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Physical properties of gelatin based solid emulsions

Effects on drug release in the GI tract

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Preface

The laboratory work in this MSc thesis was performed at the Norwegian Biopolymer Laboratory (NOBIPOL), Department of Biotechnology, Norwegian University of Science and Technology (NTNU), Trondheim, from August 2012 to April 2013.

It has been a laborious year with a steep learning curve, but I have received excellent support and advice from my supervisor, Professor II Kurt Ingar Draget and my co-supervisor, Ph.D Magnus Nergård Hattrem, to whom I wish to express my gratitude. Special thanks should be directed to Magnus, who always had time for my questions and provided valuable input throughout the thesis work. I would also like to thank staff engineer Ann-Sissel Ulseth for performing the SEC-MALLS analysis for me, and the rest of Kurt's research group at NOBIPOL.

To Sigurd; thank you for always being there.

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Abstract

Oral administration of pharmaceuticals is a convenient and simple route for drug delivery. Due to challenges concerning patient compliance and bioavailability, there has been exerted a great effort the last couple of years to optimize different types of oral delivery vehicles. Gelled emulsion-based systems have shown great promise as delivery system. They are chewable, thereby increasing patient compliance, and they are able to “trap” drugs, either in the gelled or dispersed phase, and thus provide a controlled and prolonged release of the drug, which reduce the possibility of the drug being inactivated in the stomach.

The purpose of this study was to investigate how gelatin type and strength, as well as oil content, influences the physical properties of gelatin-based solid emulsions.

Filled gel emulsions stabilized by 160 g or 260 g Bloom gelatin type A or B were prepared with varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%) and subjected to rheological characterization. The obtained results showed an increase in storage modulus, gelling and melting temperatures and viscosity with increasing amount of dispersed phase. The increases were more pronounced for both 160 g and 260 g gelatin type A, than for either types of gelatin B. Gelled emulsions were investigated using longitudinal deformation and there was a clear increase in Young’s modulus for gelatin A when the oil content was raised. For 260 g Bloom gelatin A and B, there was also a clear trend towards a decrease in force and strain at break with increasing oil content, but again, this was more pronounced in gelatin type A. *In vitro* dissolution studies in a near gastric environment were performed on a filled gel emulsion (40 wt.% corn oil) stabilised by either gelatin type A or B (260 g Bloom). Here there was also seen a clear correlation between the rheological characteristics of the filled gel emulsions and dissolution time, where gelatin type A had a prolonged dissolution profile compared to gelatin type B. According to these results, there is a possibility to use different gelatin types and oil content to modify the properties of filled gel emulsions for a variety of pharmaceutical applications.

Sammendrag

Administrering av legemidler oralt er en praktisk og enkel leveringsmetode. På grunn av utfordringer med hensyn til biotilgjengelighet og brukervennlighet har det de siste årene blitt lagt ned store mengder arbeid i å utvikle og optimalisere nye typer vehikler for oral administrering. Gelede, emulsjonsbaserte systemer er lette å tygge, noe som gjør det lettere for pasientene å innta legemiddelet. De har også muligheten til å «fange» legemiddelet i emulsjonsmatriksen, noe som vil muliggjøre en kontrollert og forlenget frigjøring av legemiddelet. Dette reduserer muligheten for at legemiddelet brytes ned prematurt i det sure miljøet i magesekken.

Målet med denne studien var å kartlegge hvordan gelatintype og –styrke i tillegg til innhold av olje påvirker de fysiske egenskapene til faste, gelatinbaserte emulsjoner.

De reologiske egenskapene til emulsjonsgelene (0, 10, 20, 40 og 50 wt.% maisolje) stabilisert av gelatintype A eller B, med Bloomverdi 160 g eller 260 g ble karakterisert. Resultatene viste en økning i lagringsmodulus, gelings- og smeltetemperatur og viskositet med økende innhold av dispergert oljefase. Økningene var tydeligere for emulsjonsgelene stabilisert med gelatin type A (både 160 g og 260 g Bloom) enn for gelatin type B. De gelede emulsjonene ble også undersøkt med kompresjonsmålinger, noe som viste en klar økning i Youngs modulus hos gelatin type A, med økende oljeinnhold. Det var også en klar nedgang i kraft og deformasjon ved brudd hos gelatin type A og B med Bloomverdi 260 g når oljeinnholdet ble økt. Også her var trenden tydeligst i gelatin type A. Oppløsningsstudier ble også gjennomført *in vitro*, i et nær gastrisk miljø, på emulsjonsgeler (40 wt.% maisolje) stabilisert med 260 g Bloom gelatin type A eller B. Det ble også her observert sammenheng mellom de reologiske egenskapene til emulsjonene og oppløsningstiden. Gelatin type A hadde en lengre oppløsningsprofil i forhold til gelatin type B.

På bakgrunn av disse resultatene kan man si at det er mulig å bruke ulike gelatintyper og oljeinnhold til å modifisere egenskapene i emulsjoner til forskjellige farmasøytiske applikasjoner.

List of abbreviations

A	area
Bloom	measured gel strength under standard conditions
CCx	Concordix
°C	degrees Celsius
E	Young's modulus
F	force
G'	storage modulus
G''	loss modulus
GIT	gastro-intestinal tract
IEP	isoelectric point
L_0	original length
ΔL	change in length
M_n	number average molecular weight
MQ	MILLI-Q water
M_w	weight average molecular weight
N	number of replicates
PI	polydispersity index
rpm	revolutions per minute
SEC-MALLS	size exclusion chromatography, coupled to multi-angle laser light scattering
Type A	gelatin exposed to acidic pretreatment
Type B	gelatin exposed to alkaline pretreatment
T_g	gelling temperature
T_m	melting temperature
wt.%	weight percentage

δ	phase angle	$\dot{\gamma}$	shear rate
η	viscosity	**ν**	Poisson ratio
γ	strain	**ω**	angular frequency
σ	stress		

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1 Introduction

1.1 Background and aim of thesis

One of the most preferred methods of drug delivery is oral administration. Tablets and/or liquids are easy to administer, confers no pain for the patient and are very versatile. In addition, there is no need for sterile conditions during production, so they are less expensive to manufacture. Despite all these advantages, there are also several disadvantages and challenges associated with oral drug administration. One of these challenges is patients suffering from *dysphagia*, or difficulty in swallowing. This is a disease affecting all age groups, and is associated with a number of disorders, but it is especially pronounced in elderly people. (Sastry, Nyshadham, & Fix, 2000). A study performed on Norwegian patients in 1995, revealed that of a patient population of 6158; 1576, or 26 % had problems swallowing tablets. The most common complaint was the size of the tablet, followed by the surface, form and taste (Andersen, Zweidorff, Hjelde, & Rodland, 1995). Oral administration also places great demands on the physical and chemical properties of the drug in question. When taken orally, the drug enters the gastrointestinal tract (GIT), which is comprised of the mouth, throat, stomach and the upper and lower intestines. The drug has to be chemically stable to stay unmodified during the encounter with gastric acid and digestive enzymes. In addition, it is essential that the drug has a certain balance of water versus lipid solubility to ensure that the drug passes through the cells lining the intestinal or gut wall. A very hydrophilic drug will not be able to pass through the fatty cell membranes, and a very lipophilic drug risks being dissolved in fat globules, causing poor contact with the gut wall (Patrick, 2009).

The poor solubility of newly developed drugs is thus a reoccurring problem in the pharmaceutical industry. Between 40 and 70 percent of all new chemical entities that enters drug development programs does not possess the sufficient aqueous solubility to ensure that a sufficient amount of the drug is absorbed from the GIT (Hauss, 2007). Lipid-based formulations have the ability to facilitate gastro-intestinal absorption of poorly soluble drugs. Hence, there is a need to further develop such delivery systems that are able to encapsulate, protect and release bioactive and functional lipophilic components. There are a number of applications for such delivery systems, such as control and increase lipid bioavailability and targeted delivery within the gastrointestinal tract. (Hauss, 2007; McClements & Li, 2010).

The *Concordix* (CCx, patent number US 2011/0268770 A1) technology was developed by NOBIPOL (Norwegian Biopolymer Laboratory) and Ayanda AS, as an answer to the demand for improved drug delivery formulations (Seternes, Draget, & Haug, 2009). It is an emulsion-based oral delivery system for nutraceuticals and drugs, where lipid droplets are dispersed in a gelled water phase, providing a soft and chewable gel matrix. The CCx technology is currently on the market as a delivery system for PUFAs (polyunsaturated fatty acids), and it has demonstrated increased bioavailability for the omega 3 (ω-3) fatty acids DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid), compared to traditional soft gel capsules (I. J. Haug et al., 2011). It is also desired to utilize the CCx technology for oral delivery of pharmaceuticals, due to its easy to swallow properties and increased patient compliance, as well as its potential for increasing the solubility of the drug and thus increase bioavailability.

The aim of this study was to investigate and characterize the physical properties of solid gelatin-based filled emulsions, and how these changed in response to varying amounts of corn oil. The thesis focuses on identifying the rheological characteristics of gelatins of different types (type A and B) and Bloom values (160 g and 260 g) by using small strain oscillatory, viscosity and longitudinal measurements. Dissolution studies were also performed to investigate how the filled gel emulsions behaved in an environment resembling the GIT.

1.2 Gelatin

Gelatin is considered one of the most versatile biopolymers, due to its large number of uses in different applications, ranging from making jelly in the kitchen to usage in the pharmaceutical and cosmetic industry. The origin of gelatin is the collagen protein, the protein class that occurs most frequently in both animals and humans. There exist 27 different types of collagen, and type I is the most widely occurring, existing primarily in connective tissue, like skin, bone, and tendons. (Schrieber & Gareis, 2007) A collagen molecule consists of a right-handed triple helix of individual α-chains (Babel, 1996; Smidsrød & Moe, 2008). The triple helix is stabilised by hydrogen bonds between the peptide chains. The general amino acid sequence of the α peptide chains is Gly-X-Y, where X tends to be proline, and Y often is hydroxyproline (Smidsrød & Moe, 2008). Collagen is treated with alkali or acid to break up the secondary and higher structures of the protein, which will yield type A or type B gelatin, respectively (Haug & Draget, 2009). Table 1.2.1 shows the amino acid content of collagen type I and gelatin type A and B.

Table 1.2.1: Amino acid composition of collagen and gelatin – Residues per 1000 residues.

Amino Acid	**Type I collagen (bovine)**	**Type A Gelatin[a]**	**Type B Gelatin[b]**
Alanine	114	112	117
Arginine	51	49	48
Asparagine	16	16	-
Aspartic Acid	29	29	46
Glutamine	48	48	-
Glutamic Acid	25	25	72
Glycine	332	330	335
Histidine	4	4	4
4-hydroxyproline	104	91	93
ε-Hydroxylysine	5	6	4
Isoleucine	11	10	11
Leucine	24	24	24
Lysine	28	27	28
Methionine	6	4	4
Phenylalanine	13	14	14
Proline	115	132	124
Serine	35	35	33
Threonine	17	18	18
Tryptophane	-	-	-

Tyrosine	4	3	1
Valine	22	26	22

[a]Type A gelatin: Acid pre-treated pig skin gelatin; [b]Type B gelatin: Alkali pre-treated bone gelatin (Babel, 1996).

1.2.1 Gelatin Production

Gelatin can be produced from any collagen-containing raw material, but skin, hides and bones from mammalian sources (porcine, bovine) are preferred. Several steps are included in the manufacturing process; first the raw material is cleaned, followed by either acid or alkali pre-treatment, to break up the intermolecular bonds. The acid pretreatment yields type A gelatin, and is mainly used in pigskin gelatin production. To acquire material suitable for gelatin production, 24 – 48 hours of acid treatment at pH 1 – 2 is required. Alkali treatment gives type B gelatin, and the raw materials are usually bone and hide splits. The procedure requires 8 – 12 weeks of treatment with calcium hydroxide (pH 12 – 13) at 15 – 20 °C, so-called “liming”. This process extracts alkali-soluble non-collagenous proteins, remove undesirable components of connective tissue, hydrolyses residual fat and inter- and intramolecular crosslinks in the collagen molecule. After the pre-treatment, the raw material is washed to remove excess acid or alkali, followed by gelatin extraction. Residual salt is then removed by a simple ion exchange process. It is preferred that the ash content does not exceed 1 %. After extraction, the gelatin is filtrated to improve clarity, then concentrated by evaporation and filtered again. The two last steps are sterilization and drying. The finished gelatin has a moisture content of 9 – 13 % (Babel, 1996).

A point of interest is the difference in isoelectric point (IEP) between gelatin A and gelatin B. This is due to the alkaline treatment of gelatin B, which almost completely converts asparagine and glutamine to aspartic and glutamic acid, respectively. Hence, gelatin B has an IEP at pH 4,8 - 5,5, while the IEP of gelatin A lies around pH 8 – 9. The isoelectric point of gelatin A is identical to that of the parent collagen molecule (Babel, 1996; Schrieber & Gareis, 2007).

1.2.2 Gelatin structure and gelling mechanism

The collagen molecule is made up of three parallel α-chains, forming a right-handed triple helical rod stabilized by intramolecular hydrogen bonds (M. Djabourov, Lechaire, & Gaill, 1993). It measures about 300 nm, has a diameter of 1,5 nm and molecular weight of 300,000

Da. Pre-treatment of collagen followed by extraction provides the final gelatin product, which is a mixture of polypeptides with varying compositions and molecular weights. Three dominating fragments are found in gelatin; α, β and γ. The α-chains are the "building" blocks of β and γ chains, which consist of two and three covalently bound α-chains, respectively. Single α-chains have a molecular weight of 90-110 kDa ($M_{w\alpha}$), β-chains are 180-220 kDa ($M_{w\beta}$) and γ-chains are 270-300 kDa ($M_{w\gamma}$) (Haug & Draget, 2009). Gelatin is hence a polydisperse protein, and the molecular weight distribution ranges from 10,000 to a few million Daltons, depending on the raw material, type of pre-treatment and extraction process (Babel, 1996).

An aqueous solution of gelatin is thermo-reversible, which means that it is able to interconvert between a sol and a gel, dependent on the temperature. A gelatin solution with a concentration >1 % (Djabourov & Papon, 1983) will increase its viscosity drastically when cooled to 35-40 °C, and form a gel when cooled further. It is assumed that the gelling process involves three steps: 1) there is an association of free dissolved α-chains, through the hydrophobic interaction between structural elements containing glycine, proline and hydroxyproline. This causes the formation of ordered helical structures. 2) Two or three of these ordered segments associates and forms crystal structures, and 3) the structure is stabilized through local formation of hydrogen bonds within and between the helices. Additionally incorporated water molecules will enhance the stability of the gel by forming hydrogen bonds with the hydrophilic OH-groups of the corresponding amino acids (Babel, 1996).

1.2.3 Physical properties of gelatin gels

Gelling and Melting Temperature: It has been shown that there is a correlation between the thermal stability of gelatin gels and the amount of the imino acids; proline and hydroxyproline. It is suggested that hydroxyproline located in the third position of the Gly-X-Y triplet is the main stabilizer of the triple helix, because of its ability to bind hydrogen. Fish gelatins from cold water species are poor gelling agents, because they contain low amounts of imino acids, compared to warm water fish species and warm blooded mammals. (Haug & Draget, 2009). Table 1.2.1 summarizes some physical features of mammalian and fish gelatin gels.

Table 1.2.2: Physical properties of different gelatin gels (Haug & Draget, 2009)

Property	Mammalian gelatin 220 g Bloom	Cold water fish gelatin	Warm water fish gelatin 220 g Bloom
Gelling temperature	26 – 27 °C	4 – 8 °C	21 – 22 °C
Melting temperature	33 – 34 °C	14 – 16 °C	28 – 29 °C
Solubility	> 40 °C	> 22 °C	> 35 °C

The helix-to-coil temperature is a critical parameter for both collagen and gelatin. It may also be called the denaturation temperature, and it is mainly determined by the pyrrolidine content. It lays between 15 – 20 °C for cold water fish gelatin and around 36 °C for mammalian gelatins, because of the differences in pyrrolidine content (Djabourov & Papon, 1983). Above the helix-to-coil temperature the individual peptide chains will not interact to a large degree. However, by lowering the temperature below the helix-to-coil temperature the peptide chains will form intermolecular hydrogen bonds, causing the partially regaining of the collagen structure. A gelatin solution with a concentration above the critical overlap concentration (c*), will form a thermo reversible, viscoelastic gel upon cooling, because the gelatin molecules reassemble into triple helices, forming a network. The critical overlap concentration and gel strength of a gelatin is dependent on different factors, like molecular weight distribution, co-solutes and the pH of the solution (Haug & Draget, 2009).

