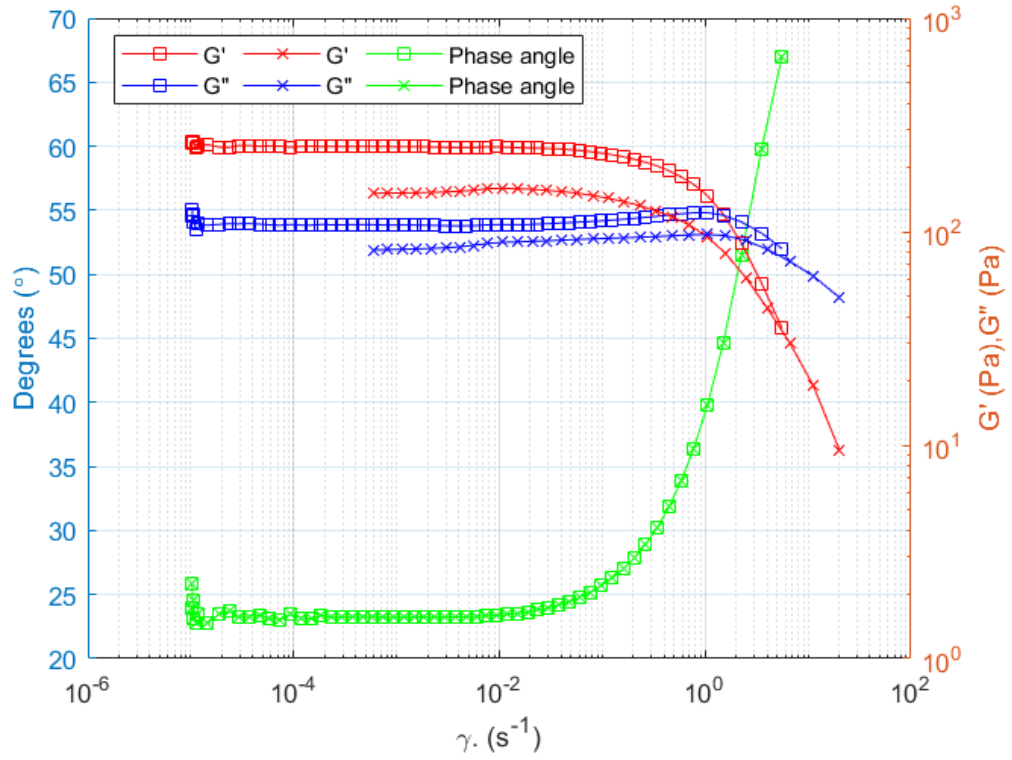
Frequency sweep measurement



Amplitude sweep down measurement



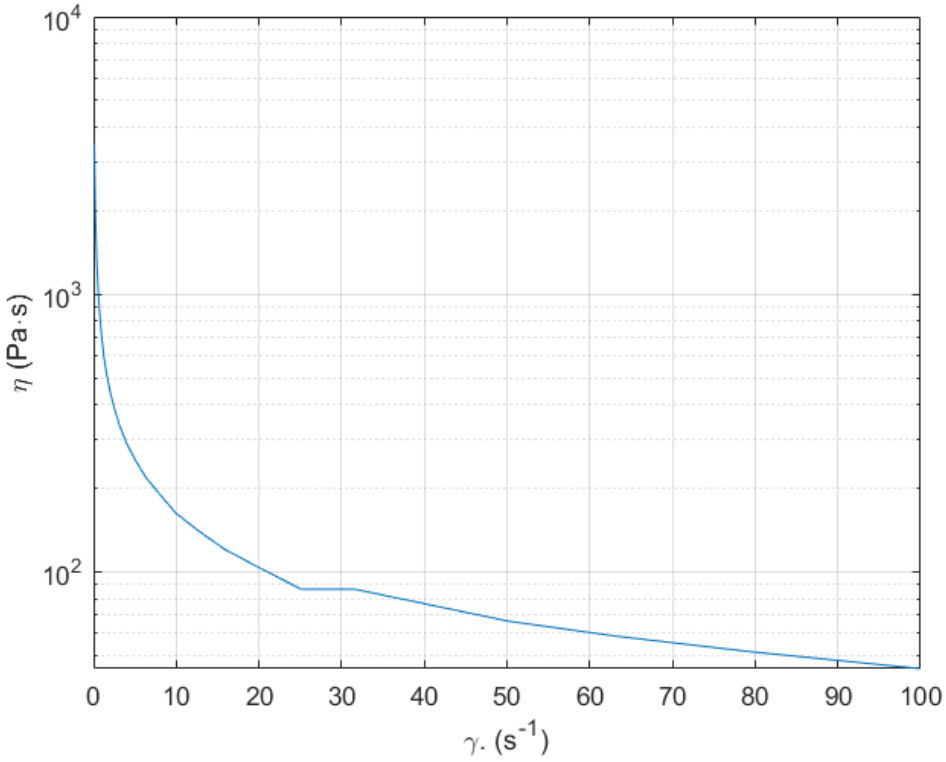
Amplitude sweep up measurement



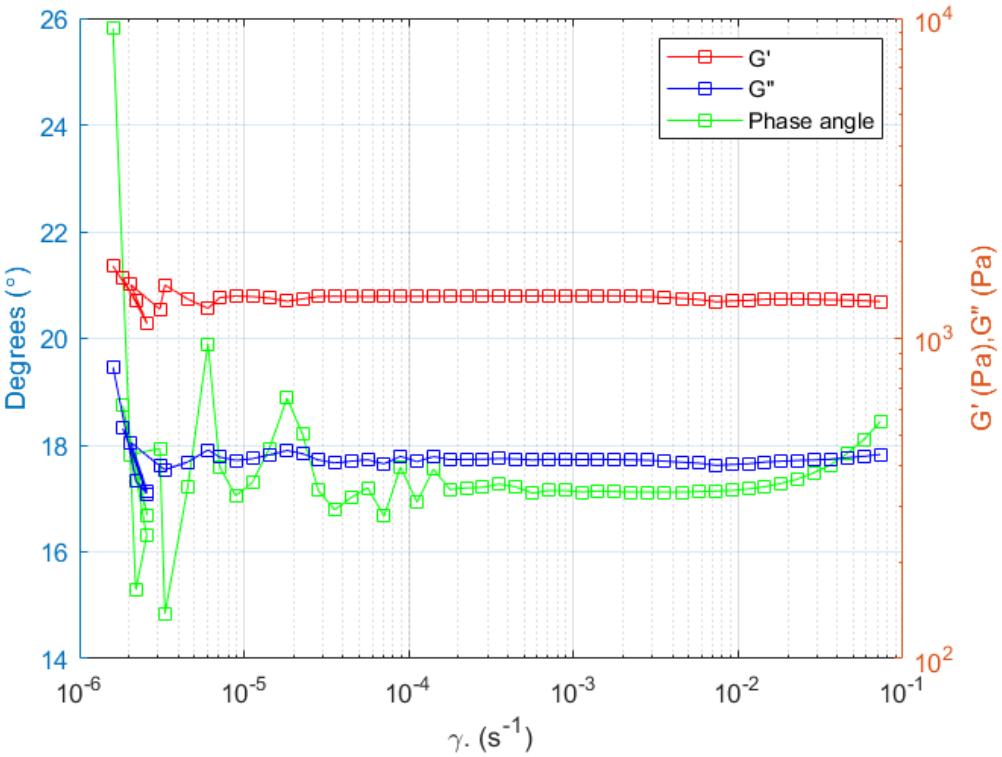
Amplitude sweep up and down combined

ChCl:glycerol + 5 wt% Carbopol 974P NJ

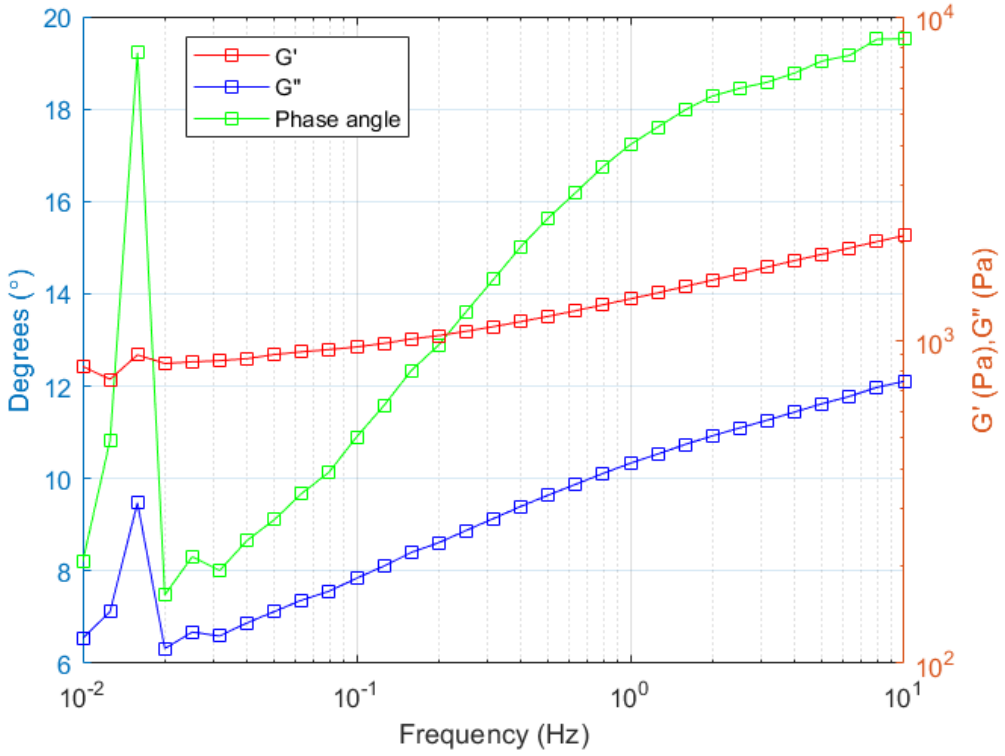
Day 1



Viscosity measurement

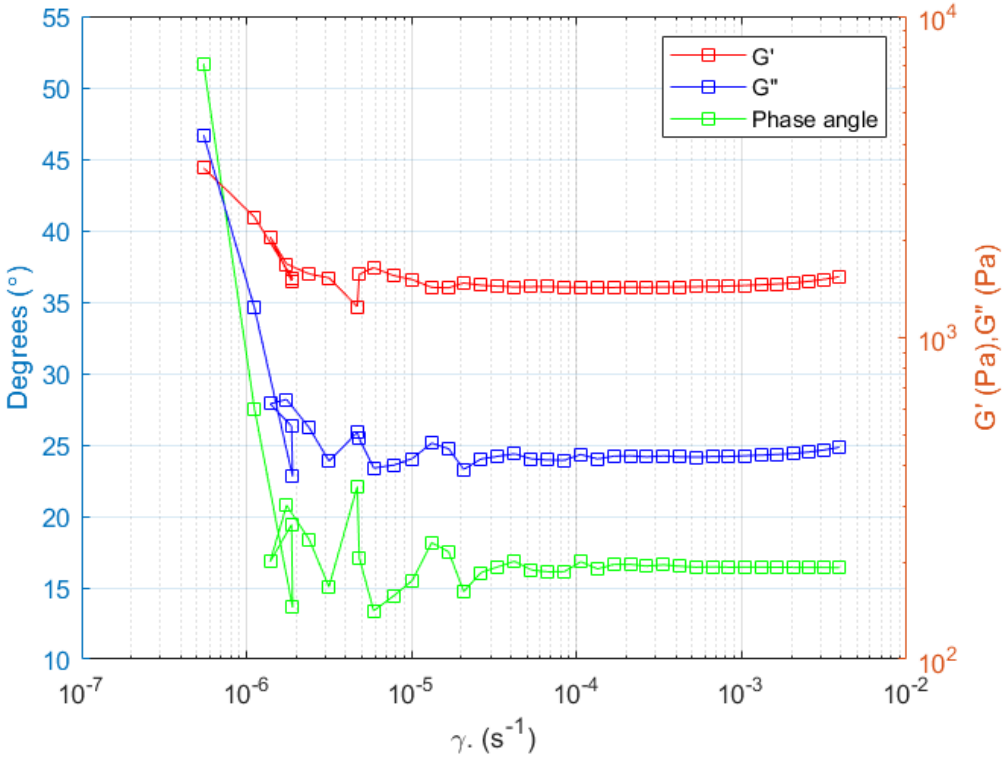