The helix-to-coil temperature should not be confused with the gelling or melting temperature. For a pure gelatin system, the gelling and melting temperature will always be lower than the helix-to-coil transition temperature. Hysteresis exist between the gelling and melting, and this is caused by associations of helices in gel state. The strength of a gelatin gel will increase with time, independent of temperature (Haug & Draget, 2009). The coil-helix transition process is showed in Figure 1.2.1.

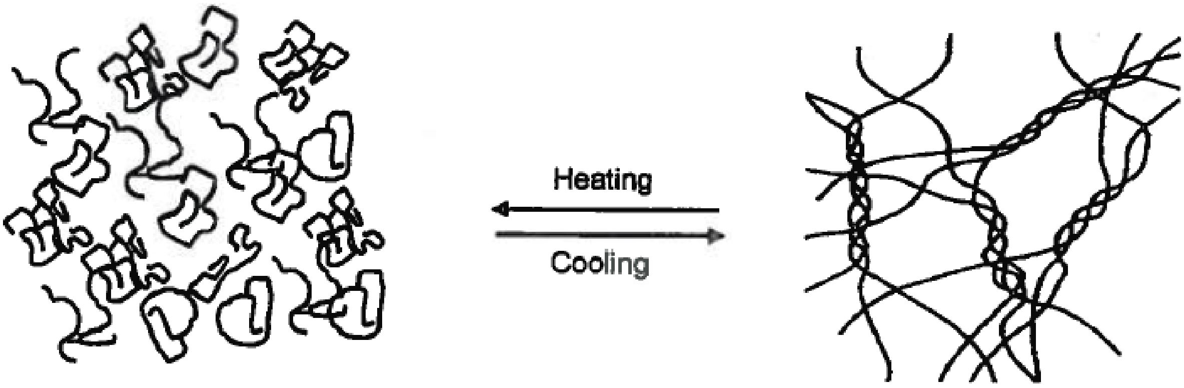

Figure 1.2.1: The thermo reversible gelling process for gelatin (Haug & Draget, 2009).

Gel strength: The Bloom value is the analytical measure of gel strength, and it is named after the American scientist Oscar T. Bloom, who developed a "Machine for Testing the Jelly Strength of Glues, Gelatines, and the Like". The Bloom value is defined as the weight in grams required for a specified plunger to depress the surface of a standard, thermostatted gel to a defined length under standard temperature and pressure. Commercial gelatins usually have a Bloom value within a range of 50 – 300 Bloom. Gelatins with higher Bloom values usually have higher melting and gelling temperatures, shorter gelling times, lighter color and a more neutral taste and odor (Schrieber & Gareis, 2007). In a pH range of 4 – 10, the gel strength for both fish and mammalian gelatins is rather independent of the pH value. Gelation will be markedly inhibited outside this pH-range, probably because the chains will carry a high net positive or negative charge, which again will cause electrostatic forces to inhibit the ability of the chains to enter suitable positions for the formation of junction zones (Haug & Draget, 2009).

Rheology: As a dilute solution, gelatin is a Newtonian fluid with a purely viscous behavior. This changes upon cooling, as hydrophobic and hydrophilic interactions are formed between the gelatin molecules. When the solution is further cooled, the structure-forming process continues until it reaches the phase-transition point and the gelling starts. A permanent gel is formed with further reduction in temperature, and this will have a viscoelastic behavior giving the system the nature of a solid material (Schrieber & Gareis, 2007).

1.2.4 Applications of gelatin

Gelatin has several unique properties, giving it many and diverse applications as an important component in food, pharmaceutical, cosmetic, medical and photographic products. It is a good protein source and has high nutritional value. The most important properties of gelatin, making it so useful in several areas is its ability to form thermo reversible gels, its texture, thickening and water binding capacity (Haug & Draget, 2009). As many other proteins, gelatin possess amphipathic properties and can thereby participate in the formation of air/water and oil/water phase interfaces. It will further provide stability by the formation of thin films at the oil-water interphase. This property can be used to stabilize foams and emulsions for different purposes, as in food products like marshmallows and mayonnaise or in medical applications. The gelatin may also increase the stability of an oil-in-water emulsion by gelling of the aqueous phase of the emulsion (Babel, 1996).

1.3 Emulsions

1.3.1 General properties

Emulsions are dispersions of two immiscible liquids, for example oil and water, where one of the liquids is dispersed uniformly as droplets in the other liquid. There are many important applications for emulsions, among others in the pharmaceutical industry and in different food products, like milk, mayonnaise, margarines etc (Butt, Graf, & Kappl, 2006; Norde, 2003). The most important emulsions are oil-in-water (O/W) emulsions, where oil is the dispersed phase and water is the continuous phase, and water-in-oil (W/O) emulsions, where water is dispersed in oil (McClements, 2002). The size of the droplets usually ranges between 0,1 and 10 µm, and they are called the *dispersed* or *internal phase,* while the liquid they are dispersed in is called the *continuous* or *external phase* (Butt et al., 2006). The type of emulsion formed depends on many factors, like the type of surfactant, volume fraction of oil and water, temperature etc. Bancroft's rule states that the phase where the emulsifier is most soluble, will usually be the continuous phase after emulsification (Malmsten, 2002).

1.3.2 Emulsion stability

An emulsion is a thermodynamically unstable system, as it will form only if energy is applied, and need to be kinetically stabilised in order to be stable over time (Butt et al., 2006; McClements, 2002). An emulsion is usually prepared by high-speed homogenization, during which the droplets continuously breaks up and recoalesce. The final droplet size is determined by the timescale of the breakup and recoalescence processes. Coalescence is the process where the "film" between two droplets rupture, causing them to fuse into a larger droplet due to the Laplace pressure (Norde, 2003). If an emulsion of pure oil and water is left to stand, coalescence between the dispersed droplets will reduce the surface area, eventually leading to the two immiscible liquids separating into a low-density phase (oil) and high density phase (water) as showed in Figure 1.3.1. This phenomenon is called *phase separation* or *demulsification*, and leads to a lower Gibbs free energy of the system. (Butt et al., 2006; McClements, 2002)

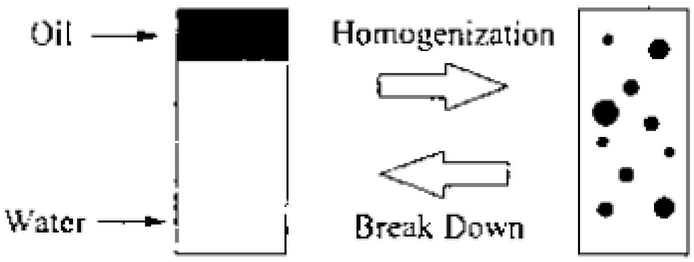

Figure 1.3.1: Phase separation. Over time, a thermodynamically unstable system like an emulsion will revert back to the individual oil and water phases. (McClements, 2002)

Other mechanisms of emulsion destabilisation are sedimentation and creaming, flocculation/aggregation, coalescence and Ostwald ripening, as shown in Figure 1.3.2 (Norde, 2003). *Creaming* and *sedimentation* occurs when the dispersed droplets have a higher or lower density than the surrounding medium. If the density is lower than the continuous phase, the droplets will move upwards and “cream”, while droplets that are heavier will sink to the bottom, causing sedimentation. Most liquid oils have a lower density than water, and thus O/W emulsions will tend to cream, and W/O emulsions will sediment. *Flocculation* and *coalescence* occurs because of the constant fluctuation of the dispersed droplets. This movement is caused either by thermal energy, gravitational forces or mechanical influence. If two droplets collide, they will either move apart or become aggregated, and the relative magnitude of the attractive or repulsive forces between the them influences whether this happens or not (McClements, 2002). The interaction potential, *V(s),* between two droplets with separation *s*, is given as a sum of various repulsive and attractive interactions, as shown in equation 1.1;

$$V(s) = V_{VDW}(s) + V_E(s) + V_{SR}(s) \qquad (1.1)$$

$V_{VDW}(s)$, $V_E(s)$ and $V_{SR}(s)$ is van der Waals forces, electrostatic and short range interaction potentials, respectively. Van der Waals forces are normally attractive and act over long droplet separations, while electrostatic interactions between two similarly charged droplets are repulsive and may be of short or long range, depending on the ionic strength of the emulsion. Short range interactions, like steric, thermal fluctuation and hydration forces, are important when the droplets of an emulsion come very close together, and are very repulsive and hinders coalescence (Demetriades, Coupland, & McClements, 1997). Flocculation is commonly observed in emulsions; here two or more droplets form an aggregate, while retaining their individual integrity (McClements, 2002). Flocculation usually occurs when the van der Waals forces dominates in the solution. However, if the electrostatic interactions dominate, there will usually be no aggregation (Demetriades et al., 1997). Another form of coalescence is *ostwald ripening,* which is defined as large droplets growing at the expense of smaller droplets. This phenomenon is due to the fact that the solubility of the dispersed phase increases as the size of the droplet decreases. (McClements, 2002)

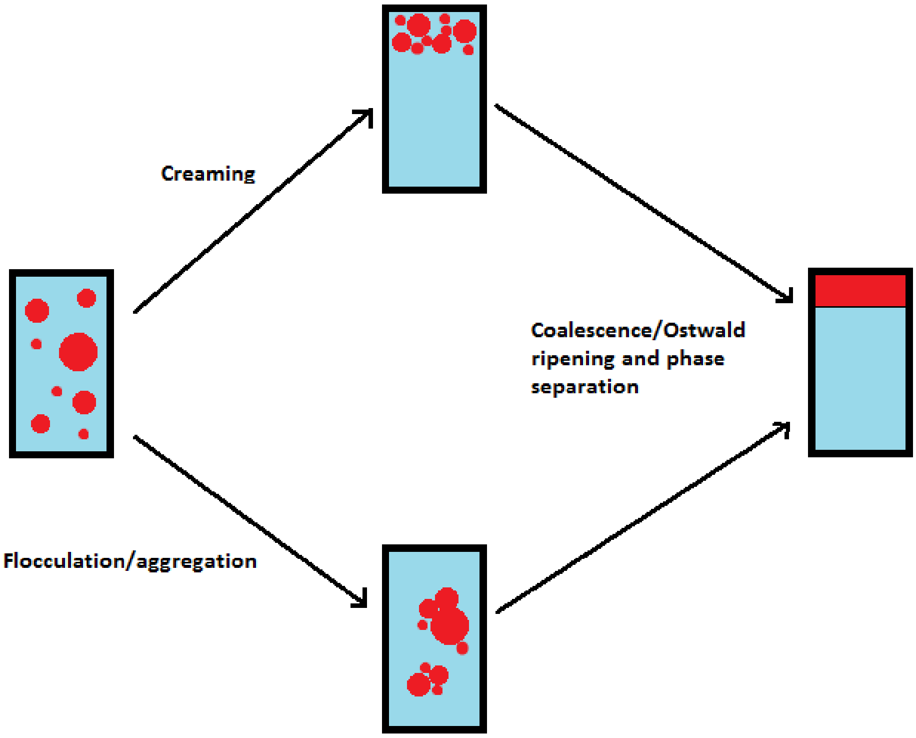

Figure 1.3.2: Demulsification processes in emulsions. In creaming (or sedimentation, not shown), the dispersed droplets migrate in the gravitational field and increase in concentration in either top or bottom of the vessel. In flocculation or aggregation, the dispersed droplets form loose clusters without the droplets loosing their integrity. Both mechanisms may further lead to coalescence and/or Ostwald ripening and phase separation (Butt et al., 2006).

1.3.3 Stabilization

Emulsions are generally thermodynamically unstable, and will thereby eventually phase separate into two liquids. In order to achieve a sufficient shelf-life, the droplets must be kinetically stabilised, which can be achieved by addition of a stabilising agent. The stabilising agent will adsorb strongly to the liquid-liquid interfaces and stabilise it through the different droplet interactions mentioned earlier (Butt et al., 2006; Demetriades et al., 1997). It is normal to distinguish between two different types of ingredients in formulation of emulsion systems; the emulsifier and the stabiliser (Dickinson, 1992). The emulsifier promotes emulsion formation and short-term stabilisation through action on the liquid-liquid interfaces. The emulsifying agents used in food processing can be divided into two broad classes; the small-molecule surfactants (monoglycerides, polysorbates, lecithin etc); and the macromolecular emulsifiers, like proteins from egg and milk (Dickinson, 2003).

Surfactants are amphiphilic molecules, consisting of a hydrophilic head group and a lipophilic tail group, and can be represented by the general formula RX. Here X is the hydrophilic head and R is the lipophilic tail. The head group may be anionic, cationic, zwitterionic or nonionic, while the tail consists of one or more hydrocarbon chains, with 10 – 20 carbon atoms in each chain. These chains can be saturated or unsaturated, linear or branched, and aliphatic and/or aromatic (McClements, 2002). Surfactants can be characterized by a HLB number, which represents the hydrophile-lipophile balance of the surfactant. The HLB number is defined so that HLB = 7 represents a compound that have equal solubility in the aqueous and lipid phase. Thus, if an w/o emulsion is desired a surfactant with HLB < 7 is used, and for an o/w emulsion a surfactant with HLB > 7 will be used (Norde, 2003).

A *stabiliser* is defined as a chemical component that provides long-term emulsion stability. Stabilisers are normally biopolymers, like polysaccharides or proteins (Dickinson, 2003). Biopolymers acts as stabilisers by adsorption at the interphase and providing electrostatic and/or steric stabilisation, and they have a tendency to form thick, viscoelastic membranes highly resistant to rupture (McClements, 2004a). Polysaccharides usually stabilise through viscosity modification, or gelation of the aqueous continuous phase. Proteins form stabilising layers around oil droplets by adsorbing to the oil/water interface, and thus accomplish both the emulsifying and stabilising roles. A biopolymer working effectively as an emulsifier must be surface-active, giving it a capacity to lower the interfacial tension at the oil-water interface. To be surface-active, a biopolymer must have an amphiphilic character, and an ideal emulsifier should then typically be composed of species with relatively low molecular mass, with both aqueous and lipid solubility. The pH and ionic strength of aqueous solutions affects the properties of emulsions stabilised by charged biopolymers. If the pH values are close to the IEP of proteins, or at high ionic strengths, the electrostatic repulsions between the droplets may not be sufficient to prevent droplet aggregation (McClements, 2002).

1.4 Filled gel emulsions

Many food, cosmetic and pharmaceutical products are regarded as macromolecular gels that contain dispersed particles (droplets), or fillers. These particles can influence the rheological properties of a gel quite dramatically, depending on the concentration and mechanical properties of the particles, and on the extent of interaction between the filler and the gel matrix (van Vliet, 1988). Filler particles may contribute to the network matrix in different ways, and are usually classified as either “active” or “inactive”. Active fillers will interact strongly with the gel matrix, providing a mechanical reinforcement of the gel network, and thus increase the gel strength. To the contrary, inactive fillers have a low chemical affinity for the matrix polymer, and will not strengthen the gel. The surface properties of the filler particles, especially the nature of the interfacial layer, determine whether a filler is active or not (Dickinson & Chen, 1999). Gelatin is frequently used in filled gels, and the level of reinforcement from active fillers depends on the volume fraction of the filler and on the shape of the filler particles (Stainsby, 1987).

1.5 Rheological characterization

Rheology is the study of how a material flows and responds to deformation by an applied force. In this scientific discipline, the flow and the elastic properties of a matter is characterized (Norde, 2003; Smidsrød & Moe, 2008). There are three different ways that a solid or liquid can respond to an applied deformation. It can exhibit *elastic* or *viscous* properties, and if both these properties are displayed at the same time, the material is referred to as being *viscoelastic* (Smidsrød & Moe, 2008).

First, a few definitions are required: Stress (σ) is defined as the force (F) divided by the area (A) to which it is applied (equation 1.2).

$$\sigma = \frac{F}{A} \tag{1.2}$$

When performing a deformation, a material is exposed to stress. Strain (γ) is defined as the relative deformation, which is deformation (ΔL) per unit length (L_0) (equation 1.3) (Goodwin & Hughes, 2008).

$$\gamma = \frac{\Delta L}{L_0} \tag{1.3}$$

1.5.1 Viscosity

Viscosity is a measure for a fluid's resistance to deformation (Holmberg, Jönsson, Kronberg, & Lindman, 2003) and can be defined as the ratio of stress to shear rate. Viscosity is linked to energy loss, because the energy needed to deform the material, is lost as heat. Liquids can be roughly divided into two different types; Newtonian or non-Newtonian. An ideal fluid has Newtonian properties, meaning it has a linear correlation between applied stress and the rate of deformation, as shown in Figure 1.5.1.

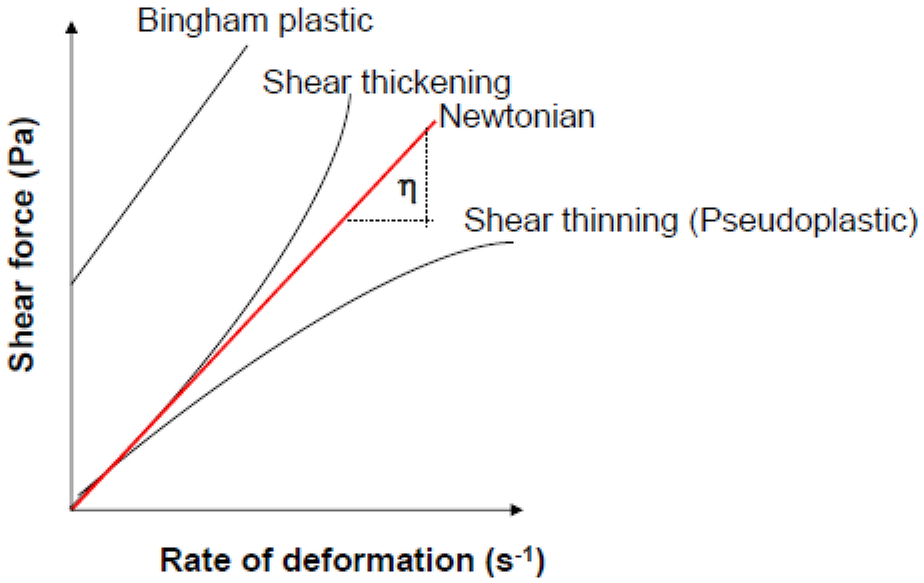

Figure 1.5.1: Newtonian and different variations of non-Newtonian viscosities. The curves show the relationship between shear force and shear rate for Newtonian and non-Newtonian fluids. The red line depicts the linear relationship between stress and shear rate of an ideal fluid, while the curved lines show how non-ideal fluids can react when shear and stress is applied (Smidsrød & Moe, 2008).

The proportionality constant obtained from the force/rate ofdeformation plot is referred to as viscosity of the fluid, with the abbreviation η. There are many examples of fluids showing Newtonian behavior; among them are syrups, water and many synthetic and vegetable oils. Non-Newtonian fluids do not have a linear correlation between applied stress and rate of deformation, and thus they have no constant viscosity, but a viscosity dependent on either the rate of deformation or the time of observation. Table 1.5.1 shows different types of non-Newtonian behavior (Smidsrød & Moe, 2008).

Table 1.5.1: Different types of non-Newtonian behavior (Smidsrød & Moe, 2008).

Behavior with increasing :	**Rate of shear**	**Time**
Thinner	Shear thinning (pseudoplastic)	Thixotropic
Thicker	Shear thickening (dilating)	Rheopectic

If the viscosity is dependent on shear rate, the fluid is said to show either shear thinning or shear thickening behavior, where the viscosity of the fluid is reduced or increased with increasing rate of deformation, respectively. It is also worth mentioning Bingham plastics, where the shear force needs to reach a certain threshold before the material starts to flow. The different flow behaviors are shown in Figure 1.5.1 (Smidsrød & Moe, 2008).

Materials showing time dependent viscosity are referred to as being either thixotropic or rheopectic. A thixotropic material exposed to a constant shear rate will exhibit decreased viscosity over time. If the shear is removed, the viscosity of the material will increase again with time. Rheoplastic materials show the opposite behavior of thixotropic materials; because the viscosity of a rheopectic material will increase with time (Doran, 1995).

Viscosity can be measured using a viscometer, which yields a shear rate dependent viscosity, η, or with a rheometer which gives a frequency dependent (dynamic) viscosity, η*. Cox and Merz depicted an empirical correlation between steady flow and dynamic viscosity measurements (Cox & Merz, 1958), given in equation 1.4 (Kulicke & Porter, 1980):

$$\eta \cdot \dot{\gamma} = \eta^* \cdot \omega \tag{1.4}$$

γ is the rate of deformation and ω is the angular frequency. The Cox-Merz rule states that there is a relationship between linear and non-linear viscoelastic properties, which makes it possible to obtain shear viscosity information when only dynamic viscosity (linear) data are available and vice versa (Kulicke & Porter, 1980).

1.5.2 Elasticity

For an ideal elastic material there is a linear correlation between stress and deformation of the sample. This is valid at the initial stage of deformation. Deformation can further be applied to an elastic solid as simple shear, compression or bulk, and the proportionality constant obtained from the initial part of these stress/strain measurements is referred to as the shear, Young's and bulk modulus, respectively. The different formulas are given in Table 1.5.2.

Table 1.5.2: Rheological parameters for different types of deformations of elastic solids (McClements, 2004b)

Deformation	Stress	Strain	Elastic modulus
Simple shear	$\sigma = \frac{F}{A}$	$\gamma = \frac{\Delta l}{l} = \tan\phi$	$G = \frac{\sigma}{\gamma} = \frac{F}{A\tan\phi}$
Simple compression	$\sigma = \frac{F}{A}$	$\gamma = \frac{\Delta l}{l}$	$Y = \frac{\sigma}{\gamma} = \frac{Fl}{A\Delta l}$
Bulk compression	$\sigma = \frac{F}{A} = P$	$\gamma = \frac{\Delta V}{V}$	$K = \frac{\sigma}{\gamma} = \frac{PV}{\Delta V}$

Note: G = shear modulus, Y = Young's modulus, K = bulk modulus, P = pressure, F = force, A = area, V = volume, l = height

For an ideal elastic material, the deformation energy is stored in the body, which will regain its original shape when the force of deformation is removed, while in an ideal rubber elastic polymer network, all energy will be stored as reduced *entropy* in the network, and the expression for G is given in equation 1.7;

$$G = nkT = \frac{cRT}{M_c} \tag{1.7}$$

where M_c is the average molecular weight of an elastic chain. In a real network, with molecules of not infinite lengths, there will be some non-elastic loose ends, and G will then be given by equation 1.8;

$$G = \frac{cRT}{M_c}\left(1 - \frac{2M_c}{M_w}\right) \tag{1.8}$$

where M_w is the weight-average molecular mass. The equation for an ideal rubber elastic body predicts that the modulus (G) will increase with temperature. This does not apply for most biopolymer gels. Biopolymer networks have stiff chains and physical cross-links, and the stress applied is used for bending of bond-angles and –lengths, in addition to distortion of junction zones in the network. This type of energy storage is *enthalpic*, and results in a decrease in modulus with increasing temperature.

It is possible to relate Young's modulus, E to the elastic modulus, G through the Poisson ratio, ν, which is defined as the ratio between contractile and tensile strain. The relationship between E and G can thus be given through equation 1.10:

$$E = 2G\left(1+\upsilon\right) \tag{1.10}$$

For an incompressible material, ν = 0,5, and thus E = 3G. The Poisson value for rubber is close to 0,5, but most solids have values lower than this (0,25 < ν < 0,33). Poissons ratio assumes isotropy and is not applicable to viscoelastic materials (Goodwin & Hughes, 2008).

1.5.3 Rheological properties of emulsions

The rheology of emulsion systems is of general interest in both the food and pharmaceutical industry (Malmsten, 2002; McClements, 2004b). The rheological properties of a food emulsion are directly related to its sensory attributes, and thus it is important for food manufacturers to consistently produce emulsions with the desired rheological properties (McClements, 2004b). Qualitatively speaking, emulsions can range from milk-like Newtonian liquids with low viscosity, through shear-thinning liquids with higher viscosity, to thick, cream-like materials (Barnes, 1994). The type and concentration of the ingredients in an emulsion influences its rheological behavior, as well as processing and storage conditions (McClements, 2004b). Following is a list of the major factors that influence emulsion rheology.

Disperse phase volume fraction

The viscosity of an emulsion is highly dependent on the volume fraction of the dispersed phase. The viscosity increases as the presence of the dispersed droplets increase the energy dissipation that is associated with fluid flow. When the droplet concentration is low, the viscosity will increase linearly with volume fraction, but the viscosity increase will be steeper at higher droplet concentrations. The viscosity's dependence on volume fraction is mainly determined by the type of colloidal interaction between the dispersed droplets. If, for example, there is a relatively strong repulsion or attraction between emulsion droplets, their *effective* volume fraction may be much larger than their *actual* volume fraction (McClements, 2004b).

Rheology of component phases

At lower droplet concentrations, where the dispersed oil droplets are sufficiently separated from each other, the overall emulsion rheology will mainly be determined by the behavior of the aqueous continuous phase. If the continuous phase of an emulsion has low-viscosity and the volume fraction of the dispersed phase is not too high, the emulsion will display a liquid-like flow behavior. However, if the continuous phase has viscoelastic properties like a biopolymer solution or gel, the emulsion itself will be viscoelastic (Dickinson, 2012).

Droplet size

Droplet size has a large influence on the rheology of an emulsion. By comparing fine emulsions versus coarse emulsions with the same weight fraction of dispersed phase, the fine emulsions will usually have higher viscosities and storage moduli than coarse emulsions. In fine emulsions, the shear thinning effect will also be stronger (Pal, 1996). The mean droplet size and polydispersity will have a significant impact on the rheology of a highly concentrated emulsion. Polydisperse droplets will be able to pack together more efficiently, and thus be able to lower the viscosity in a concentrated polydisperse emulsion, compared to an emulsion with monodisperse droplets (McClements, 2004b).

Colloidal interactions

One of the most important factors determining the rheological behavior of an emulsion is the nature of the colloidal interactions between the dispersed droplets. When long range repulsive interactions exist between emulsion droplets, the dispersed phase may exhibit a significantly larger effective volume fraction. In addition, if the droplet interactions are sufficiently attractive, droplet flocculation may occur. This may lead to an increased effective volume fraction of the dispersed phase, due to water binding between the flocculates which gives a higher volume, or the non-spherical shape of the flocculates. Both cases will cause an increase in the emulsion viscosity. Therefore, the rheological properties of an emulsion will depend on the range and relative magnitude of the attractive forces (van der Waals, hydrophobic, depletion) and the repulsive forces (electrostatic, steric, thermal fluctuation) (McClements, 2004b). The rheological properties of an emulsion can effectively be controlled by manipulating the colloidal interactions between droplets. For example; the viscosity of an oil-in-water emulsion can be increased by promoting droplet flocculation (Dickinson, 1998). One approach to increase droplet flocculation in a protein-stabilised emulsion can be to alter the pH or ionic strength of the emulsion. If the pH in the emulsion is close to the isoelectric point of the protein, the charge on the dispersed droplets is low and hence the electrostatic repulsion is weak. If the ionic strength of the solution is increased, the electrostatic repulsion will be lowered because electrolytes in the aqueous phase will reduce the electrostatic double layer (Demetriades et al., 1997).

Droplet charge

The adsorption of charged surface active molecules at the oil-water interphase causes many droplets to have an electrical charge. This can influence the rheology of the emulsion in different ways. 1) There is the *primary electroviscous effect*: A charged droplet moving through a fluid will distort the cloud of counterions floating around it. Due to the attraction between the charged droplet and the lagging cloud of counterions, the movement of the droplet will be opposed, causing an increase in solution viscosity. 2) There is the *secondary electroviscous effect*, where electrostatic repulsion hinders two charged droplets to approach each other closely. This increases the effective diameter of the droplets, which again will increase the emulsion viscosity. When the Debye screening length is of the same order of magnitude as the droplet radius ($\kappa^{-1} \sim r$), the secondary electroviscous effect can have a large impact on the rheological properties of an emulsion. 3) The *tertiary electroviscous effect* can have a large effect on emulsions stabilised by thick of charged biopolymers like some proteins and polysaccharides. The thickness of this adsorbed layer may change with the ionic environment (pH, ionic strength). If the ionic strength or pH is changed, the electrostatic interactions between the adsorbed biopolymer chains will change. This can lead to either a decrease or increase in the thickness of the layer. As mentioned in the last section, if the pH or ionic strength of an electrostatically stabilised system is changed, so the electrostatic repulsion between droplets is not sufficiently high enough to counteract the attractive interactions, the droplets will flocculate and the viscosity increase (McClements, 2004b).

2 Materials and Methods

2.1 Materials

2.1.1 Gelatin

Gelatin gels and filled gel emulsions were prepared by using type A and B gelatins, with Bloom values 160 g and 260 g. All the gelatins were provided by GELITA® (Germany). Information about the different gelatins is listed in Table 2.1.1.

Table 2.1.1: Information on the different types of gelatin used.

Type	Source	Bloom (g)	Batch number
A	Pig skin	160	629586
B	Limed bovine bone	160	630473
A	Pig skin	260	628305
B	Limed bovine bone	260	630407

2.1.2 Milli-Q water

Milli-Q (MQ) water (de-ionized water) was used in all experiments, and had an electrical conductivity of 18,2 mΩ (Millipore, Norway)

2.1.3 Other materials

All other materials are listed in Table 2.1.2.

Table 2.1.2: List of all materials used for different purposes throughout the study.

Material	Manufacturer	Country	Batch number
Corn oil	Sigma-Aldrich	Switzerland	MKBH4894V
Polysorbate 80	Sigma-Aldrich	Switzerland	BCBG3820V
Sodium chloride	Merck KGaA	Germany	K42150704 116
Acetic acid	VWR International	Norway	K36296763 628
Trizma base®	Sigma-Aldrich	USA	108K54151
Acetaminophen	Sigma-Aldrich	USA	078K0032
Hydrochloric acid	Merck KGaA	Germany	K42069317

2.2 Introduction to methods

2.2.1 Size exclusion chromatography – Multi-angle laser light scattering (SEC-MALLS)

Theory

Size exclusion chromatography separates molecules based on size. This is achieved by flushing a sample of molecules through a column containing porous beads, where the molecules will move into the beads by passive diffusion. The smaller molecules will to a larger extent diffuse into the pores than the larger molecules. The molecules become delayed (retained) inside the beads, and will not move along the column until they diffuse out of the beads. The retention depends on molecule size, and thus the smaller molecules will be more delayed than the larger ones. A light scattering detector that monitors up to 18 different angles simultaneously measures the amount of light scattered. Another detector that is concentration sensitive measures the refraction index or absorbance. The amount of light scattered is directly proportional to the molecular mass multiplied with the concentration, and thus the molecular weight distribution of the gelatins can be determined. (Christensen, 2010)

Molecular weight distribution in biopolymers

By performing SEC-MALLS measurements, the molecular weight distribution may be determined. The molecular weight distribution of a biopolymer is very important for its physiological properties. There are different methods to determine the molecular weight of a biopolymer, and the most common ways to present the molecular weight is by using the number average (M_n) or the weight average (M_w), as shown in equation 2.1 and 2.2.

$$M_n = \frac{\sum_{i=1}^{n} n_i M_i}{\sum_{i=1}^{n} n_i} = \frac{\sum_{i=1}^{n} W_i}{\sum_{i=1}^{n} \frac{W_i}{M_i}} \tag{2.1}$$

$$M_w = \frac{\sum_{i=1}^{n} n_i M_i^2}{\sum_{i=1}^{n} n_i M_i} = \frac{\sum_{i=1}^{n} n_i M_i}{\sum_{i=1}^{n} n_i} \tag{2.2}$$

A biopolymer sample where all the molecules exhibit the same weight is called *monodisperse*, and $M_n = M_w$, while a *polydisperse* sample will have molecules with different weights, and $M_n < M_w$. The polydispersity index (PI) is the ratio between M_n and M_w and is given in equation 2.3, and is used as a crude measure of the polydispersity in a sample (Smidsrød & Moe, 2008).

$$PI = \frac{M_w}{M_n} \tag{2.3}$$

2.2.2 Small strain oscillatory measurements

The rheological properties of viscoelastic materials can be determined using small strain oscillatory measurements, which will yield information on storage modulus (G'), loss modulus (G''), and gelling and melting temperatures (T_g and T_m respectively) (Ross-Murphy, 1984). The material is subjected to a harmonic oscillating deformation with angular frequency ω, while the force is measured. As the material undergoes deformation (strain, γ) it responds with stress (σ), as shown in equation 2.4 and 2.5:

$$\gamma = \gamma_0 \sin(\omega t) \tag{2.4}$$

$$\sigma = \sigma_0 \sin(\omega t + \delta) \tag{2.5}$$

The ratio between the elastic and the viscous response in a material is called the phase angle (δ). The phase angle is defined as tan δ = G''/G' and corresponds to the displacement between strain and stress, where γ_0 is the amplitude of the strain and σ_0 is the amplitude of the stress. This is demonstrated in Figure 2.2.1.