LVE range measurement

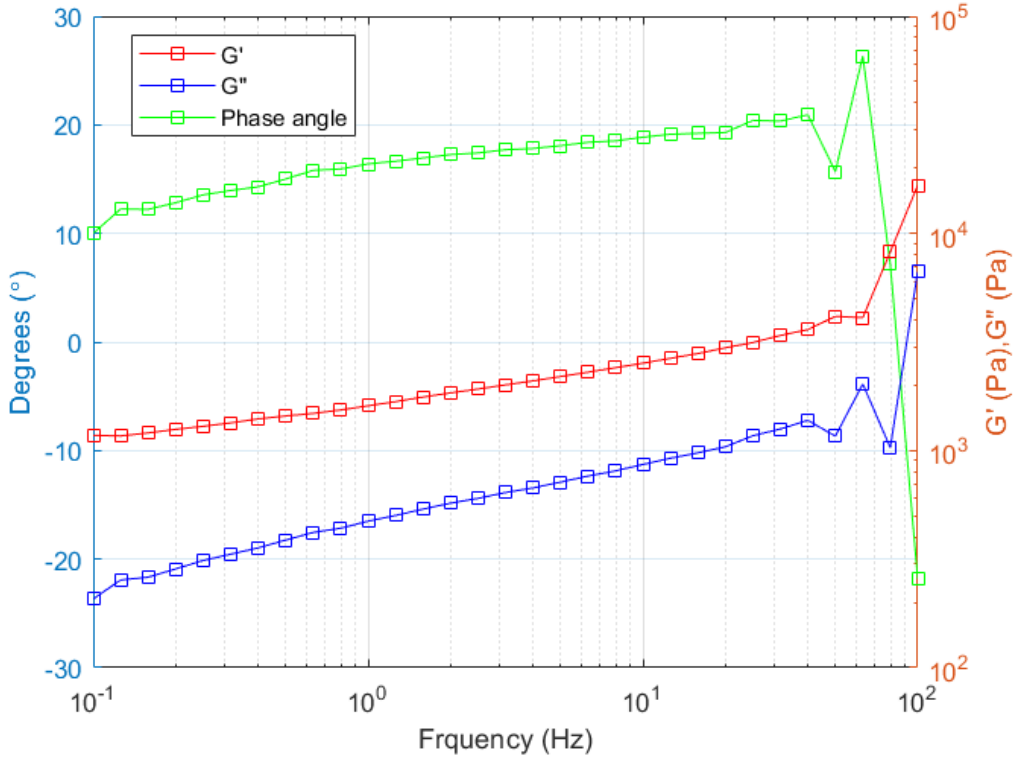


Frequency sweep measurement

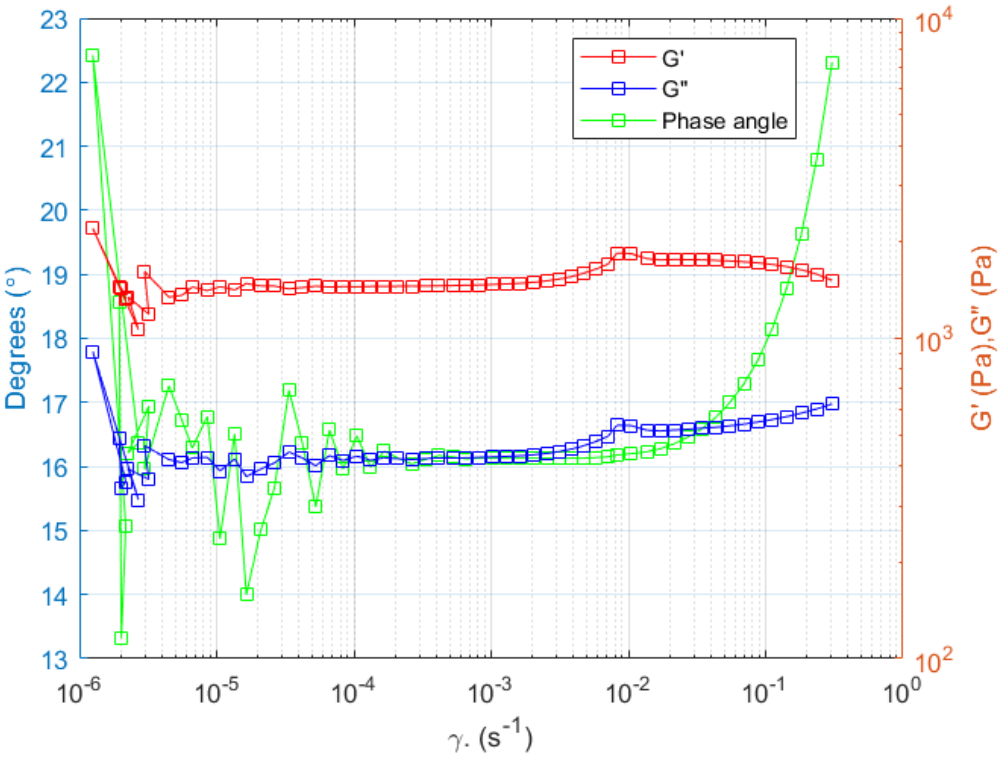
Day 2



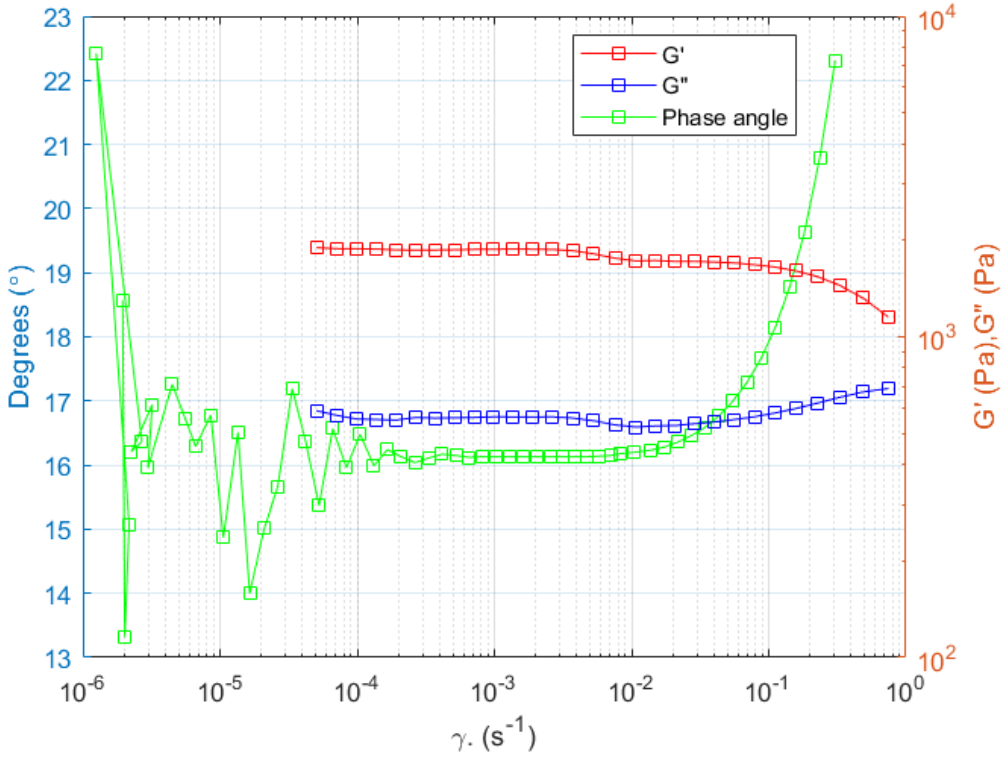
LVE range measurement



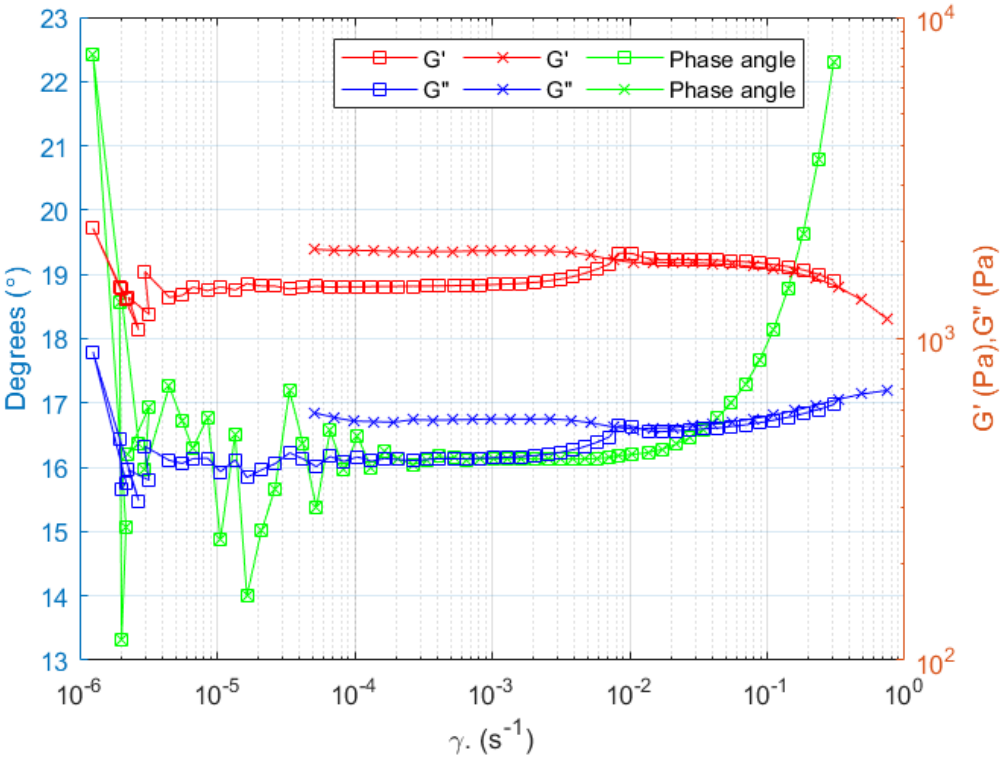
Frequency sweep measurement



Amplitude sweep down measurement



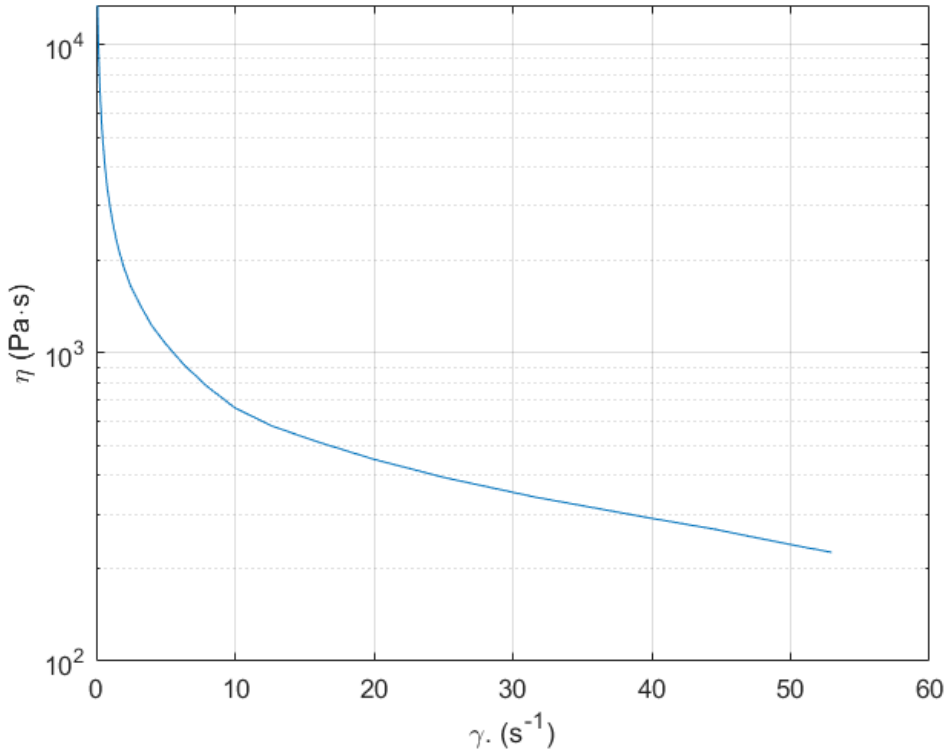
Amplitude sweep up measurement



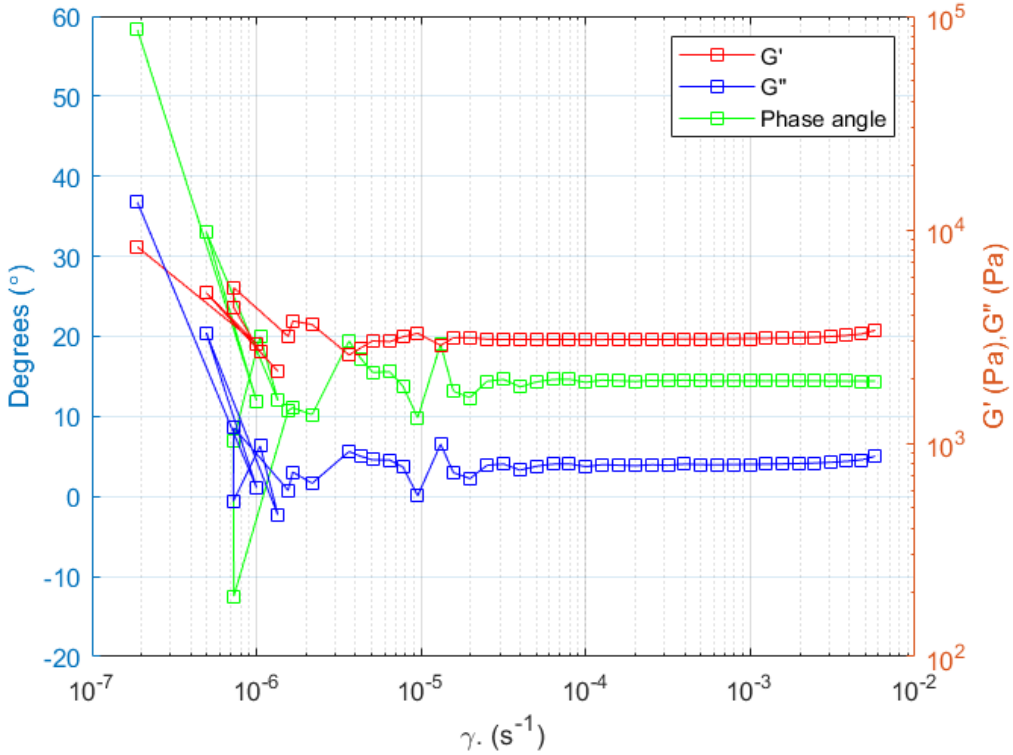
Amplitude sweep up and down combined

ChCl:glycerol + 10 wt% Carbopol 974P NJ

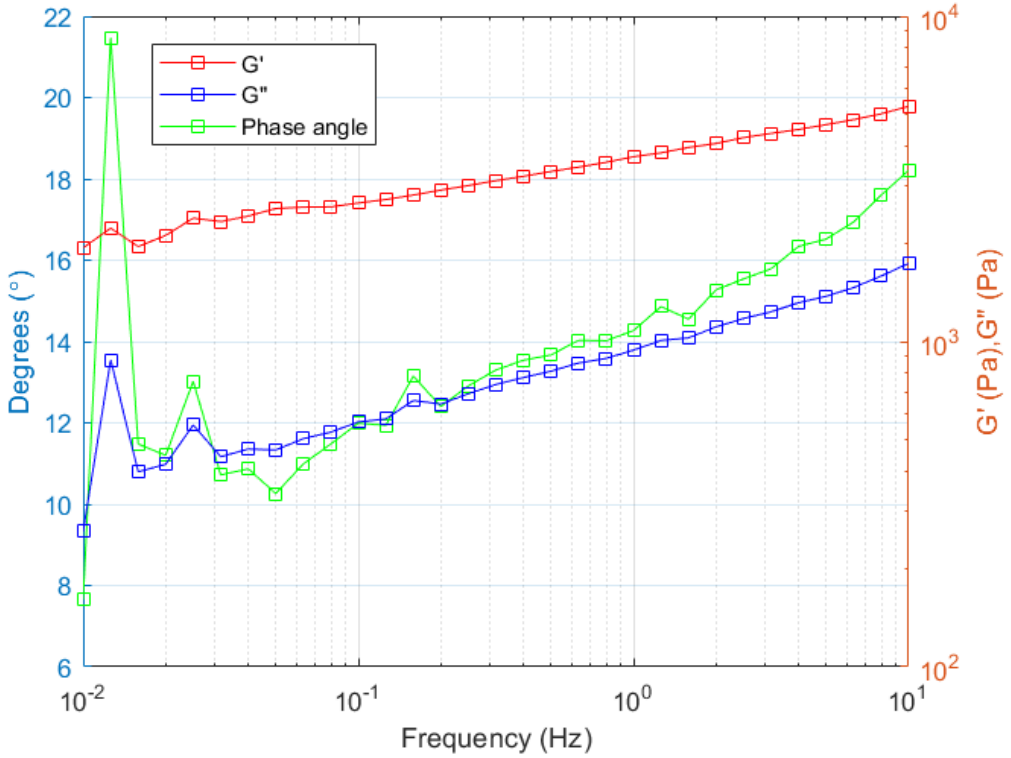
Day 1



Viscosity measurement

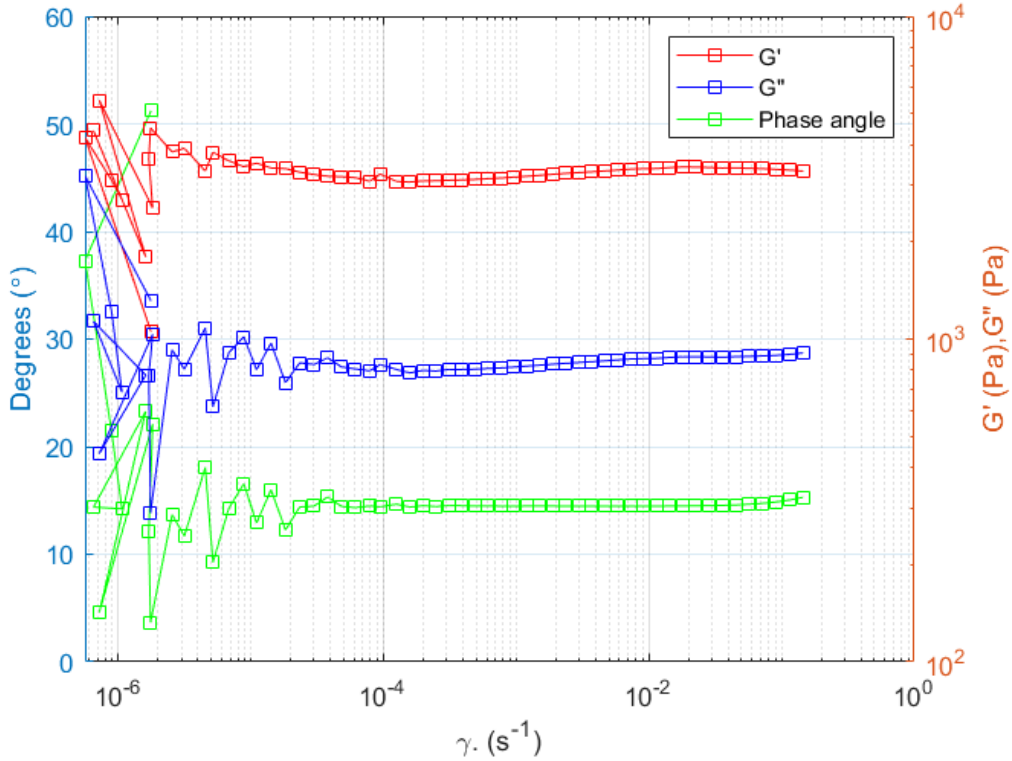


LVE range measurement

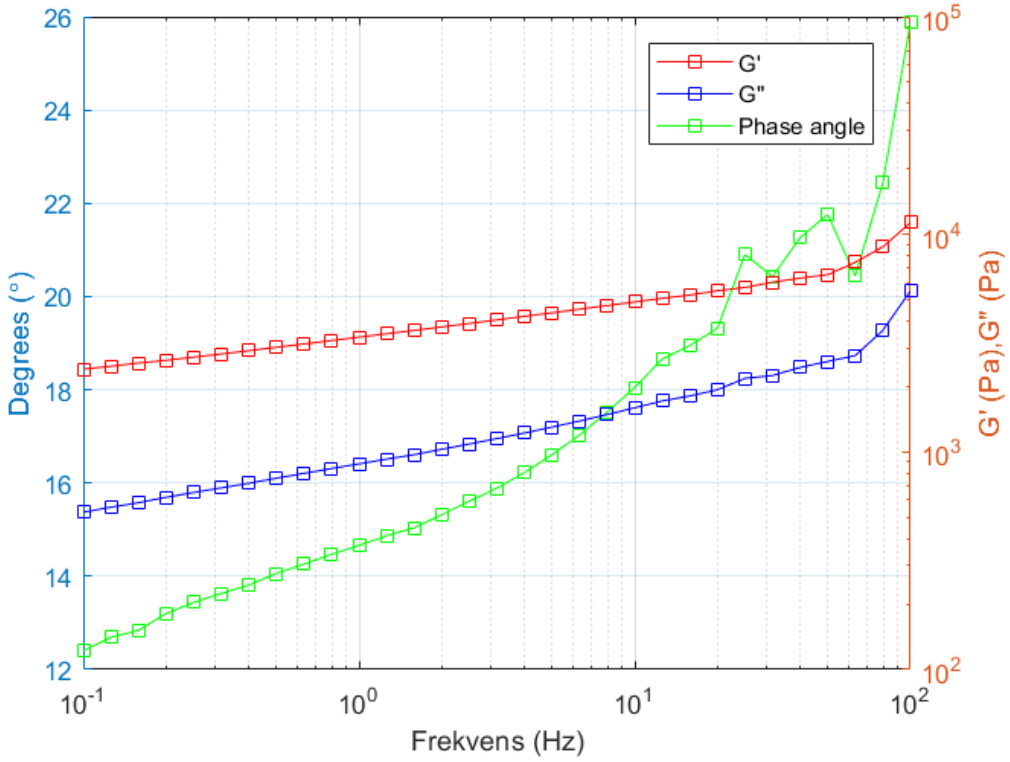


Frequency sweep measurement

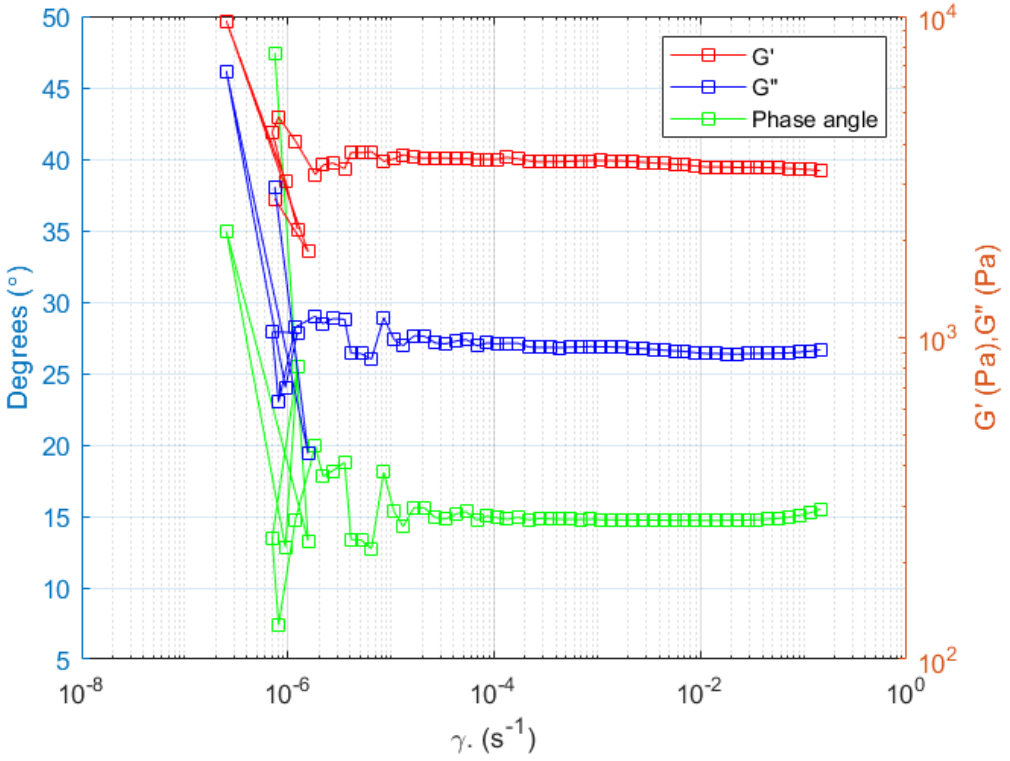
Day 2



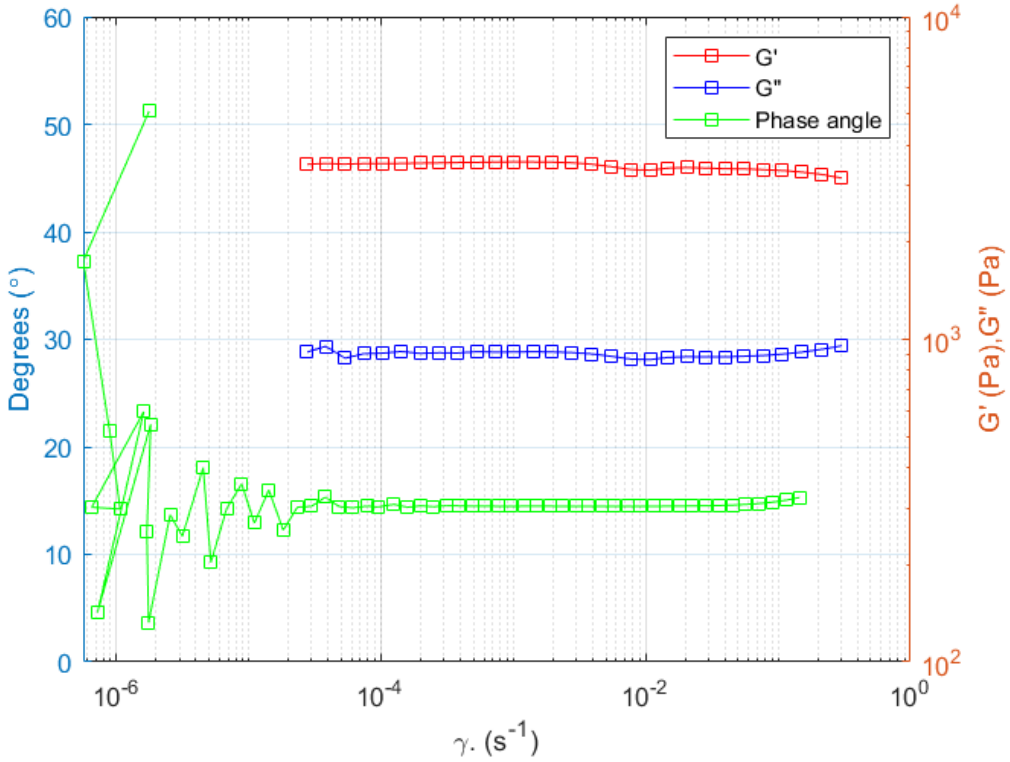
LVE range measurement