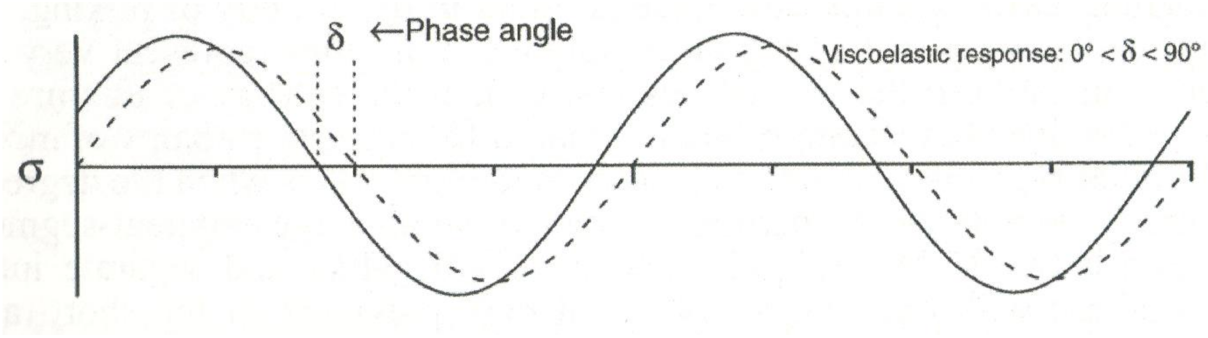

Figure 2.2.1: Small strain oscillatory measurement of a viscoelastic material with G' = 0,9 and η' = 0,4. The strain (γ) applied to the material is presented by the solid line and the response stress (σ) by the stapled line. The phase angle (δ) is defined as the displacement between the two lines (Smidsrød & Moe, 2008).

A perfectly elastic material has δ = 0° and an ideal Newtonian fluid has δ = 90°. All other fluids have δ between 0° and 90°. The viscoelastic properties of a material is described by the storage (G') and the loss (G'') moduli, as shown in equation 2.6 and 2.7 (Smidsrød & Moe, 2008):

$$G' = \frac{\sigma_0}{\gamma_0}\cos\delta \tag{2.6}$$

$$G'' = \frac{\sigma_0}{\gamma_0}\sin\delta \tag{2.7}$$

The relationship between G', G'', δ and its corresponding viscoelastic behavior is shown in Table 2.2.1.

Table 2.2.1: The relationship between G', G'', δ and viscoelastic behavior (Smidsrød & Moe, 2008)

G', G''	δ	Behavior
G' < G''	> 45°	Viscoelastic liquid
G' = G''	= 45°	Transition state
G' > G''	< 45°	Viscoelastic solid

2.2.3 Droplet size determination (Mastersizer 3000)

A particle size analysis instrument (Mastersizer 3000, Malvern, UK) measures particle size distribution by detecting laser light scattered off the particles. The angle of the scattered laser light is inversely proportional to particle size (Ryżak & Bieganowski, 2011). The instrument consists of a main optical unit that transmits red (wavelength = 633 nm) and blue (wavelength = 466 nm) laser light, one or more dispersion units (wet or dry) and a measurement cell (Malvern, 2011). The dispersion unit is equipped with a stirrer, to prevent sedimentation of the sample, and to facilitate the sample flow through the measurement cell (Ryżak & Bieganowski, 2011). The measurement cell collects the light scattering pattern obtained from the particles, and this is interpreted by the Mastersizer software. The instrument software provides information on volume-weighted particle size distribution (Malvern, 2011).

2.3 Methods

2.3.1 SEC-MALLS

To determine the average molecular weight and the molecular weight distribution of the four gelatins (type A and B, 160 and 260 g Bloom) used to prepare filled gel emulsions, size exclusion chromatography multi-angle laser light scattering (SEC-MALLS) analysis was performed.

Procedure

Gelatin type A and type B (160 and 260 g Bloom) was dissolved in MQ-water to a concentration of 2 mg/mL and then mixed with the elution buffer (0,1 M Na_2SO_4, 0,02 M EDTA, 0,5 M Trizma base®, with pH 9) at a ratio 1:1. The mixture was filtered through a 1 µm low protein binding filter, and then stored at 4 °C until analysis. The SEC-MALLS was performed a packed column (TSKgel G-6000 + PWXL, Tosoh Bioscience, Japan) that was connected to a Dawn DSP multi-angle laser light scattering photometer (Wyatt Technology Corp., CA, USA), followed by an Optilab DSP refractometer, P-10 cell (Wyatt Technology Corp.). The data was analyzed using the respective software (Astra, v6.1.1.17). All the measurements were performed at 40 °C. The weight average molecular weight (M_w) and number average molecular weight (M_n) were determined from the molecular weight distribution of the gelatins.

2.3.2 Preparation of filled gel emulsions

Gelatin gels and filled gel emulsions with different amounts of corn oil (0 %, 10 %, 20 %, 40 % and 50 %) and four types of gelatin; type A, Bloom 160 g and 260 g; and type B, Bloom 160 g and 260 g; were prepared and analysed.

Preparation of gelatin gels

Gelatin matrices without oil were prepared by dissolving gelatin (25 wt.%) in MQ-water at 55 °C by stirring (150 rpm). The solutions were stored at 4 °C until use.

Preparation of filled gel emulsions with various amounts of oil

Filled gel emulsions were prepared by dissolving gelatin (25 wt.%) in MQ-water at 55 °C by stirring (150 rpm). An emulsion was prepared by homogenizing the heated gelatin solution with varying amounts of corn oil (10, 20, 40 and 50 wt.%) using a VDI 12 homogenizer (VWR International GmbH, Germany), which was equipped with a S12N-12S dispersing element.

The dispersion speed was 28000 rpm, and each sample was homogenized for approximately 3 minutes. Table 2.3.1 shows the amount of ingredients used to prepare gelatin matrices and filled gel emulsions.

Table 2.3.1: The amount of ingredients used to prepare gelatin matrices and filled gel emulsions with 0, 10, 20, 40 and 50 wt.% corn oil.

Oil wt.%	MQ-water wt.%	Gelatin wt.%
0	75	25
10	67,5	22,5
20	60	20
40	45	15
50	37,5	12,5

Preparation of filled gel emulsions with different properties

In order to study the effect of ionic strength and pH on the filled gel emulsions, a 0,1 M NaCl solution, an 0,2 M acetic acid buffer (adjusted to pH 4 by the addition of 1,0 M NaOH) and a 0,2 M Trizma base® buffer (adjusted to pH 8 by the addition of 1,0 M HCl) were prepared. The filled gel emulsions were prepared as described earlier, by dissolving the gelatin in the respective solution and homogenizing the sample with corn oil (40 wt.%), using a VDI 12 homogenizer (VWR International GmbH, Germany).

In order to study the effect of added surfactant, the gelatin emulsions (40 wt.% corn oil) with MQ-water as the continuous phase were prepared as described earlier. After homogenization, polysorbate 80 (0,5 wt.%) was added to the heated emulsion and mixed using a magnetic stirrer (50 rpm) for about 30 minutes prior to the rheological measurements.

Preparation of filled gel emulsions with acetaminophen for dissolution studies

Acetaminophen (paracetamol) was used as a marker to determine the dissolution profile of the gelatin emulsions. The emulsions were prepared with MQ-water as the continuous phase and 40 wt.% corn oil. Acetaminophen was added to the liquid emulsions by stirring with a spatula at 65 °C. The acetaminophen was added in such a quantity, that 1,5 g of the final gel would have a concentration of 0,1 mM acetaminophen when dissolved in 900 mL hydrochloric acid (0,1 M). See appendix A.1 for calculations.

Approximately 5 g of the liquid emulsion was poured into small Petri dishes with a diameter of 3,5 cm and sealed with Parafilm. The solutions were gelled and stored at room temperature (20 °C) for 24 hours prior to use.

2.3.3 Droplet size determination (Mastersizer 3000)

The droplet sizes of the filled gel emulsions containing 160 g and 260 g Bloom gelatin type A and B, with 10, 20, 40 and 50 % corn oil was measured by using a Mastersizer 3000 (Malvern Instruments, Worcestershire, UK).

Procedure

Samples for droplet size analysis were prepared by heating up the emulsion and dilute it to 1:100 in MQ-water while stirring. The solution was added to the dispersion unit (Hydro MV) until an obscuration of approximately 9 % was obtained. Obscuration means to which degree the laser light is obscured by the particles that are to be measured. (Ryżak & Bieganowski, 2011) Five replicates were measured in series for each of the samples, and the instrument software provided reports based on the average. The refractive index of corn oil is 1,47, and the absorption index was set to 0,01. MQ-water has a refractive index of 1,33.

2.3.4 Viscosity measurements

The shear viscosity of gelatin emulsions prepared from type A and type B (160 g and 260 g) gelatin was measured using a rheometer. The instrument was set up with a cone/plate geometry (cone = 4°, diameter = 40 mm). Zero gap was performed at 60 °C prior to each measurement. Approximately 1,3 mL of the gelatin emulsion was applied to the plate and excess sample was removed. The sample was covered with silicone oil (Dow Corning®, 200/10 cS fluid, UK) to prevent evaporation during the measurements. Before measurements, a pre-shear (2 1/s, 300 s) was applied to obtain temperature equilibrium in the sample. Measurements were performed at 0.6, 0.8, 1, 2, 4, 7, 10, 20, 40, 60, 80 and 100 1/s, at 60 °C.

2.3.5 Small strain oscillatory measurements

Small strain oscillatory measurements were performed on filled gel emulsions prepared from type A and type B (160 g and 260 g) gelatin by using a rheometer. Measurements were performed using a cone/plate geometry (cone = 4°, diameter = 40 mm). Zero gap was performed at 60 °C prior to each measurement. Liquid, heated gelatin emulsion (volume =

1,3 mL) was added to the plate and excess sample was removed. The sample was covered with silicone oil (Dow Corning®, 200/10 cS fluid, UK) to prevent evaporation during the measurements. The start and end temperatures were 60 °C for all the samples. The cooling and heating rate was 2 °C per minute, the curing temperature was 20 °C, and holding time was 15 minutes. The measurements were performed at a constant frequency of 1 Hz, with a 5,0 x 10^{-3} strain controlled mode.

2.3.6 Preparation of solid filled emulsion gels for longitudinal deformations

Filled emulsion gels were prepared as described in section 2.3.2. The gels were casted in steel wells (NTNU Workshop, Norway) as seen in Figure 2.3.1. The wells had a cylindrical shape, an inner diameter of approximately 16 mm and an approximate height of 18 mm.

Figure 2.3.1: Steel wells used for the casting of filled emulsion gels.

Prior to casting, the wells and silicone layer were lubricated with 1 wt.% polysorbate 80 (Sigma-Aldrich, Germany) dissolved in EtOH (96 %), to ensure easy removal of the gels after casting. The wells were dried in an incubator at 70 °C, until all the ethanol was evaporated before the gels were casted.

The liquid gelatin emulsions were added to the wells until a positive meniscus was achieved. A silicone plate was put on top of the wells, and a heavy stainless steel top plate was mounted, to exert pressure and ensure equal height for all the gels. The gels were stored at room temperature (20 °C) for 24 hours prior to measurement. After removal from the casting wells, height and diameter of the gels were measured, using a digital caliper. The size of each gel is presented in appendix F. The gels were then stored in marked zip lock bags until use.

A texture analyzer (TA.XT.-Plus Texture Analyzer from Stable Micro Systems, UK) was used to perform compression measurements, in order to determine Young's modulus and force and strain at break for the filled gel emulsions, prepared from 160 and 260 g Bloom gelatin type A and B with an oil content of 0, 10, 20, 40 and 50 wt.%. Gels with 50 % oil content were not prepared for either type of gelatin with 260 g Bloom value.

Procedure

Young's Modulus

The gels were placed in the center of the texture analyzer as seen in Figure 2.3.2. A compression of 2 mm was performed using a cylindrical probe (p/35, diameter = 35 mm) with a speed of 0,1 mm/s, using a 5 kg load cell. The data was analysed using the respective software (Exponent v5.1.2.0). Young's modulus (E) was obtained by determining the slope in the linear region of the deformation-stress plot and determined using equation 2.8 (Smidsrød & Moe, 2008),

$$E = \frac{F}{A} \cdot \frac{L_0}{\Delta L} \tag{2.8}$$

where F is the force of deformation, A is the area of the sample surface, L_0 is the height of the sample and ΔL is the deformation.

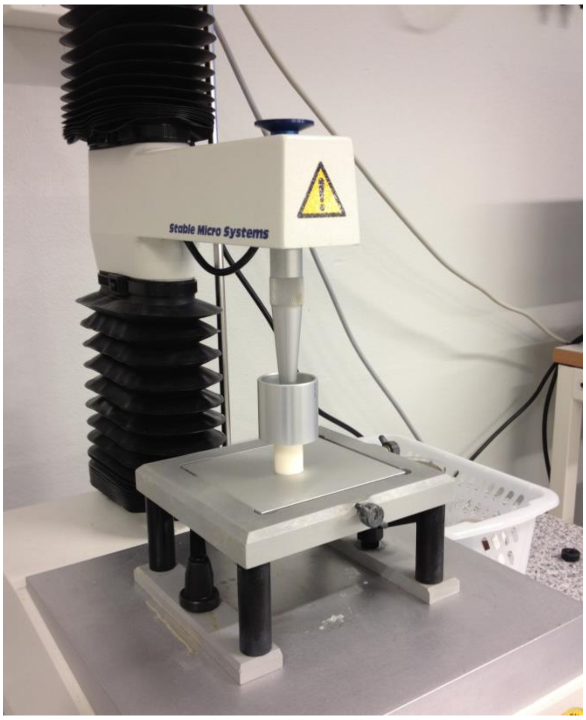

Figure 2.3.2: 2 mm compression measurement using a p/35 cylindrical probe at 0,1 mm/s with a 5 kg load cell (not in picture).

Force and strain at break

After Young's modulus was measured, the probe was exchanged with a smaller, stainless p/2 probe (diameter = 2 mm). The same gels were then compressed at 0,1 mm/s until break, using a 5 kg load cell. The Exponent software was used to determine force and strain at break. This correlated to the first break in the gel. By dividing the distance at break with the original height of the gel, strain at break was obtained.

2.3.7 Dissolution studies

Dissolution studies of 260 g Bloom gelatin type A and type B containing 40 wt.% corn oil were performed using a SOTAX dissolution unit (SOTAX USP 1,2,5,6 Manual, USA). Two independent studies were performed, both with three replicates for each type of gelatin, giving a total of six replicates. There was also performed a study with 260 g Bloom gelatin A, 40 % wt. corn oil and 0,5 % polysorbate 80, with a total of six replicates, as well as a control study with 260 g Bloom gelatin type A and B at 20 °C (three replicates per gelatin type). Filled emulsion gels containing paracetamol as a marker were prepared as described in section 2.3.2. These were cut into similar pieces of 1,50 g and placed into metal baskets that again were placed in the dissolution vessels of the SOTAX dissolution unit, as shown in Figure 2.3.3.