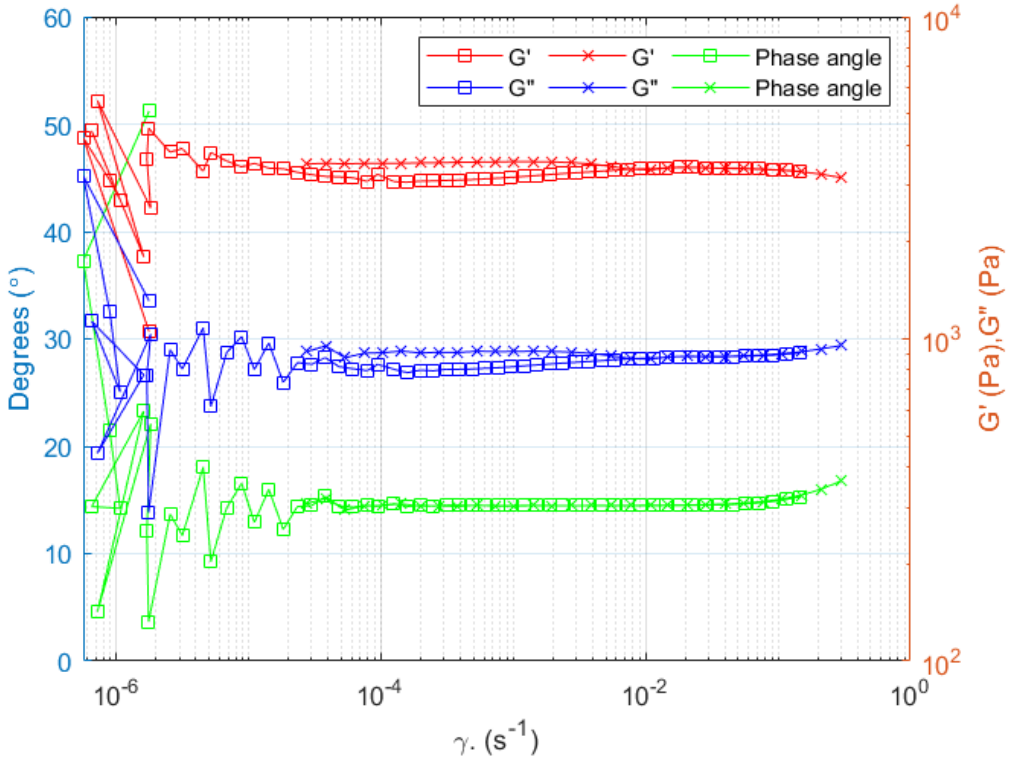
Frequency sweep measurement



Amplitude sweep down measurement



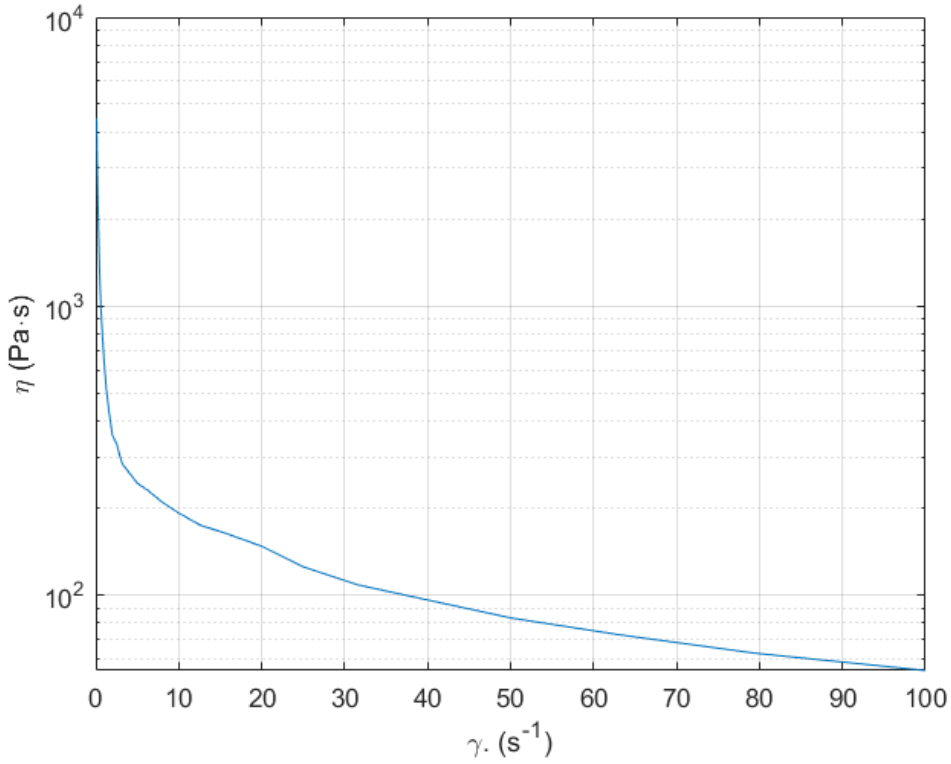
Amplitude sweep up measurement



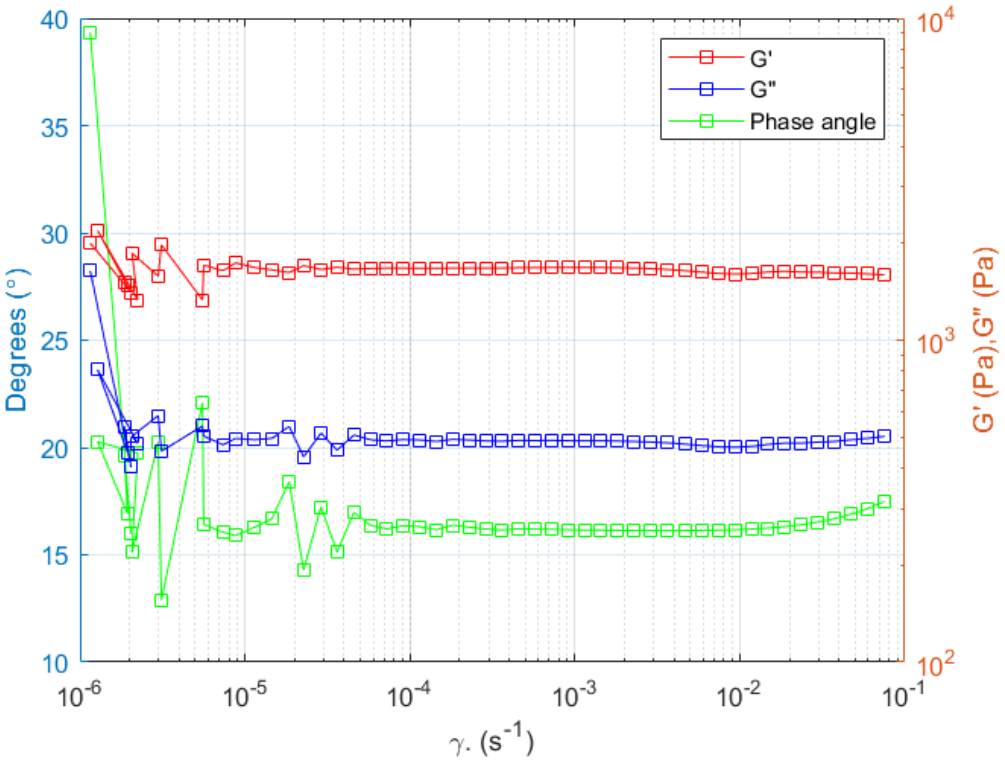
Amplitude sweep up and down combined

ChCl:glycerol + 5 wt% Carbopol 974P NJ + 0.5 wt% water

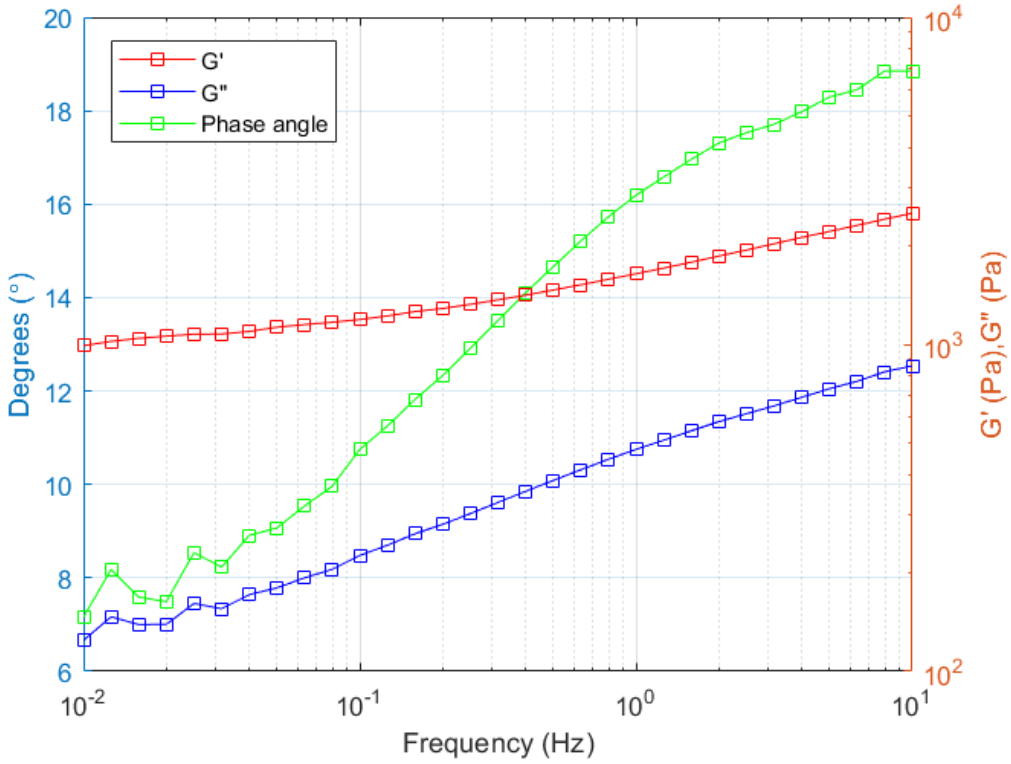
Day 1



Viscosity measurement

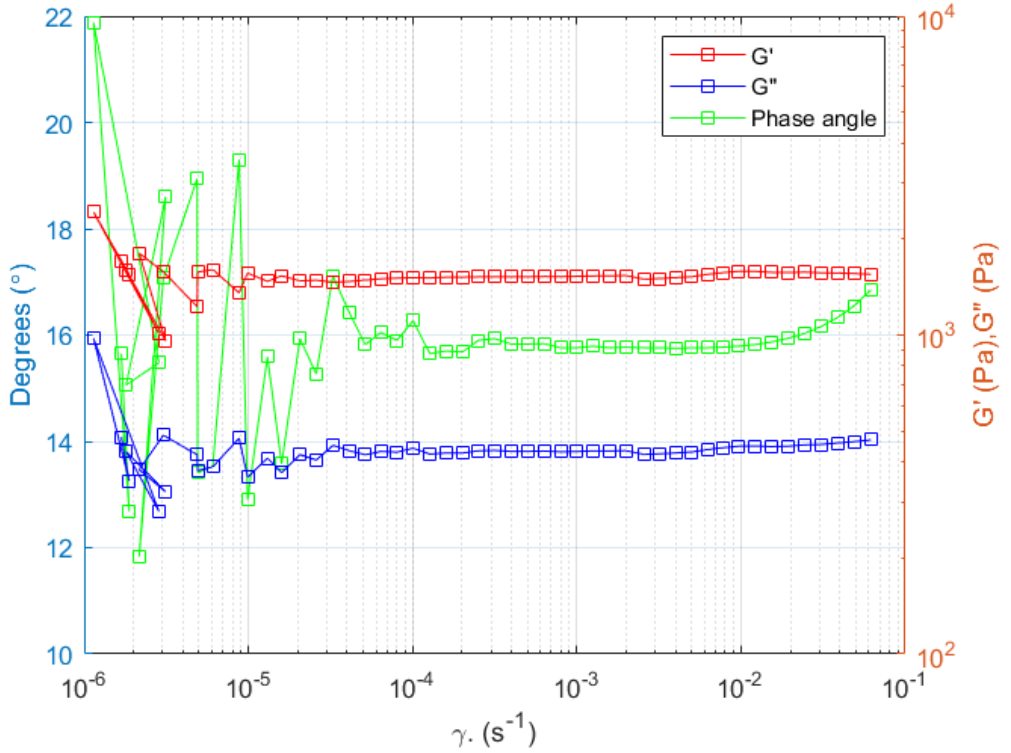


LVE range measurement

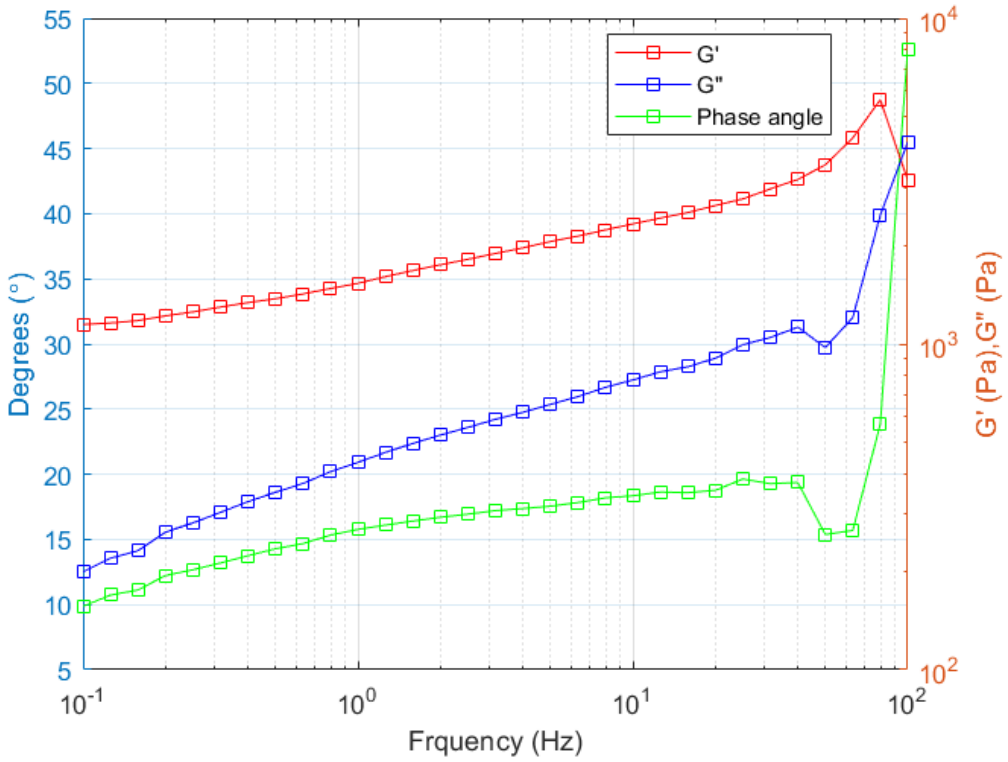


Frequency sweep measurement

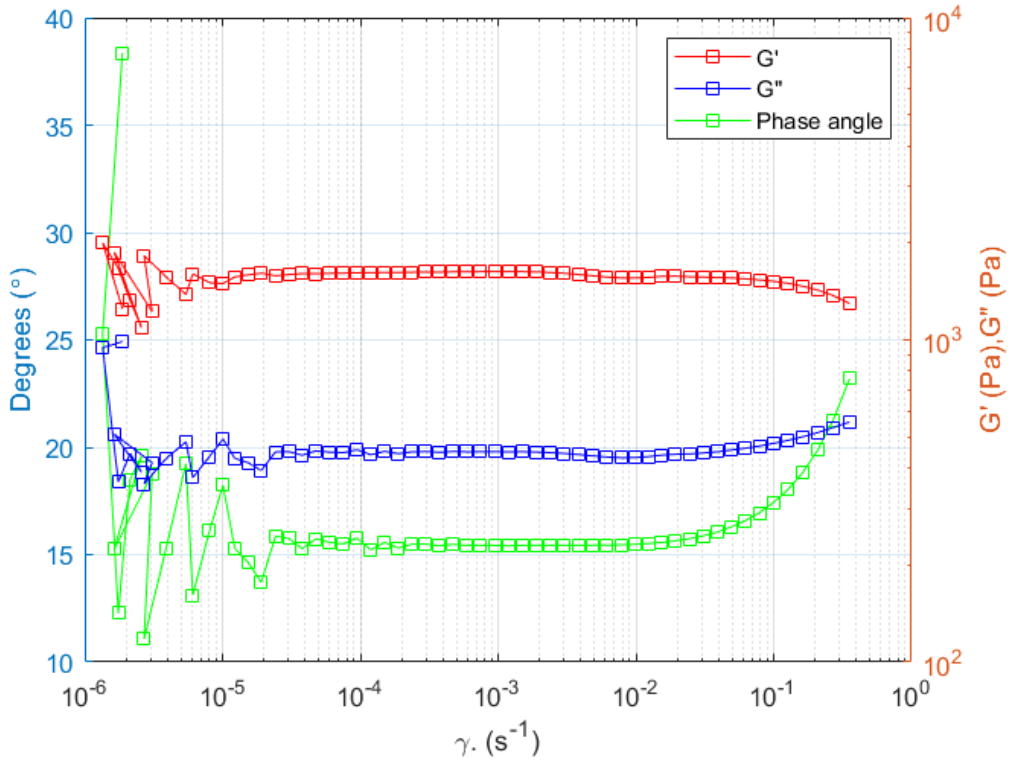
Day 2



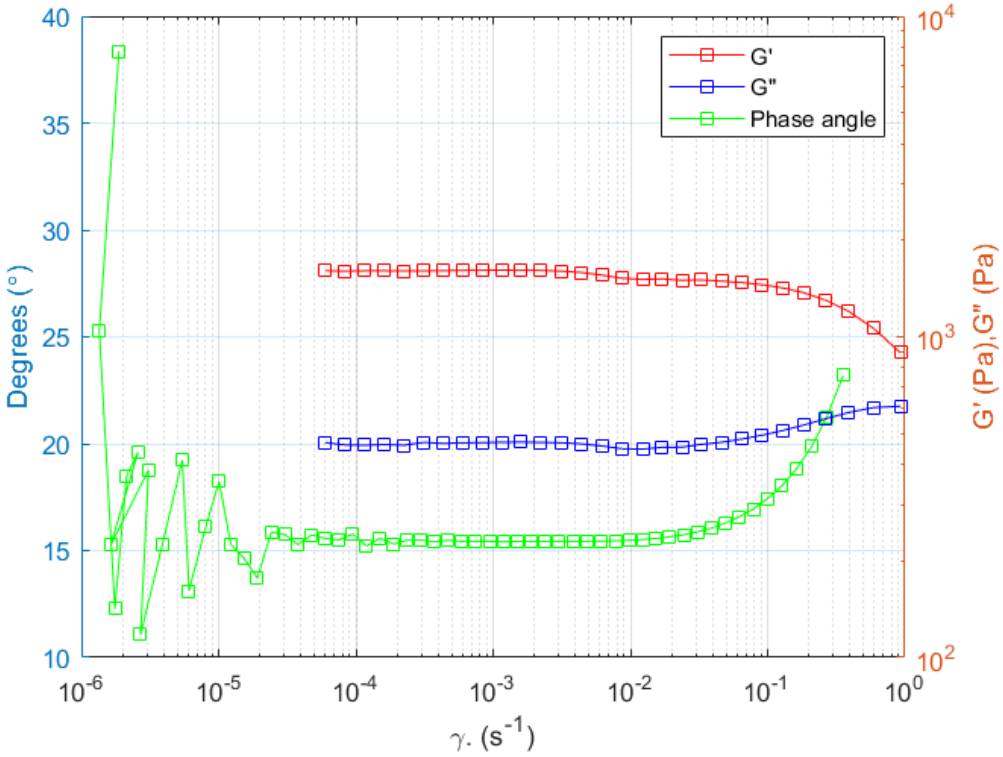
LVE range measurement



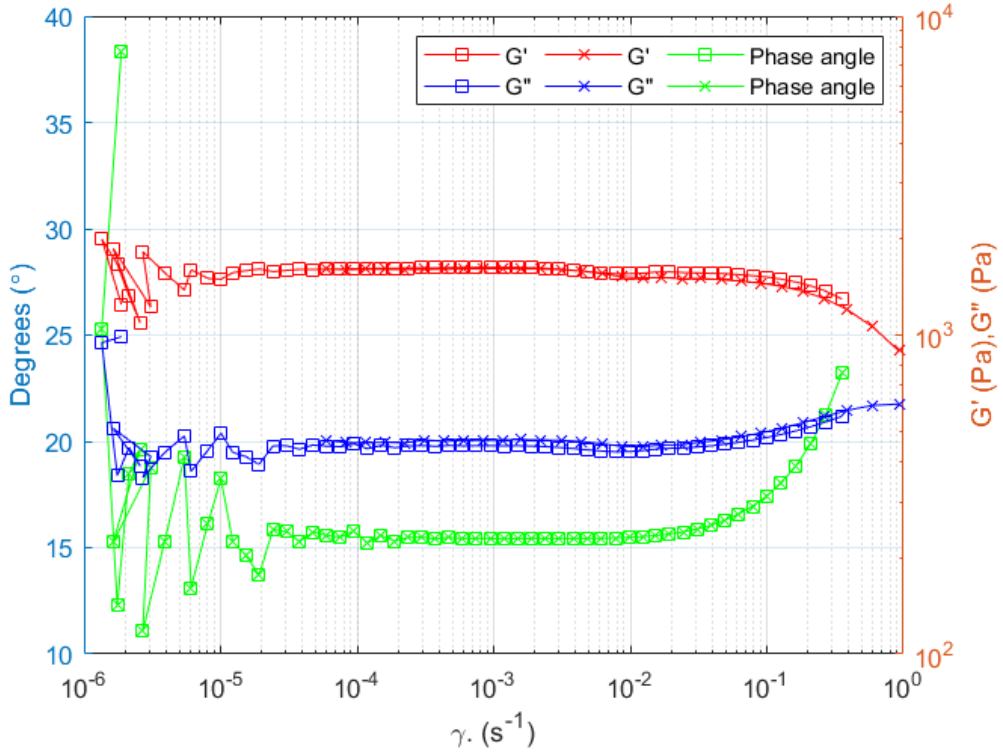
Frequency sweep measurement



Amplitude sweep down measurement



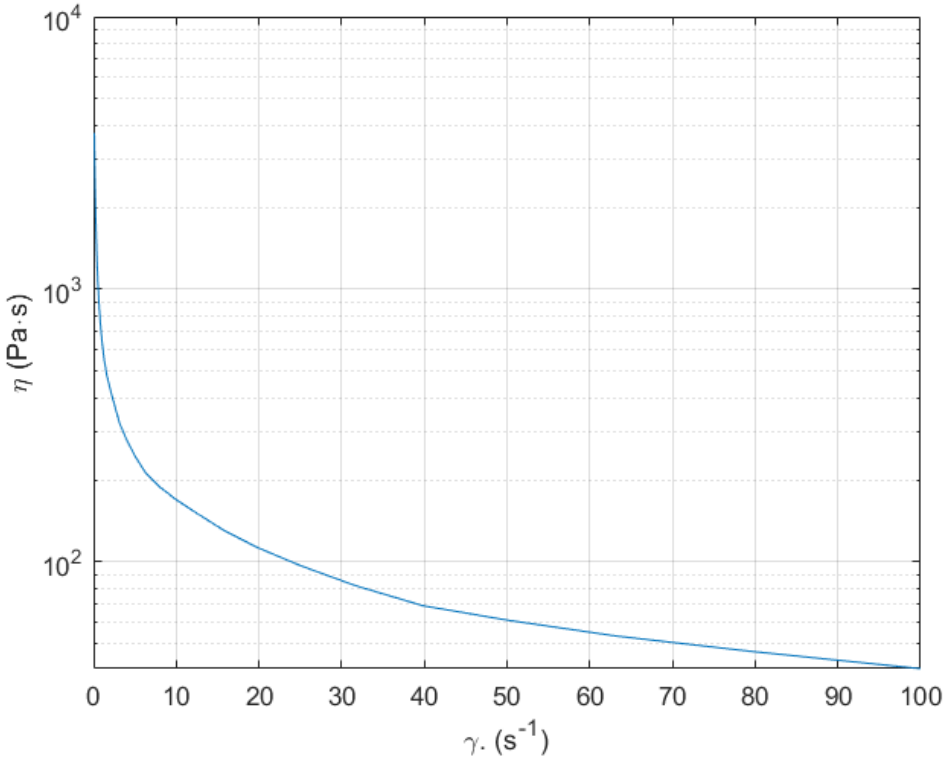
Amplitude sweep up measurement



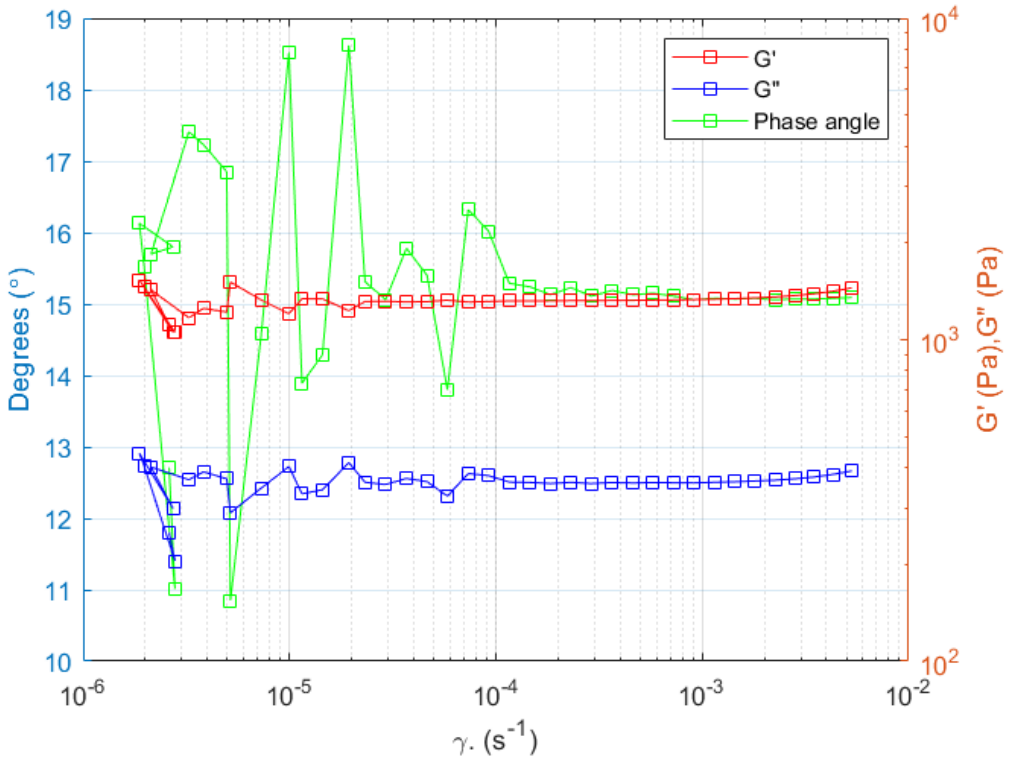