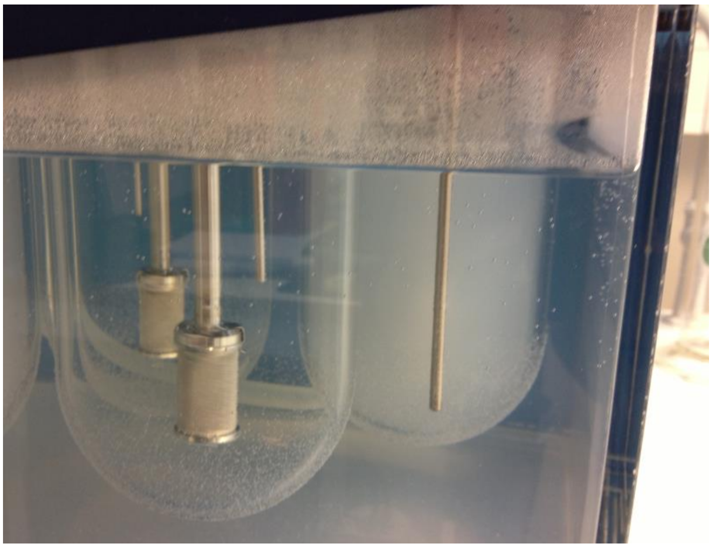

Figure 2.3.3: Dissolution beakers containing metal baskets with filled gel emulsions during the dissolution studies.

The dissolution beakers were filled with 900 mL artificial gastric acid (0,1 M HCl), and the temperature was kept at 37 °C during the studies. The baskets containing the sample rotated in the dissolution beakers with a speed of 75 rpm. Samples of approximately 4 mL were taken out at specific time points, (Table 2.3.2), by using a syringe.

After dissolution, the samples were filtrated through low protein binding filters, with a pore size of 0,2 µm. The samples were analyzed using a Lambda 25 UV/VIS spectrometer (Perkin Elmer Instruments, USA), at 243,3 nm, which is acetaminophen's known absorption maximum. 0,1 M HCl was used as blank samples. The results were compared to a standard curve with known concentrations of acetaminophen. The data was analysed using the respective software (UV WinLab) and Microsoft Excel.

Table 2.3.2: Sampling times for the different types of filled gel emulsions

Gel type	Sampling time (min)
260 g Bloom gelatin type A	2, 4, 7, 10, 14, 18, 24, 30, 60, 80, 140, 300, 1160
260 g Bloom gelatin type B	2, 4, 7, 10, 14, 18, 24, 30, 60, 80, 140, 300, 1160
260 g Bloom gelatin type A + polysorbate 80	2, 4, 7, 10, 14, 18, 24, 30, 60, 80, 100, 140
260 g Bloom gelatin type A, 20 °C	60, 120, 180, 240, 300
260 g Bloom gelatin type B, 20 °C	60, 120, 180, 240, 300

2.3.8 Morphological analysis of o/w-emulsions

The diluted gelatin A emulsions with 40 wt.% oil, with and without polysorbate 80, were examined with a conventional light microscope (Nikon Eclipse TS 100, Japan), equipped with a Nikon ELWD 0.3/OD75 camera (Japan). One droplet of dissolved and diluted emulsion (1:100) was applied to a microscope slide (VWR International, Germany) and covered with a cover glass (VWR International, Germany). The images were taken at a 400x magnification (40x objective combined with a 10x magnifier lens). The camera was connected to a computer and the software NIS Element F 3.0 was used to edit the images.

3 Results and Discussion

3.1 SEC-MALLS

Size exclusion chromatography – multi-angle laser light scattering (SEC-MALLS) analysis was performed to determine the molecular weight distribution of gelatin type A and B (160 g and 260 g Bloom) (Figure 3.1.1). From these measurements, the number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PI) were determined (Table 3.1.1).

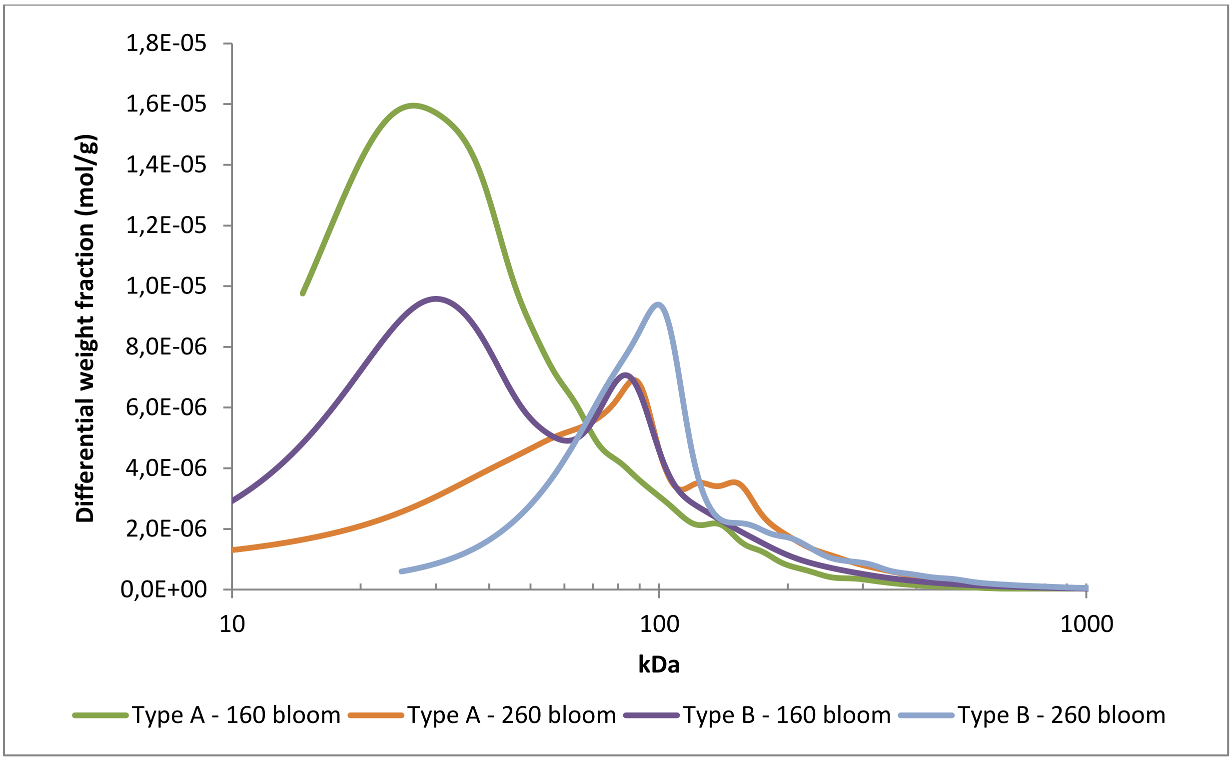

Figure 3.1.1: Measured molecular distribution of the different gelatins, determined by SEC-MALLS measurements

Table 3.1.1: Average molecular weight (M_n and M_w, kDa) determined for the different gelatin samples.

Gelatin	M_n	M_w	PI
Type A – 160 g Bloom	36,1	90,9	2,60
Type A – 260 g Bloom	88,7	191,3	2,16
Type B – 160 g Bloom	58,1	161,1	2,77
Type B – 260 g Bloom	118,8	217,2	1,86

Figure 3.1.1 shows that the different gelatin types investigated contained various amounts of high molecular (HM, >50 kDa) and low molecular (LM, <50 kDa) peptide chains. The values of M_n and M_w in Table 1.2.1 supports these results. A higher M_n and M_w was measured with increasing Bloom value for both types of gelatin, and this was caused by a higher fraction of HM peptide chains. Gelatin type B (160 g and 260 g Bloom) samples generally showed a clear shift towards a larger average molecular weight than the gelatin type A (160 g and 260 g Bloom) samples.

Very high molecular (>1000 kDa) peptide chains were found in all the gelatin types, except in 160 g Bloom gelatin type A. These heavy peptide chains gives a large weight average, and also gives a high value for the calculated PI (Table 3.1.1).

3.2 Droplet size determination in filled gel emulsions

The droplet size distributions of the filled gel emulsions (10, 20, 40 and 50 wt.%) prepared with either gelatin type A (160 g and 260 g Bloom) or type B (160 g and 260 g Bloom) are shown in Figure 3.2.1 – 3.2.4. The volume weighted average diameter and spans for all the gelatin types are displayed in Table 3.2.1 and Table 3.2.2. The data sets for all measurements can be found in appendix C.

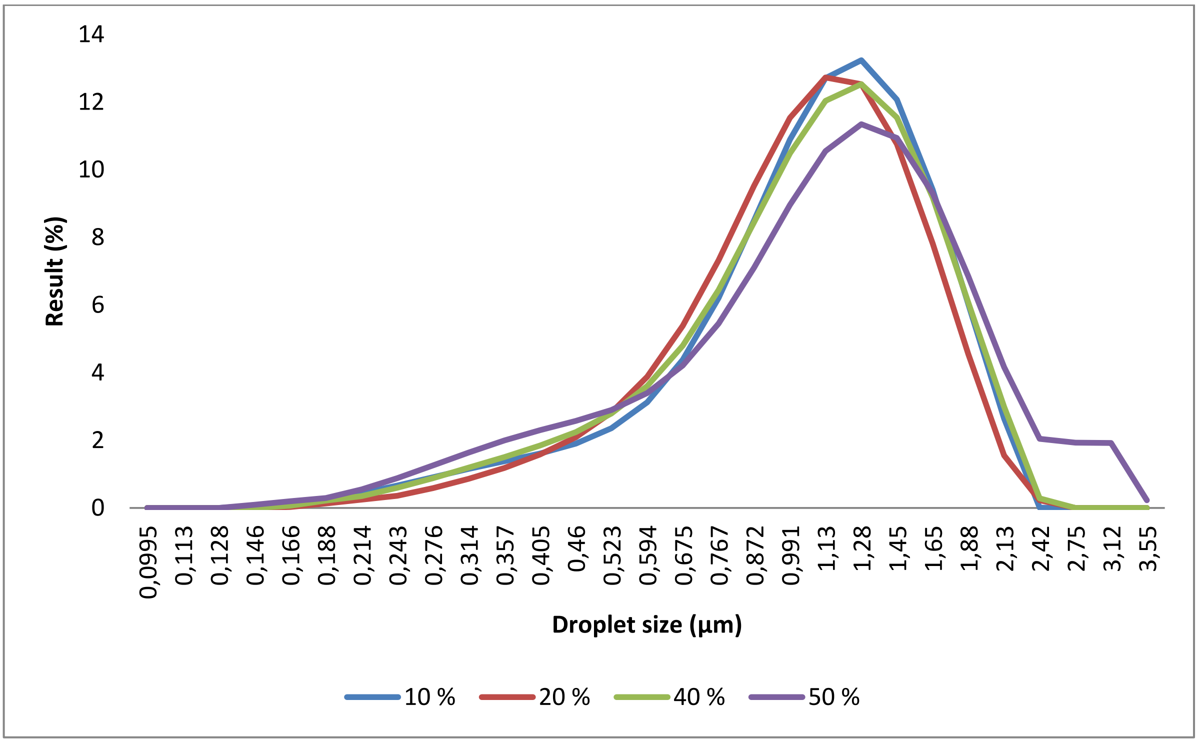

Figure 3.2.1: Droplet size distribution for dispersed oil droplets in filled gel emulsions with 160 g Bloom gelatin type A and varying amounts of corn oil (10, 20, 40 and 50 wt.%). N = 5 for each gel type.

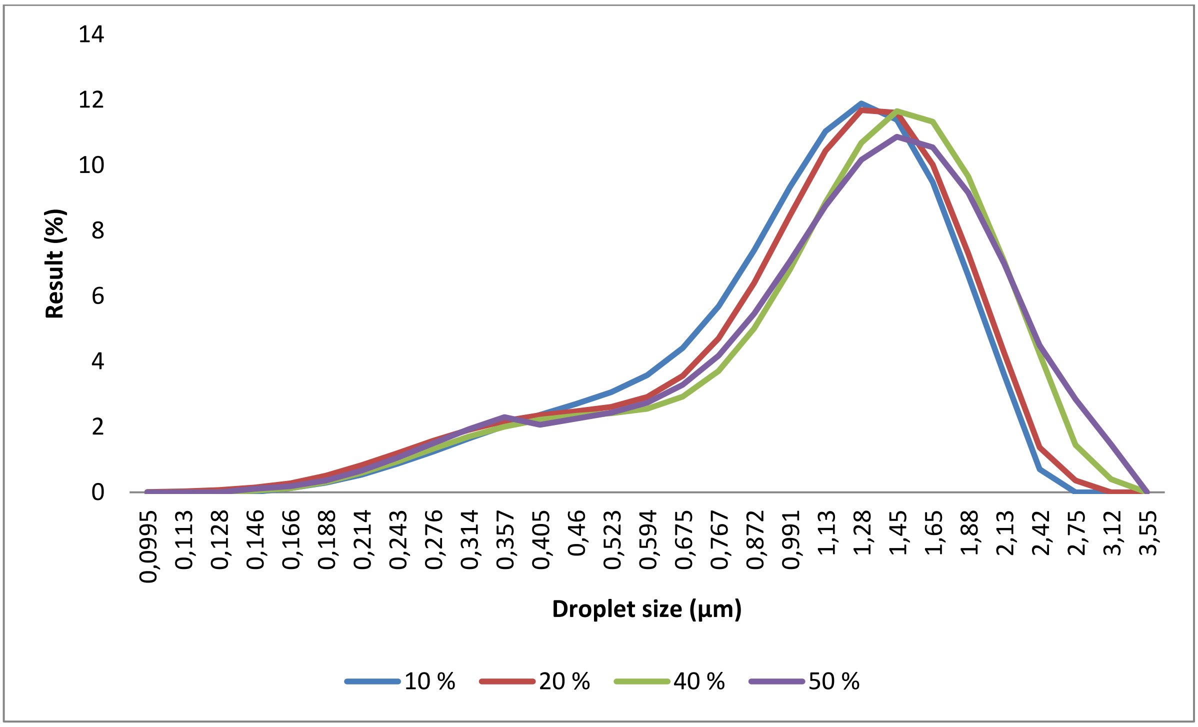

Figure 3.2.2: Droplet size distribution for dispersed oil droplets in filled gel emulsions with160 g Bloom gelatin type B and varying amounts of corn oil (10, 20, 40 and 50 wt.%). N = 5 for each gel type.