Amplitude sweep up and down combined

ChCl:glycerol + 5 wt% Carbopol 974P NJ +5 wt% water

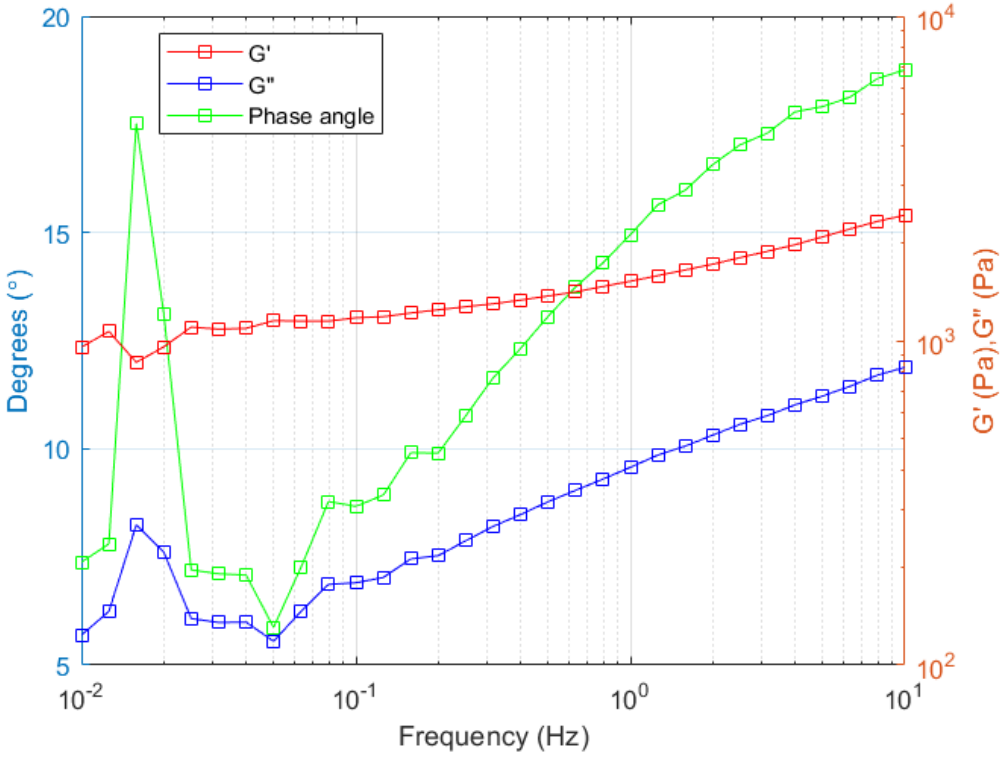
Day 1



Viscosity measurements

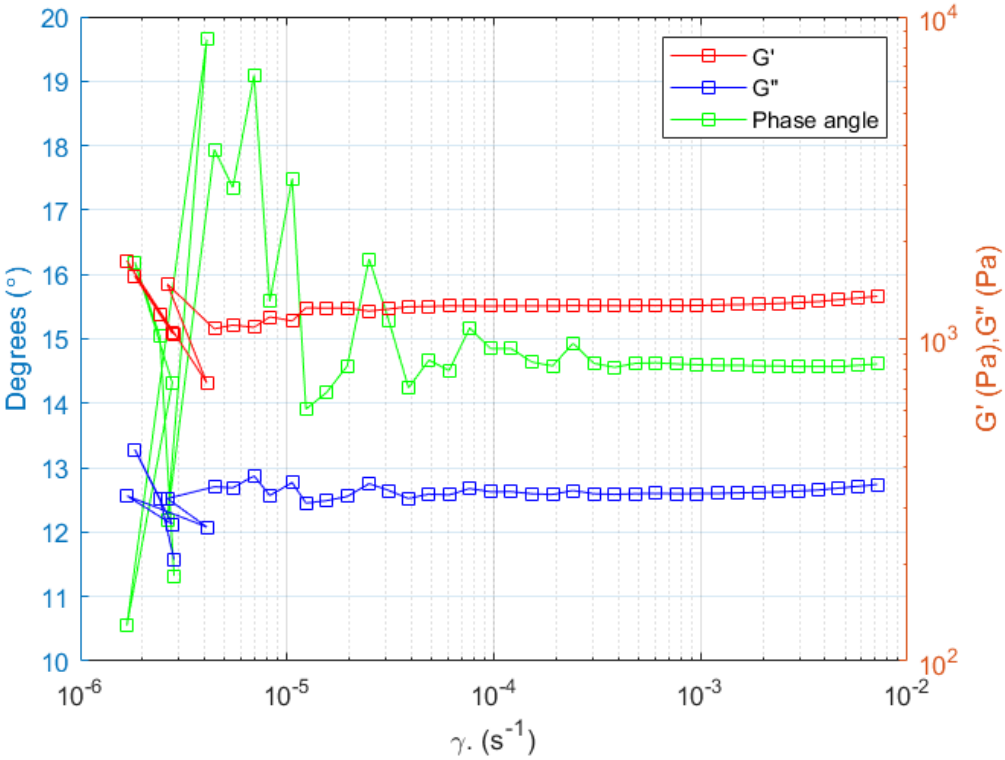


LVE range measurements

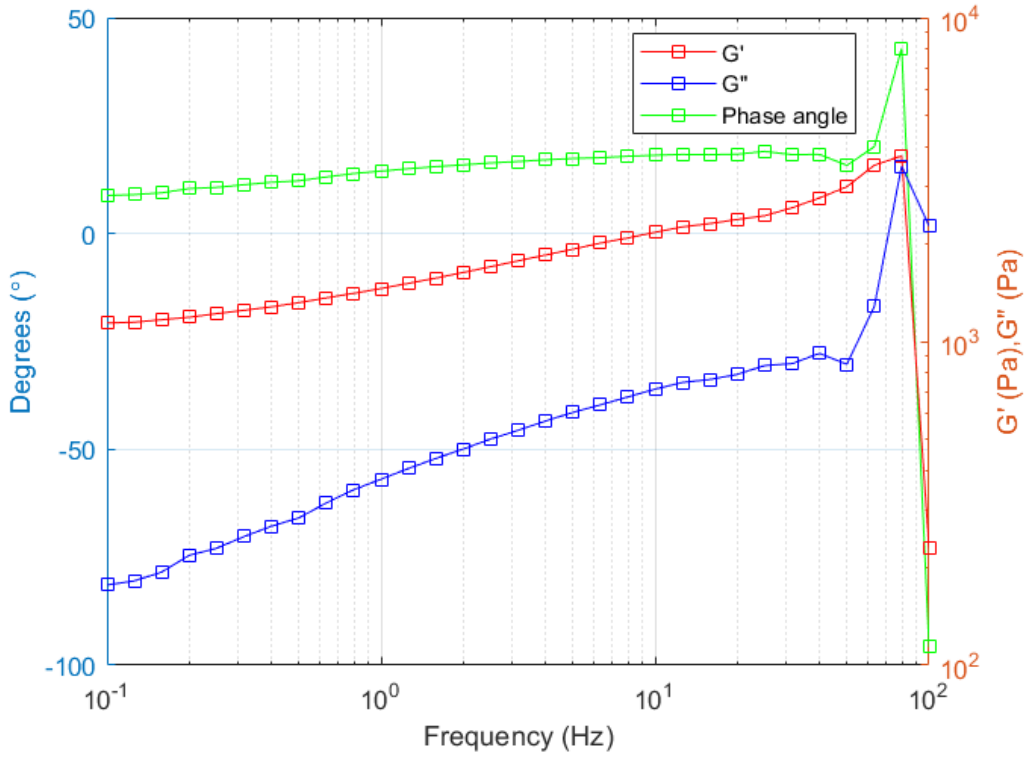


Frequency sweep measurements

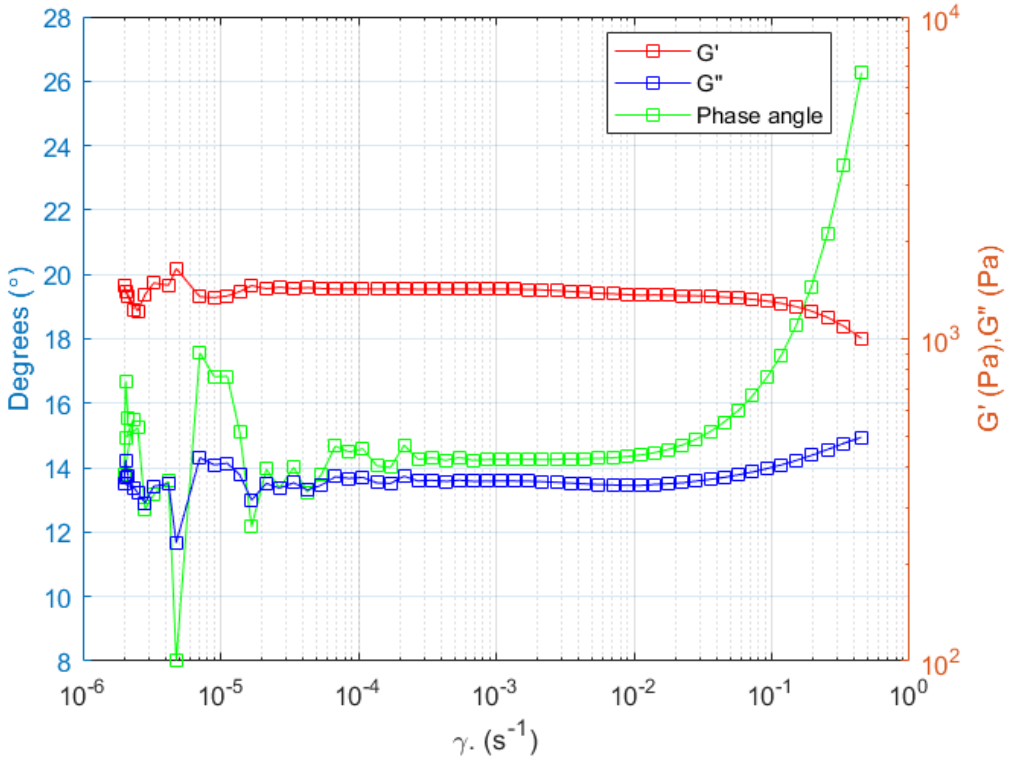
Day 2



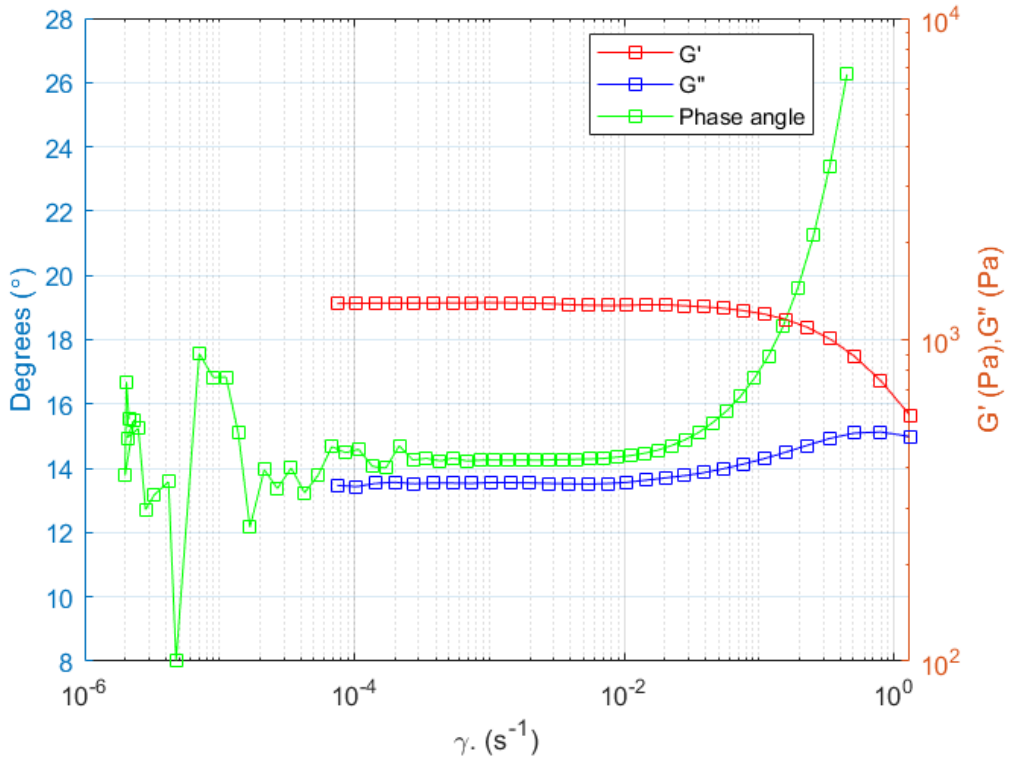
LVE range measurements



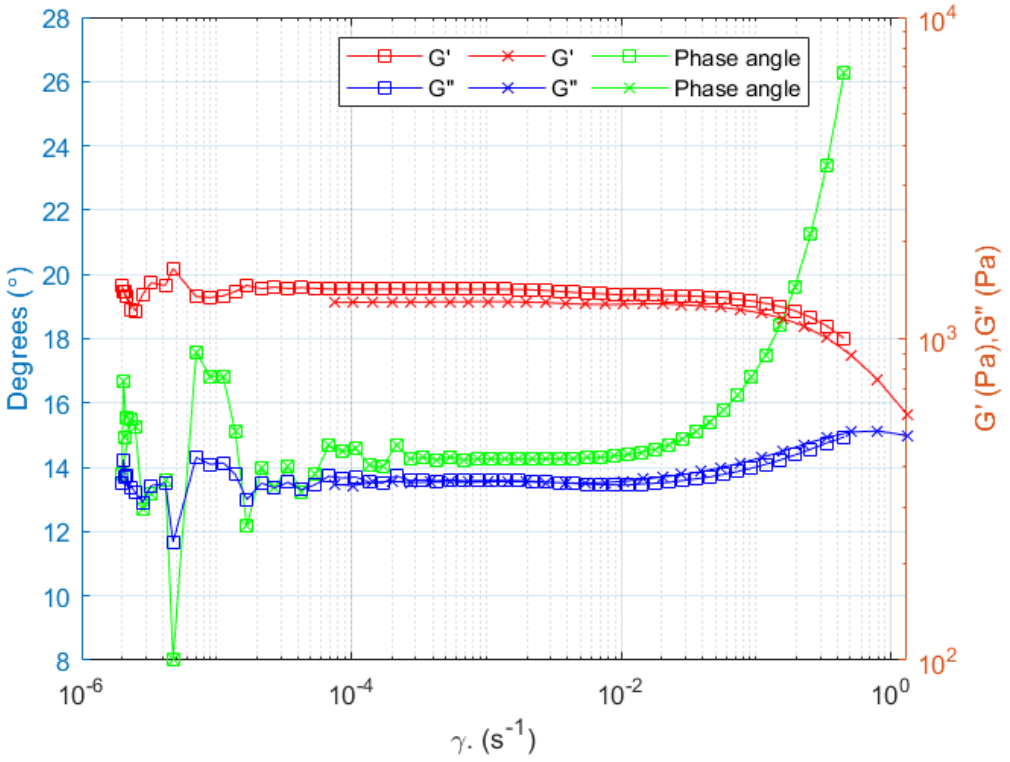
Frequency sweep measurement



Amplitude sweep down measurement



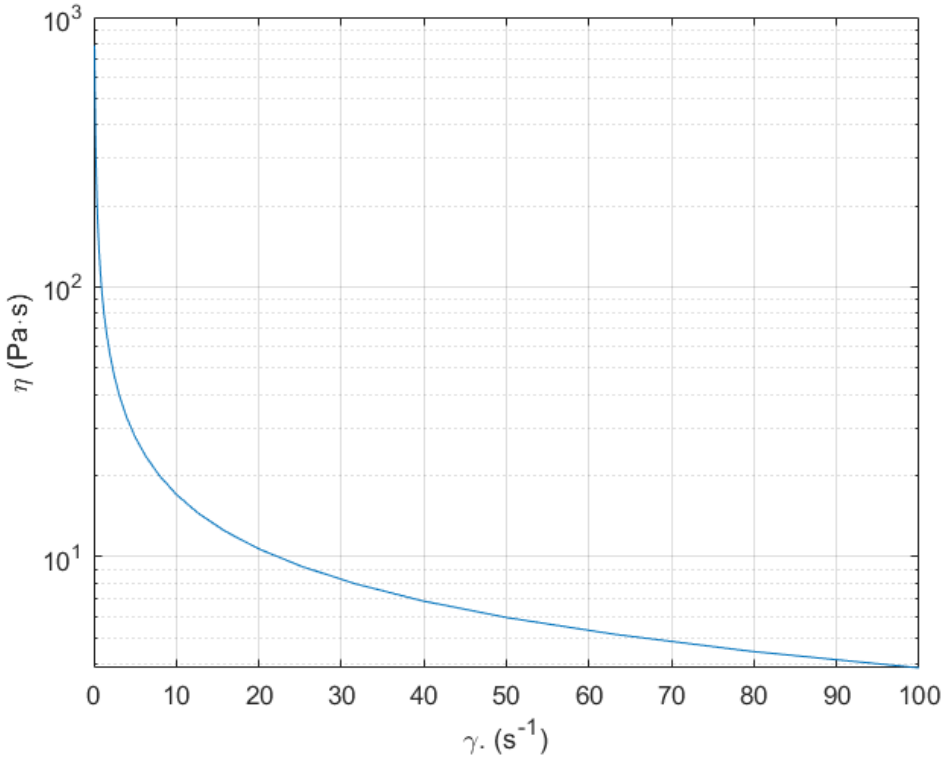
Amplitude sweep up measurement



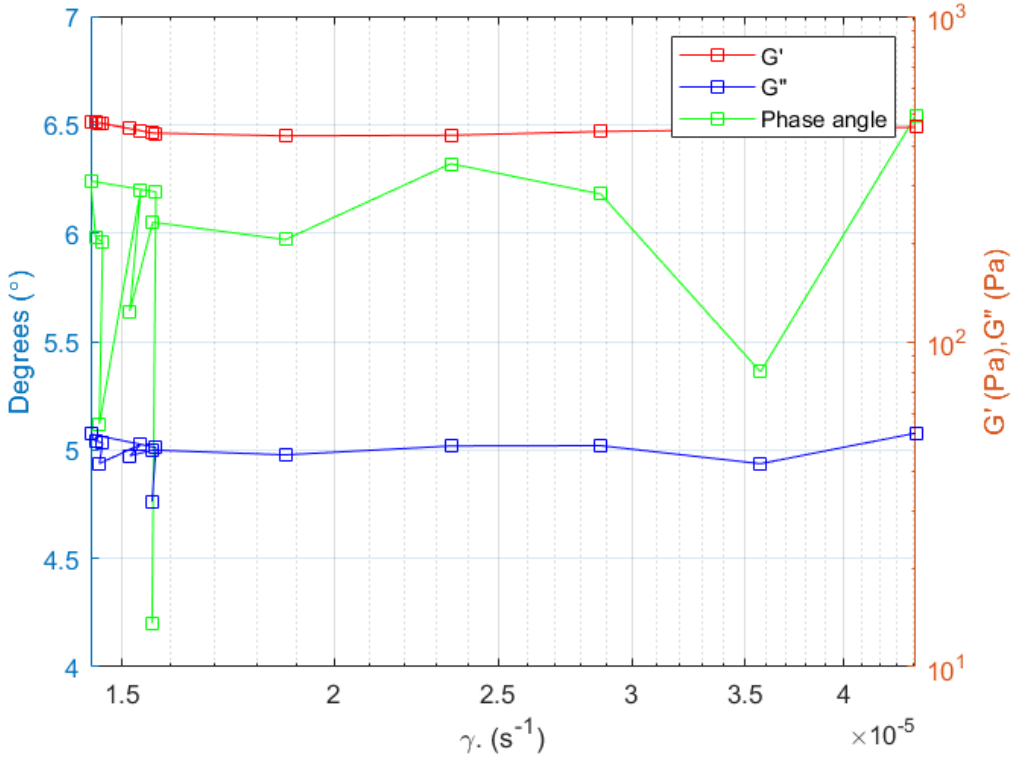
Amplitude sweep up and down combined

ChCl:glycerol + 5 wt% Carbopol 974P NJ + 50 wt% water

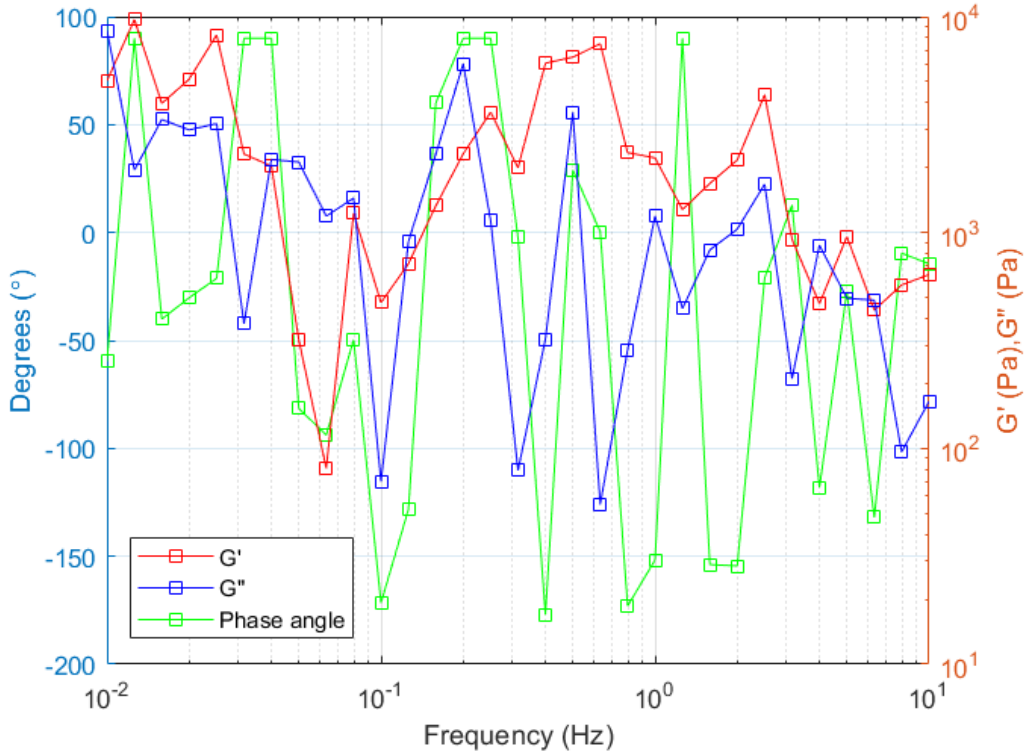
Day 1



Viscosity measurements