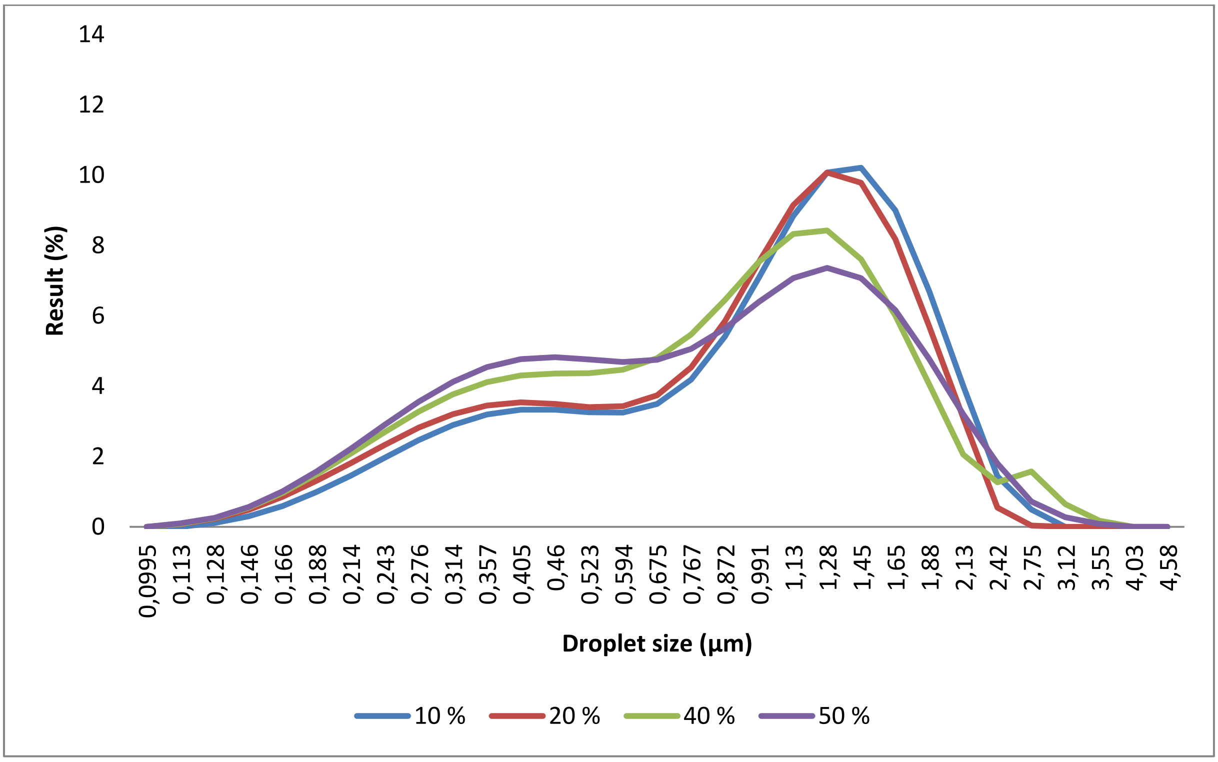

Figure 3.2.3: Droplet size distribution for the dispersed oil droplets in filled gel emulsions with 260 g Bloom gelatin type A and varying amounts of corn oil (10, 20, 40 and 50 wt.%) N = 3 for samples with 10-20 wt.% oil and N = 5 for samples with 40-50 wt.% oil.

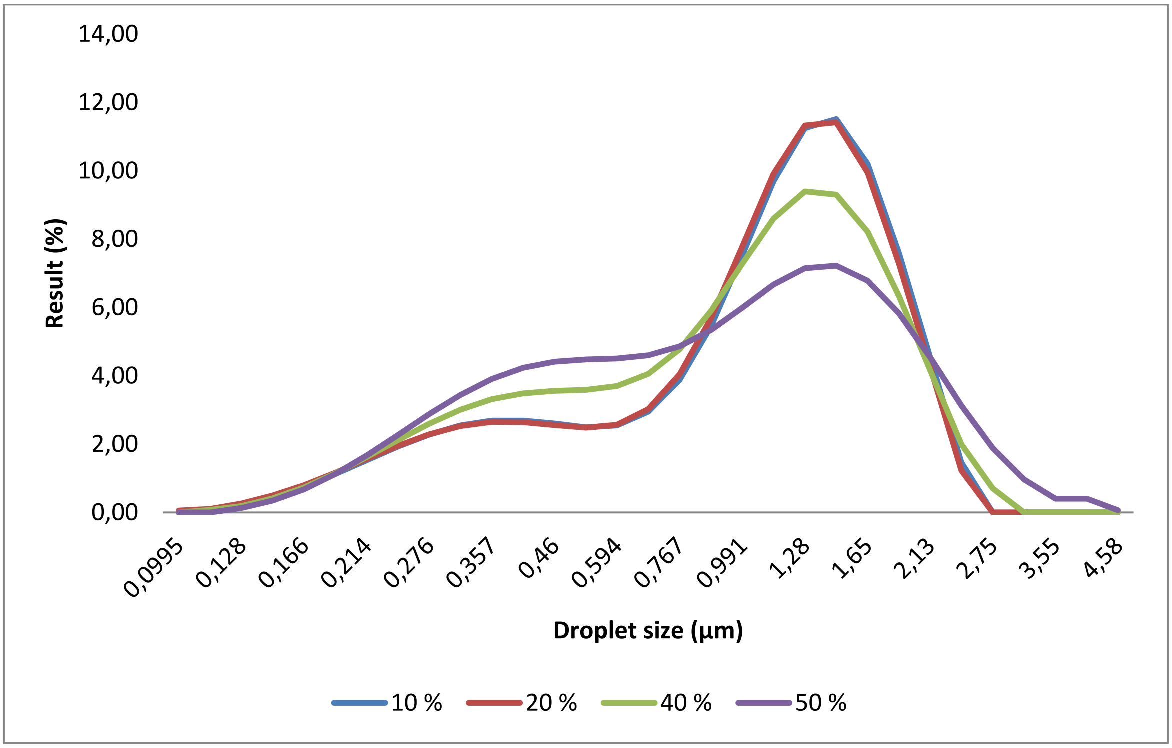

Figure 3.2.4: Droplet size distribution for the dispersed oil droplets in filled gel emulsions with 260 g Bloom gelatin type B and varying amounts of corn oil (10, 20, 40 and 50 wt.%)(N = 3).

Table 3.2.1: Volume weighted average diameter (D [4;3], µm) and distribution width (span) for dispersed droplets in filled gel emulsions made from 160 g Bloom gelatin A or B with different amounts of corn oil. The data were based on an average result created from five replicates for each gel type by the Malvern software.

160 g Bloom								
Sample	**10 wt % Type A**	**10 wt % Type B**	**20 wt % Type A**	**20 wt % Type B**	**40 wt % Type A**	**40 wt % Type B**	**50 wt % Type A**	**50 wt % Type B**
D[4;3] (µm)	1,211	1,198	1,152	1,257	1,201	1,382	1,248	1,405
Span	1,056	1,199	1,066	1,270	1,165	1,268	1,240	1,279

Table 3.2.2: Volume weighted average diameter (D [4;3], µm) and distribution width (span) for dispersed droplets in filled gel emulsions made from 260 g Bloom gelatin A or B with different amounts of corn oil. The data were based on an average result created from five replicates for each gel type by the Malvern software.

260 g Bloom								
Sample	**10 wt % Type A**	**10 wt % Type B**	**20 wt % Type A**	**20 wt % Type B**	**40 wt % Type A**	**40 wt % Type B**	**50 wt % Type A**	**50 wt % Type B**
D[4;3] (µm)	1,135	1,197	1,073	1,180	0,985	1,127	0,999	1,249
Span	1,473	1,348	1,469	1,350	1,635	1,489	1,841	1,863

As can be observed in Figure 3.2.1, Figure 3.2.2, and Table 3.2.1, the volume weighted average diameter for emulsions with 160 g Bloom gelatin type A and B ranged from 1,152 to 1,405 µm. For gelatins with 260 g Bloom (type A and B), Figure 3.2.3 and Figure 3.2.4, the volume weighted average diameter ranged from 0,985 to 1,259 µm (Table 3.2.2). By comparing the droplet size distribution for the two gelatins (Table 3.2.1 and Table 3.2.2), 260 g Bloom gelatins generally showed a lower volume weighted average diameter than the 160 g Bloom gelatins, and gelatin type A (260 g Bloom) consistently had the lowest diameters. This is probably due to the higher average molecular weight in the gelatins with higher Bloom. Higher molecular weight leads to a higher viscosity in the solution. This may lead to an increase in the stress applied on the droplets, potentially promoting droplet disruption and hence leads to a lower average measured droplet size.

The span provides information on a sample's polydispersity. A polydisperse size distribution of the oil droplets will have a higher span than a monodisperse distribution. When the weight fractions of corn oil were increased, the polydispersity of the droplets was also heightened. This effect was more pronounced for 260 g Bloom gelatins, especially type B, but observed in all the gelatin types to some extent. At higher oil content, the viscosity of the emulsions was increased, causing reduced mobility in the oil droplets. This made it more difficult to mix the emulsions evenly during preparation, and thus may have contributed to a less homogenous droplet size distribution.

Droplet size measurements were also performed on a standard system, consisting of 260 g Bloom gelatin A or B with 40 wt.% corn oil. The electrostatic properties of the system were altered by exchanging the regular MQ-water with buffers of pH 4 or 8, or a 0,1 M NaCl solution, or by adding 0,5 wt.% polysorbate 80 (Tween 80) to a standard system with MQ-water. The preparation is described in section 2.3.2. The eventual changes in diluted emulsion properties were investigated with the Mastersizer 3000 and the results are presented in Figure 3.2.5.

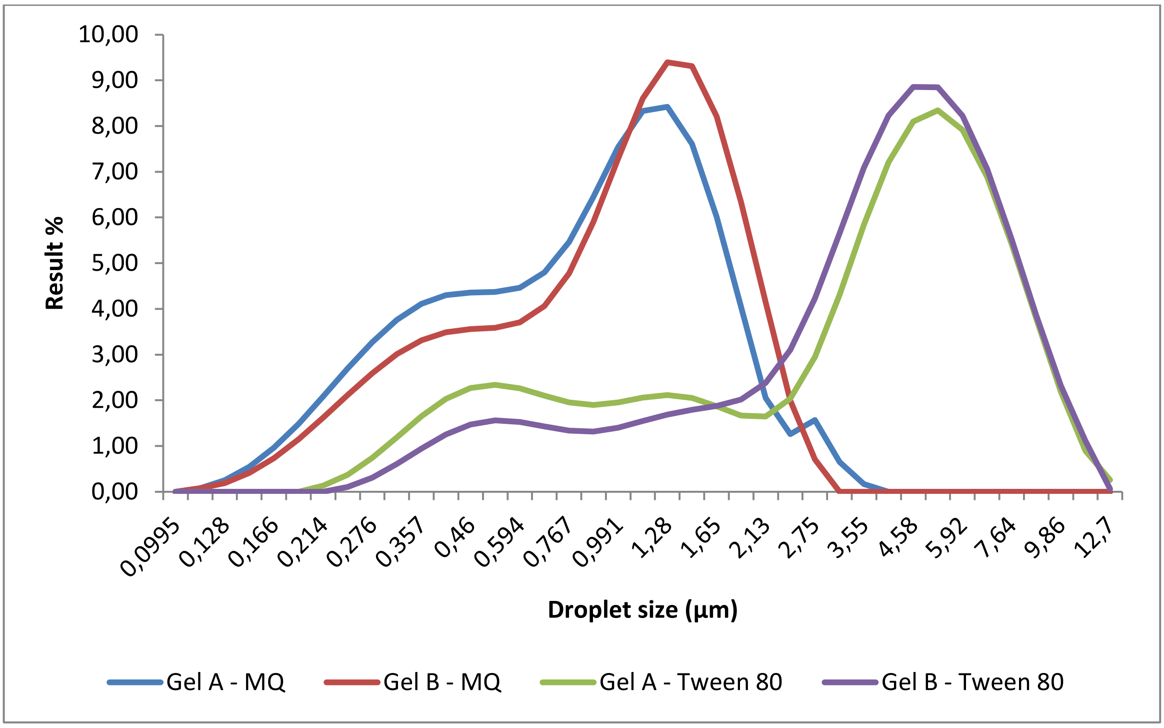

Figure 3.2.5: Droplet size distribution for the dispersed oil droplets in filled gel emulsions with 260 g Bloom gelatin type A and B, prepared with 40 wt.% corn oil and MQ-water, with and without 0,5 wt.% added polysorbate 80 (Tween 80) (N = 3).

The samples with changed pH and ionic strength are not included in Figure 3.2.5, as the results were similar to the samples without altered electrostatic properties. In Figure 3.2.5 one can observe the differences between the standard system with 260 g Bloom gelatin type A, prepared with 40 wt.% corn oil and MQ-water, and the standard systems with 0,5 wt.% polysorbate 80 added. The samples with polysorbate 80 showed a volume weighted average diameter of 4,815 µm for the gelatin type A and 4,795 µm for gelatin type B, with spans of 1,857 and 1,728, respectively. The samples with polysorbate 80 consequently exhibited larger diameters and were more polydisperse than the standard system without surfactant.

A possible reason for the large differences exhibited by the systems with added polysorbate 80, is that the surfactant displaces the gelatin molecules adsorbed to the surface of the oil droplets, and thus cause destabilization of the droplets, leading to flocculation and coalescence.

3.2.1 Morphological characterization of filled gel emulsions

Diluted filled gel emulsions (40 wt.% corn oil) prepared with 260 g Bloom gelatin type A (Figure 3.2.6A), and the same emulsion with 0,5 wt.% polysorbate 80 (Figure 3.2.6B) added to the emulsion after homogenization, were investigated and compared using a light microscope.

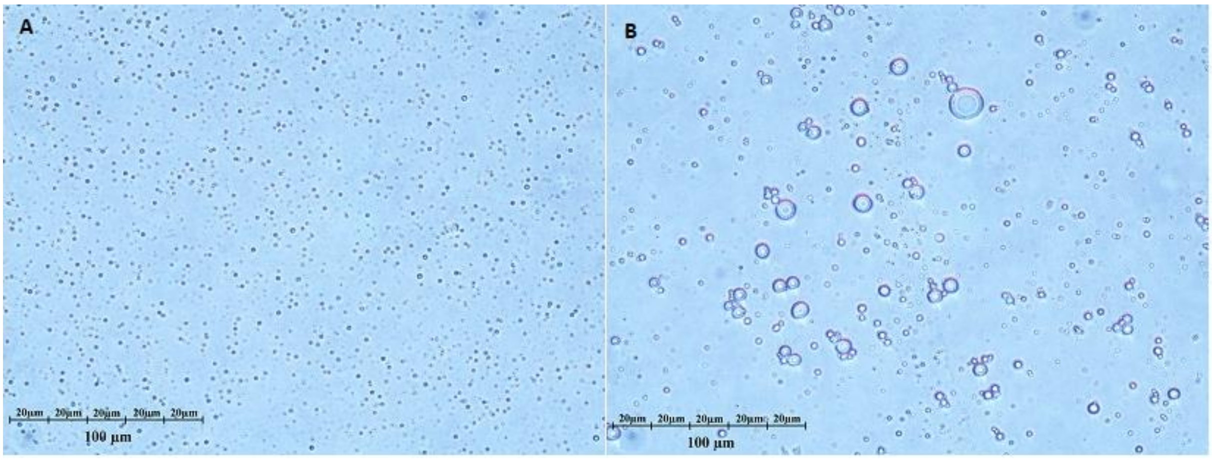

Figure 3.2.6: Microscope pictures of a standard emulsion with (A) 260 g Bloom gelatin type A and 40 wt.% corn oil and (B) 260 g Bloom gelatin A with 40 wt.% corn oil and 0,5 wt.% polysorbate 80 The magnification was 400x.

Figure 3.2.6 verifies the results showed in Figure 3.2.5. Figure 3.2.6A depicts a standard filled gelatin emulsion with evenly sized and distributed oil droplets. No flocculation is observed. In Figure 3.2.6B one can observe the effect of the addition of polysorbate 80. The dispersed oil droplets varies greatly in size and the show different degrees of flocculation. As mentioned, this is most probably caused by the surfactant displacing the gelatin molecules from the droplet surfaces, leading to destabilization, flocculation and coalescence.

3.3 Viscosity measurements of gelatin emulsions

The shear viscosity of the gelatin emulsions with 160 g or 260 g Bloom gelatin (either type A or B) containing different amounts of corn oil (0, 10, 20, 40 and 50 wt.%) was measured by performing continuous shear deformations at 60 °C and with increasing shear rate (0,6 – 100 1/s).

Figure 3.3.1 compares the viscosity of the filled gel emulsions with 160 g and 260 g Bloom gelatin (type A or B) respectively, at a shear rate of 10 1/s.