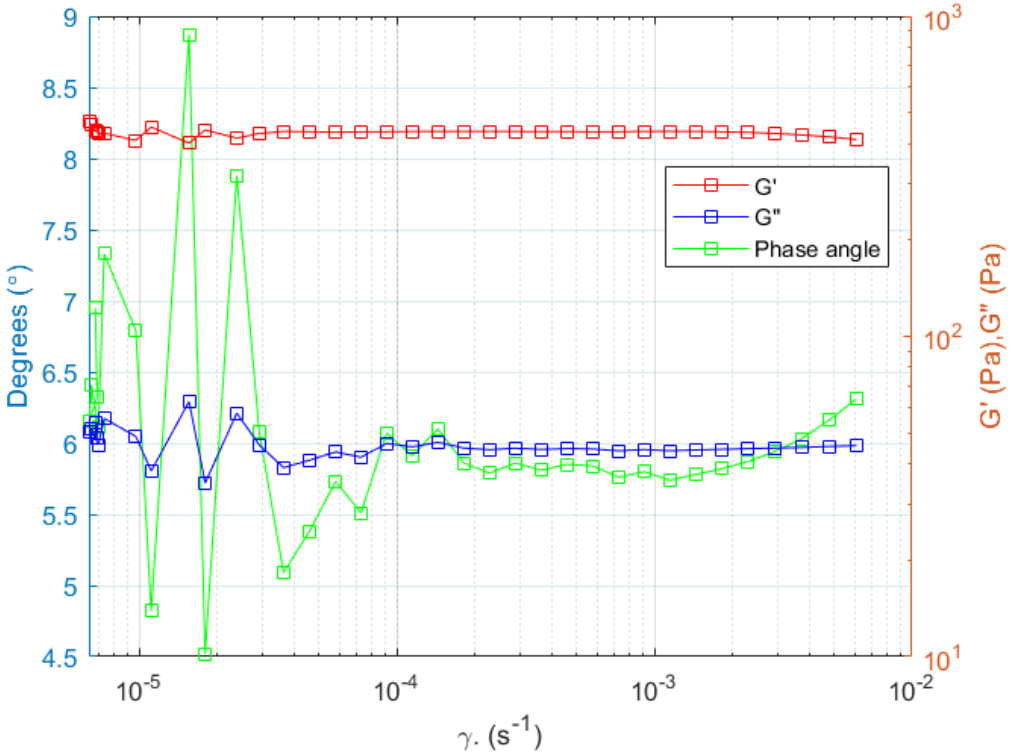


LVE range measurements

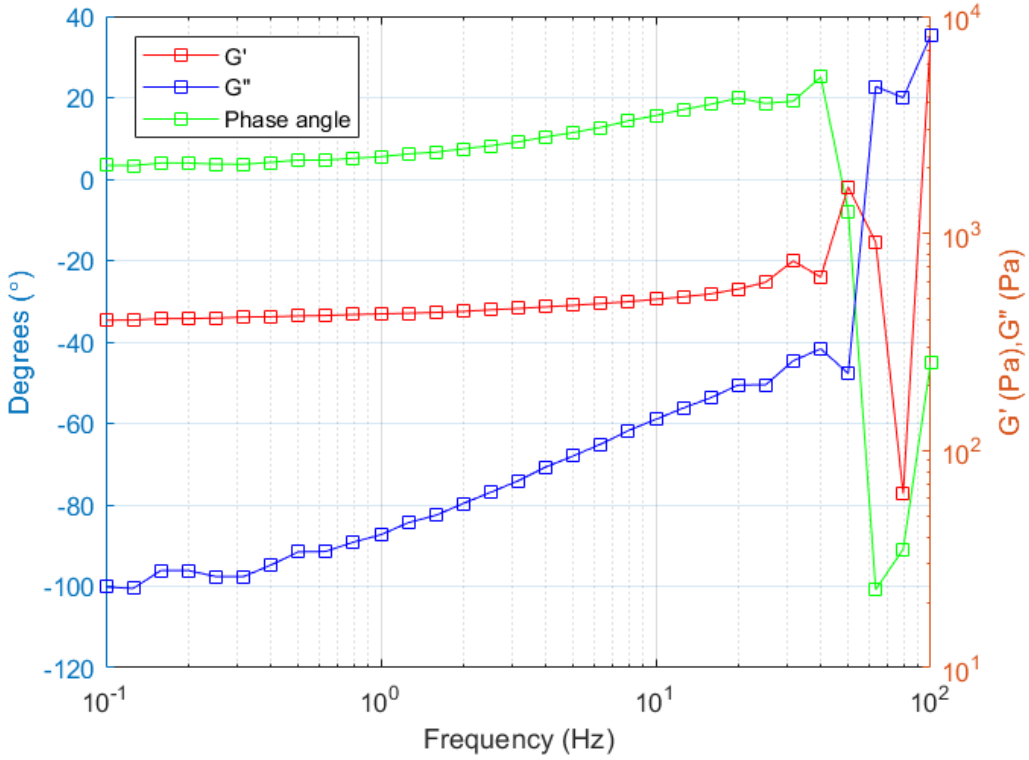


Frequency sweep measurements

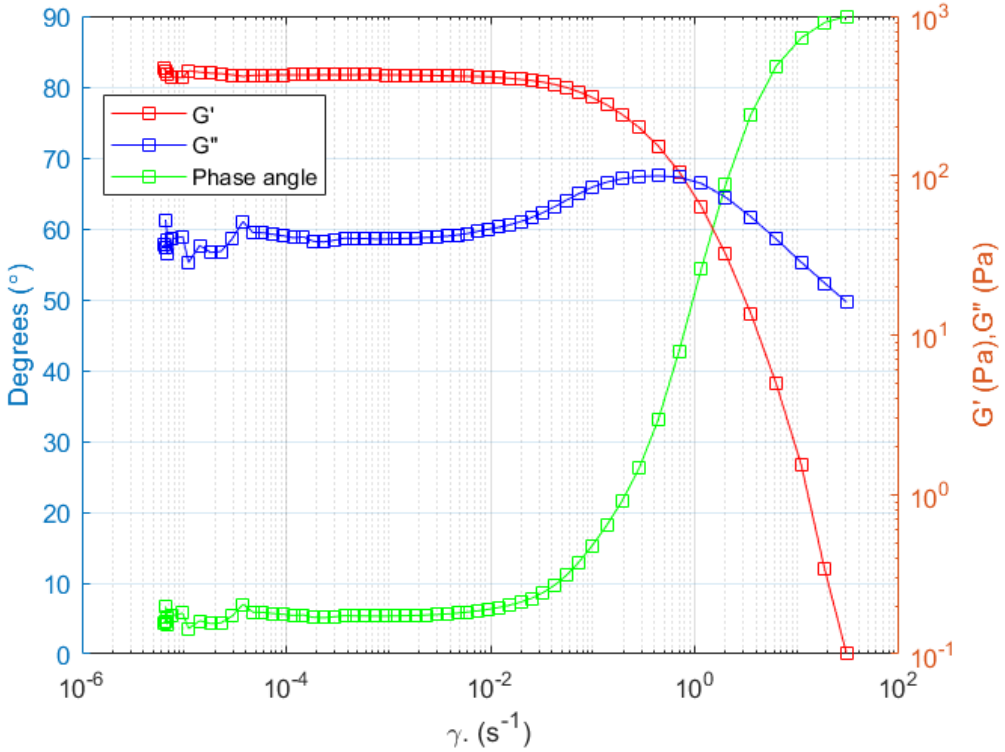
Day 2



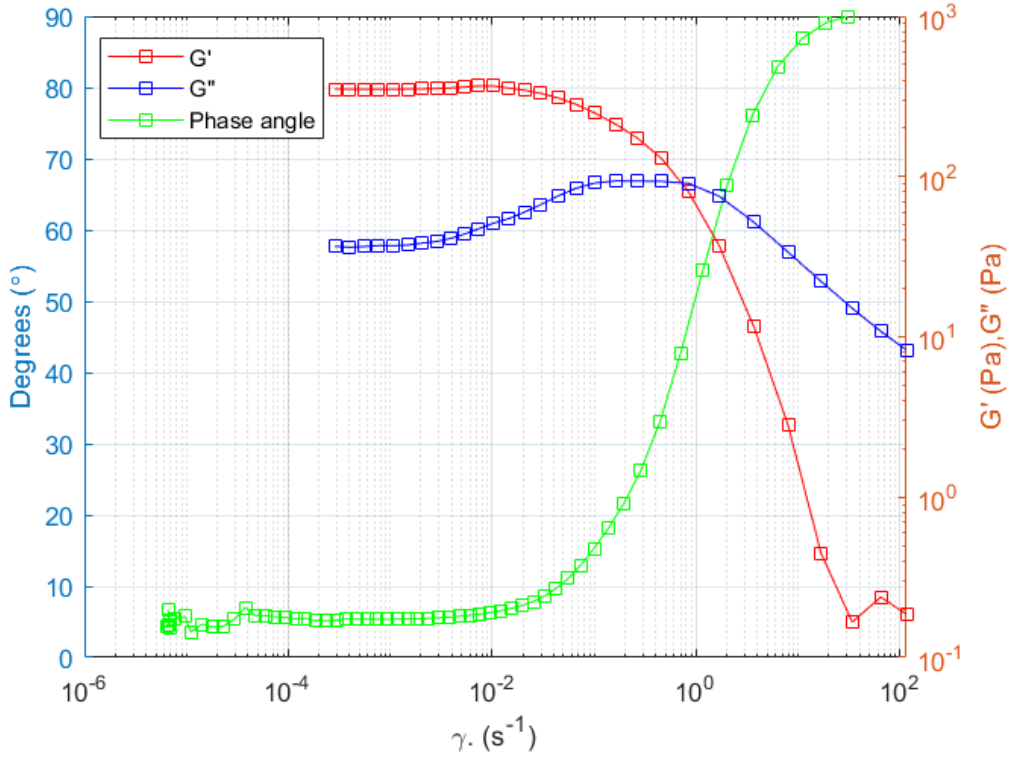
LVE range measurements



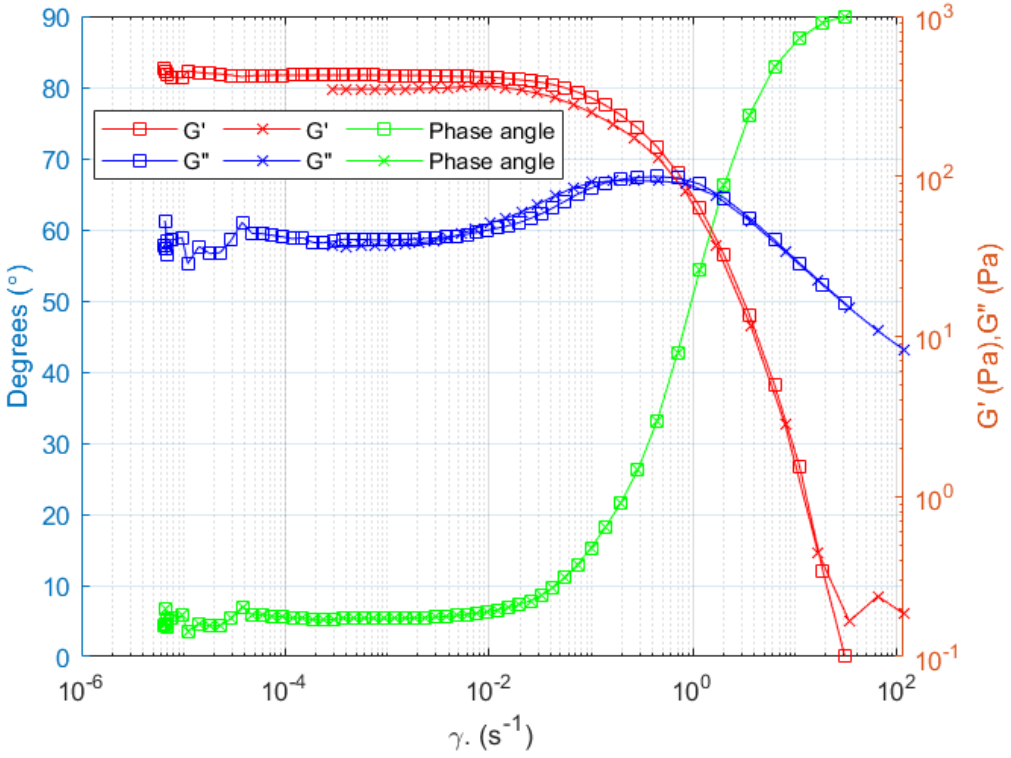
Frequency sweep measurements



Amplitude sweep down measurements



Amplitude sweep up measurements



Amplitude sweep up and down combined