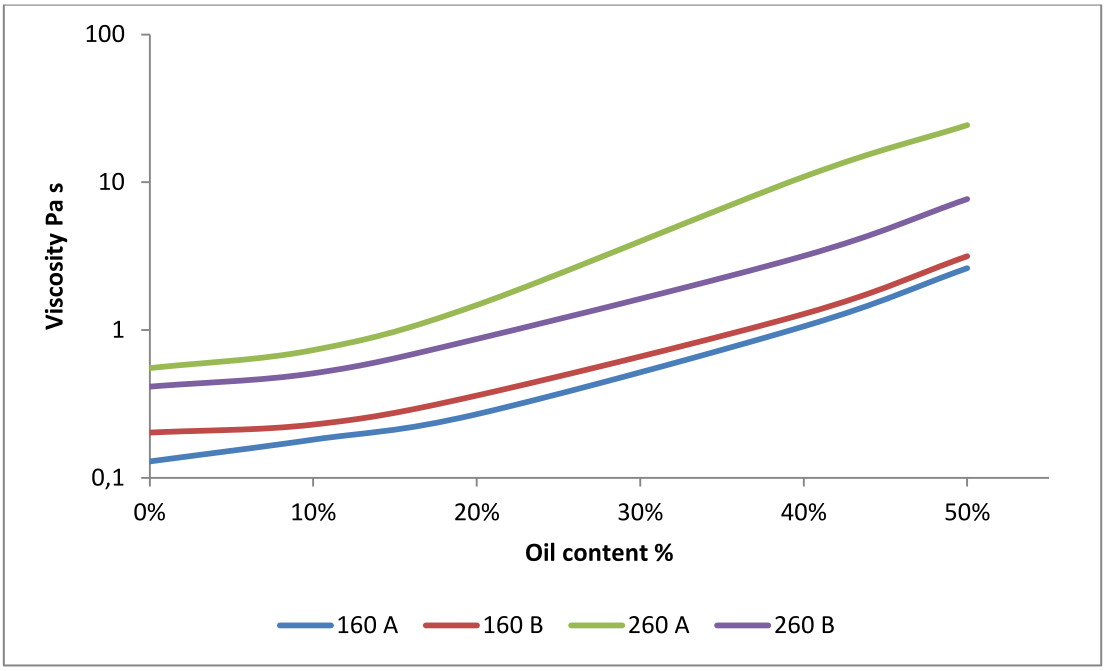

Figure 3.3.1: The viscosity of filled gel emulsions with 160 g and 260 g Bloom gelatin type A and B and varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%) at a shear rate of 10 1/s at 60 °C.

As can be observed from Figure 3.3.1, the viscosity of the emulsions with 160 g Bloom gelatins displayed a clear increase as a function of oil content. The increase is rather similar for both gelatin types. The gelatins with 260 g Bloom displayed a similar behavior, but the viscosity of gelatin A increases very strongly with increased oil fraction, compared to gelatin type B. These results are in accordance with the semi-empirical equation of Krieger-Dougherty, which shows that viscosity is increased exponentially if the weight fraction of corn oil is increased (van Aken, 2006).

As described in the introduction, increased concentration of dispersed oil droplets will increase the viscosity of an emulsion, because the oil droplets works as active filler particles, because they are bound to the gelatin polymer network. Dissipation of energy in the presence of oil droplets increases when the fraction of the dispersed phase increases, thus causing an increase in viscosity (McClements, 2004b).

Figure 3.3.2-5 shows viscosity as a function of shear rate (0,6 – 100 1/s) for 160 g and 260 g Bloom gelatin type A and B, respectively.

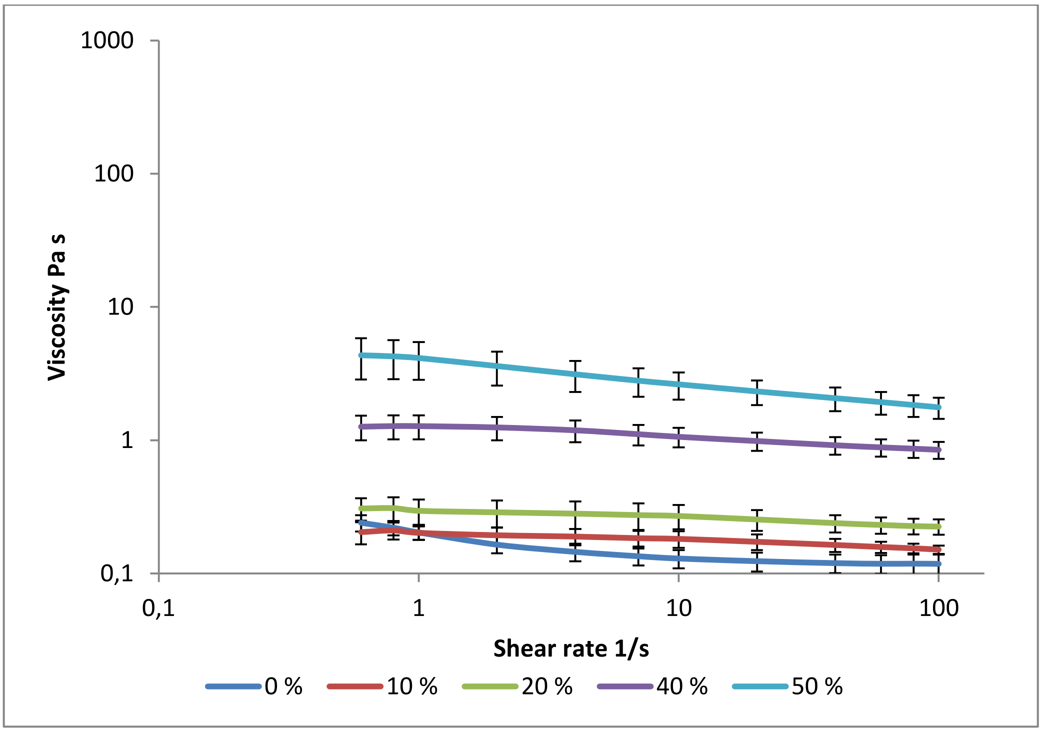

Figure 3.3.2: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 160 g Bloom gelatin type A and varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%). Measurements were performed at 60 °C. (N = 5, ± average S.D.) Raw data are found in appendix D.1.

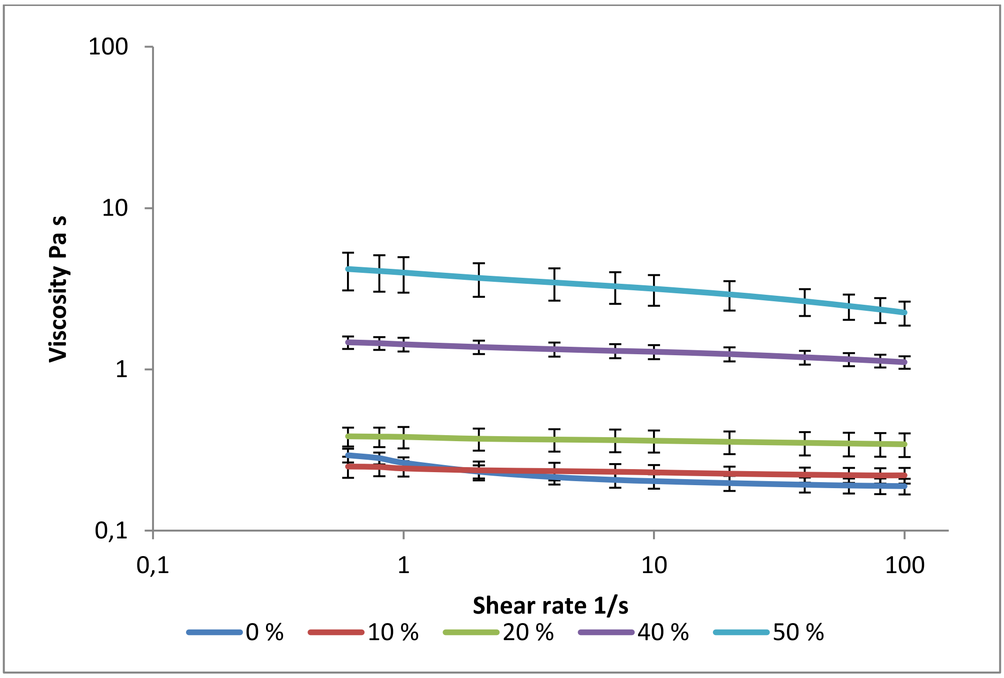

Figure 3.3.3: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 160 g Bloom gelatin type B and varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%). Measurements were performed at 60 °C. (N = 5, ± average S.D.) Raw data are found in appendix D.1.

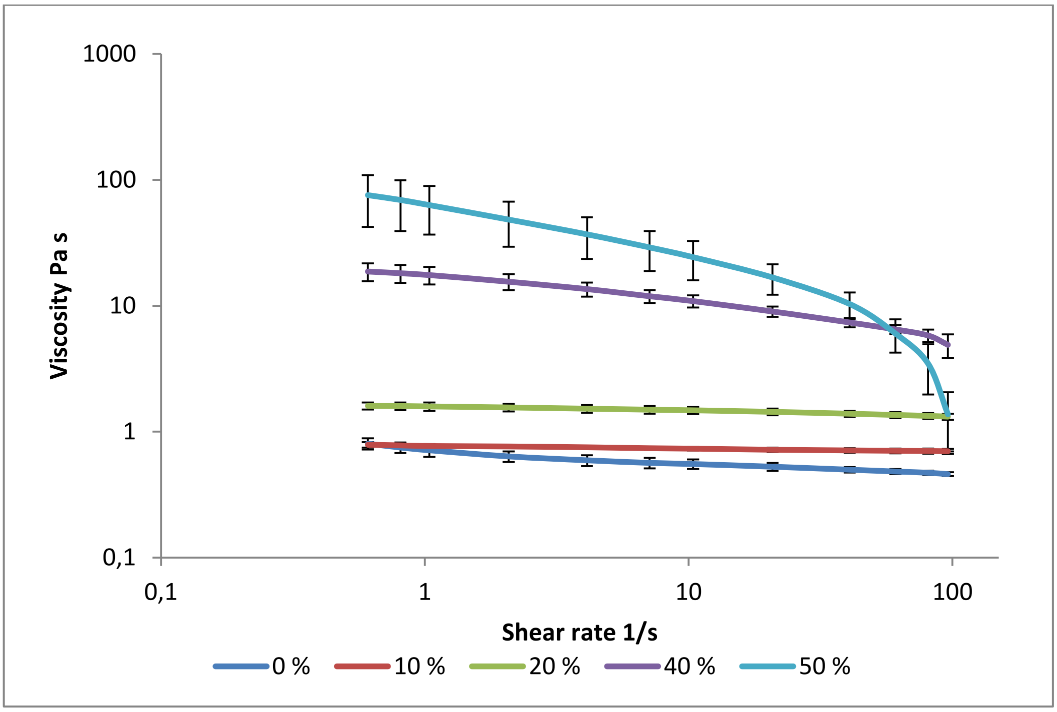

Figure 3.3.4: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 260 g Bloom gelatin type A and varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%). Measurements were performed at 60 °C. (N = 3 for 0–20 wt.% and N = 5 for 40-50 wt.%, ± average S.D.) Raw data are found in appendix D.2.

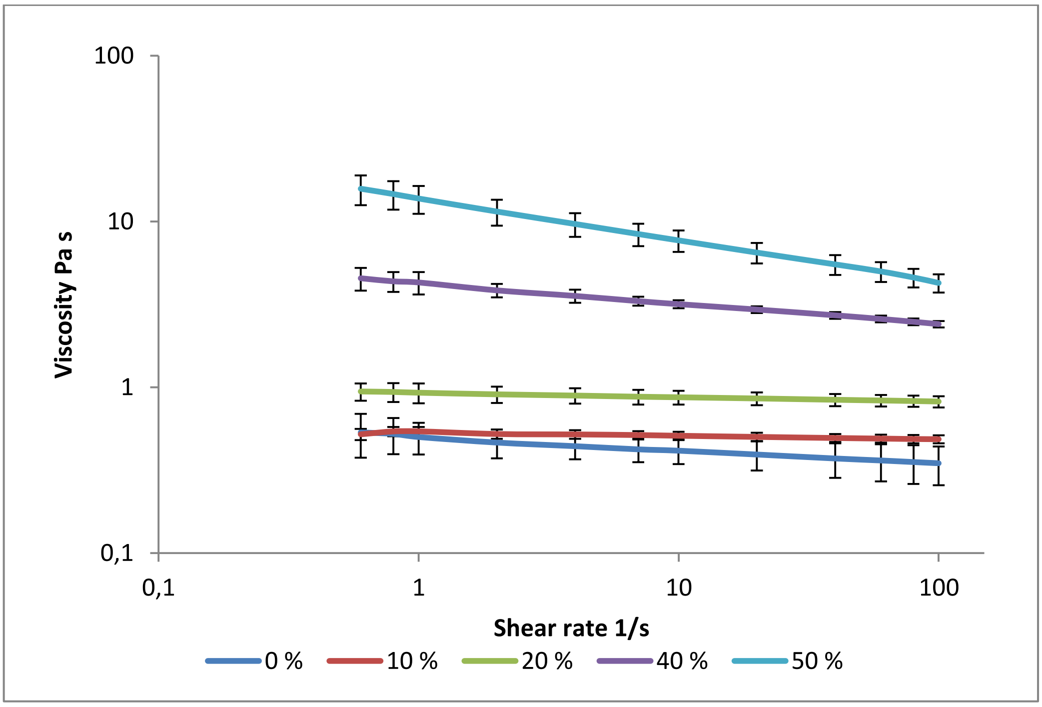

Figure 3.3.5: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 260 g Bloom gelatin type B and varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%). Measurements were performed at 60 °C. (N = 3, ± average S.D.) Raw data are found in appendix D.2.

In Figure 3.3.4-7, the filled gel emulsions containing 10 and 20 wt.% corn oil displays an almost Newtonian behavior, while at higher weight fractions, the emulsions shows a slightly shear thinning behavior. This includes both Bloom values and gelatin types. A rather strange behavior is seen for the systems without corn oil, as they seem to be slightly shear thinning, especially in the beginning of the measurements. It is assumed that this effect is due to a combination of low viscosity in the sample and low shear rate, giving a low torque, and the phenomenon can thus be considered an artifact. Concequently, the samples containing no corn oil can be described as Newtonian. The sharp drop in viscosity exhibited by gelatin type A with 50 wt.% corn oil in Figure 3.3.4, can also be considered an artifact as it is probably due to a slip effect of the cone-plate setup.

Electrostatic Interactions

It was suspected that the shear thinning behavior of the filled gel emulsions with high oil content was caused by some type of flocculation of the oil droplets. This may be caused by electrostatic interaction between the gelatin molecules on the droplet surfaces. To investigate this, filled gel emulsions (40 wt.% corn oil) were prepared with 260 g Bloom gelatin type A or B, using 0,1 M NaCl and buffered solutions with pH 4 and pH 8 as the continuous phase. Table 3.3.1 shows the average pH values for the filled gel emulsions.

Table 3.3.1: Average pH in various 260 g Bloom gelatin types with 40 wt.% corn oil. (N = 3, ± S.D.) (IEP = isoelectric point).

Buffer pH	Buffer type	Gelatin type	IEP	Average pH	± S.D.
4	Acetic acid	A	pH 8-9	4,6	0,03
4	Acetic acid	B	pH 4,8-5,5	4,9	0,08
8	Tris-buffer	A	pH 8-9	7,3	0,08
8	Tris-buffer	B	pH 4,8-5,5	7,4	0,08

Emulsions were also prepared with MQ-water and 0,5 wt.% polysorbate 80. When the pH in an emulsion is close to the isoelectric point of the stabilizing protein, there will be low netto charge on the dispersed droplets, leading to low electrostatic repulsion and thus flocculation of the droplets. (Demetriades et al., 1997). Figure 3.3.6 and Figure 3.3.7 shows the viscosity of 260 g Bloom gelatin type A and B, respectively, as a function of increasing shear rate.

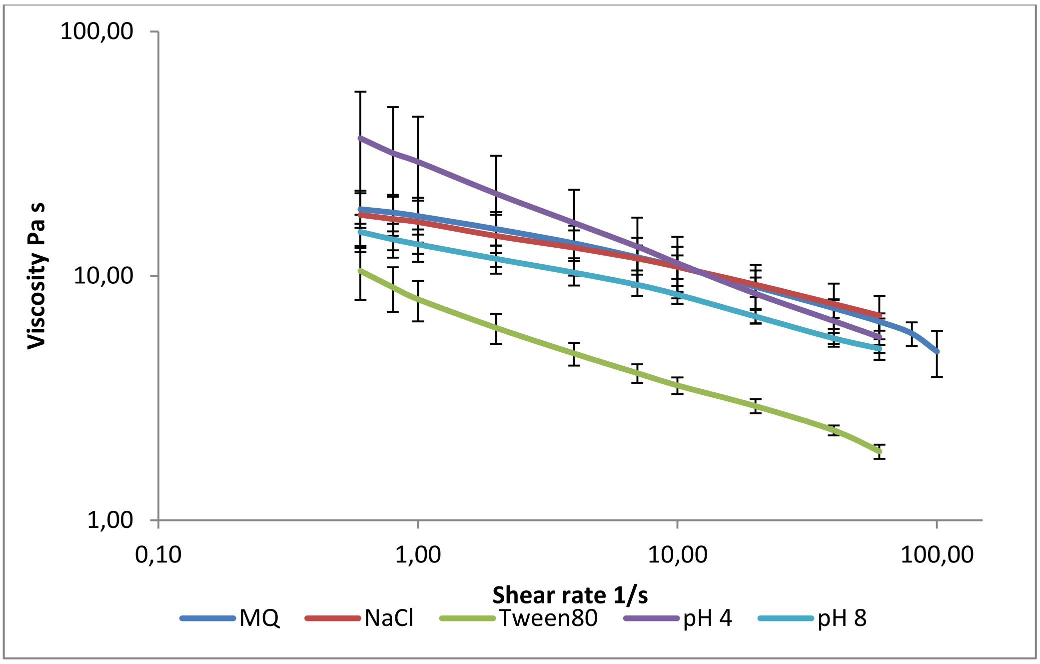

Figure 3.3.6: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 260 g Bloom gelatin type A and 40 wt.% corn oil. The standard filled emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. Measurements were performed at 60 °C. (N = 3, ± average S.D.) Raw data are found in appendix D.3.

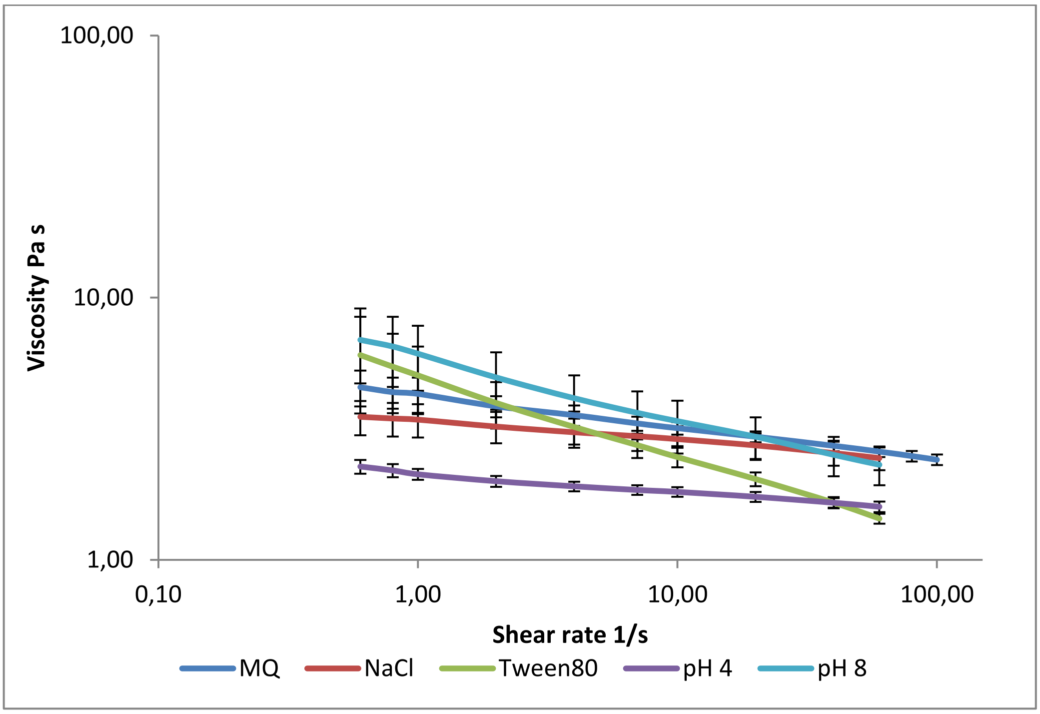

Figure 3.3.7: Viscosity (Pa s) as a function of increasing shear rate for filled gel emulsions with 260 g Bloom gelatin type B and 40 wt.% corn oil. The standard filled emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. Measurements were performed at 60 °C. (N = 3, ± average S.D.) Raw data are found in appendix D.3.

As can be observed in Figure 3.3.6 there is no change in shear thinning behavior between the standard system and the system with 0,1 M NaCl, although the charges on the droplets should be completely screened at 0,1 M, according to literature (Vu, Robblee, Werner, & Fairman, 2001). This may indicate that electrostatic interactions are not responsible for the higher viscosity of gelatin type A. As for the systems with changed pH, shear thinning behavior was observed to a great extent in gelatin type A, for both pH 4 and pH 8, and to a lesser extent in gelatin type B. Both emulsions shows the lowest viscositites at a pH value close to the isoelectric points of the protein. At this pH, the proteins will have no or low netto charge, which may cause the dispersed oil droplets to form flocs. When the emulsions are exposed to shear, the flocculates may align with the field and disrupt, causing shear thinning behavior and lower viscosity.

The samples containing polysorbate 80 exhibited very shear thinning behavior for both gelatin A and B. In gelatin A, the starting viscosity was also remarkably lower than similar samples without polysorbate. This can be explained via the microscope picture in Figure 3.2.6B, which shows that the oil droplets in the polysorbate sample were very polydisperse and flocculated. The mean droplet size and polydispersity can have a large impact on the rheology of a concentrated emulsion. Polydisperse droplets will be able to pack together more efficiently, and thus be able to lower the viscosity in a concentrated polydisperse emulsion, compared to an emulsion with monodisperse droplets (McClements, 2004b). The flocculation will also cause the solution to be shear thinning. The same behavior is shown for gelatin B, except here the starting viscosity is not very different from the starting viscosities of the other samples.

Cox-Merz

A Cox-Merz plot (Figure 3.3.8) was made by comparing the frequency sweeps of samples prepared with 260 g Bloom gelatin type A and B, 40 wt.% corn oil and 0,1 M NaCl as the continuous phase, with shear rate viscosity measurements of standard samples prepared with MQ-water.

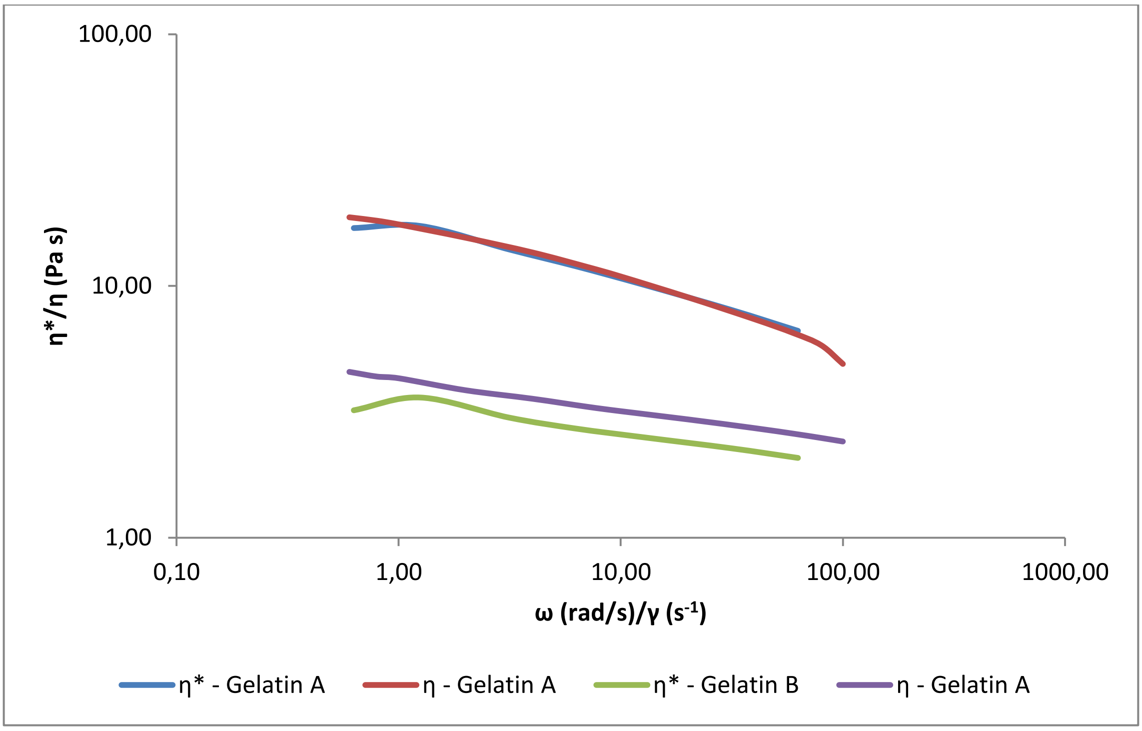

Figure 3.3.8: Cox-Merz plot of gelatin emulsion samples with 260 g Bloom gelatin type A or B and 40 wt.% corn oil.

As can be seen in the figure, there is a total overlap for gelatin A and close to overlap for type B, suggesting no long range interactions in the system and no build-up of continuous structure. The difference in viscosity for the two gelatin types is probably due to flocculation of emulsion droplets in gelatin type A, which increases the internal friction of the system compared to smaller entities with gelatin type B.

Note: Due to time issues, it was necessary to use the frequency sweeps for samples with NaCl instead of “standard” samples prepared with MQ-water. There was not enough time to prepare new standard samples and run frequency sweeps on them before the deadline of the thesis. This was not considered a problem, as the samples with NaCl showed similar behavior when exposed to shear as the samples with MQ-water, due to the lack of electrostatic interactions in the filled gelatin emulsion systems.

3.4 Small strain oscillatory measurements on filled gel emulsions

Small strain oscillatory measurements were performed on filled gel emulsions with 160 g and 260 g Bloom gelatin A and B, containing varying amounts of corn oil (0, 10, 20, 40 and 50 wt.%). From these measurements, the storage modulus (G'), gelling (T_g) and melting (T_m) temperatures were determined.

Effect of oil content and Bloom value on the storage modulus of filled gel emulsions

The storage moduli (G') of the filled gel emulsions after 15 minutes of curing at 20 °C are presented in Figure 3.4.1. Measurement data are presented in appendix E.1.

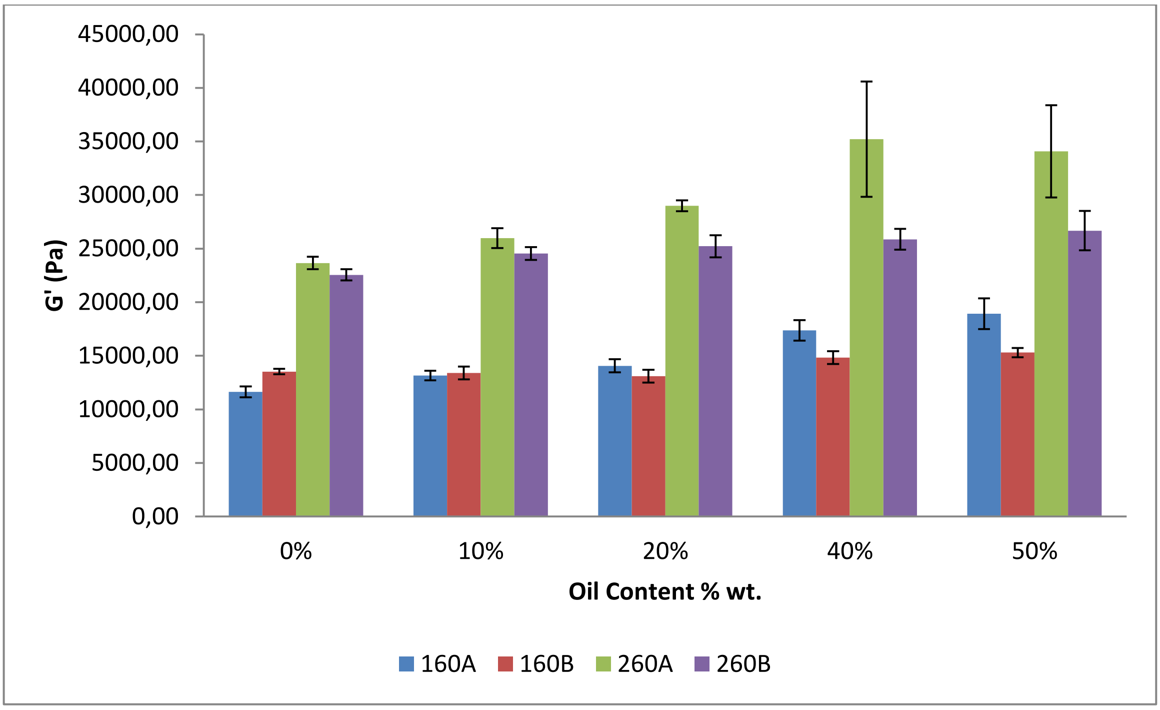

Figure 3.4.1: Storage modulus (G', Pa) of 160 g and 260 g Bloom gelatins type A or B presented as a function of oil content (0, 10, 20, 40 and 50 wt.%) (N = 5, average ±S.D. for 160 g Bloom type A and B, and 260 g Bloom type A with 40 and 50 wt.% oil. For the rest, N = 3, average ±S.D.).

An increase in storage modulus with increasing oil content was measured for all the different gelatins. The oil content generally had a more pronounced effect on the G' for type A gelatin independent on Bloom value.

As previously mentioned, the increase in modulus may be due to a filler effect. Since gelatin is a network polymer, which adsorbs to the surface of dispersed droplets, one can likely conclude that the oil droplets in this case acts as active filler particles, increasing the gel strength at increasing oil concentrations.

A similar study suggested that the difference in the increase in gel strength between gelatin type A and type B might be due to flocculation between the oil droplets in gelatin type A (Sæther, 2012). This effect may be due to the difference in IEP between the two gelatin types, where gelatin type A has an IEP at pH 8 – 9, while the IEP of gelatin type B is 4,8 – 5,5. This variation is caused by the chemical pretreatment of the gelatins. The alkaline pretreatment of gelatin type B converts the amino acids asparagine and glutamine into their carboxyl forms, aspartic and glutamic acid, respectively. Both asparagine and glutamine have weak polar side groups, able to accept and donate hydrogen bonds. This may be the reason for the suggested flocculation between oil droplets stabilized by gelatin type A; the gelatin molecules adsorbed to the droplet interface interact with each other through hydrogen bonds. The high oil content will also lead to a relatively tightly packed system, which will bring the oil droplets and their adsorbed gelatin molecules closely together, facilitating hydrogen bonding.

An earlier study performed by Dickinson et al. suggested that the size distribution of the filler particles is of considerate importance for weak gelatin gels, as the amount of gelatin required for adsorption onto filler particles causes less gelatin to be available for bulk-phase gelation. This is not as critical for stronger gelatin gels (gelatin > 5 wt.%) (Dickinson, Stainsby, & Wilson, 1985) Hence, this should not influence the results of this study to a alrge degree, as all the gelatin emulsions contained 25 wt.% gelatin. However, for 260 g Bloom gelatin type A, there is a slight decrease in storage modulus when the corn oil content is increased from 40 wt.% to 50 wt.%. This is not seen for any of the other gelatin types with 50 wt.% corn oil, so it is questionable if this is caused by this effect. Still, if the gelatin content was to be lowered considerably, there would probably be observed some effects similar to those in the Dickinson study, especially at high oil content (40 – 50 wt.%).

Research has indicated that a maximum gelling power is reached for gelatins with a molecular weight in the 100 kDa range (Schrieber & Gareis, 2007). This may explain the higher modulus exhibited by the 260 g gelatin type A and type B, compared to the 160 g

Bloom gelatins, as the 260 g Bloom gelatins had higher fractions in the 100 kDa range, according to the figure in section 2.3.1.

Effect of oil content and Bloom value on the gelling and melting temperatures of filled gel emulsions

Gelling and melting temperatures are presented in Figure 3.4.2 and Figure 3.4.3, respectively. Measurement data are found in appendix E2 and E3.

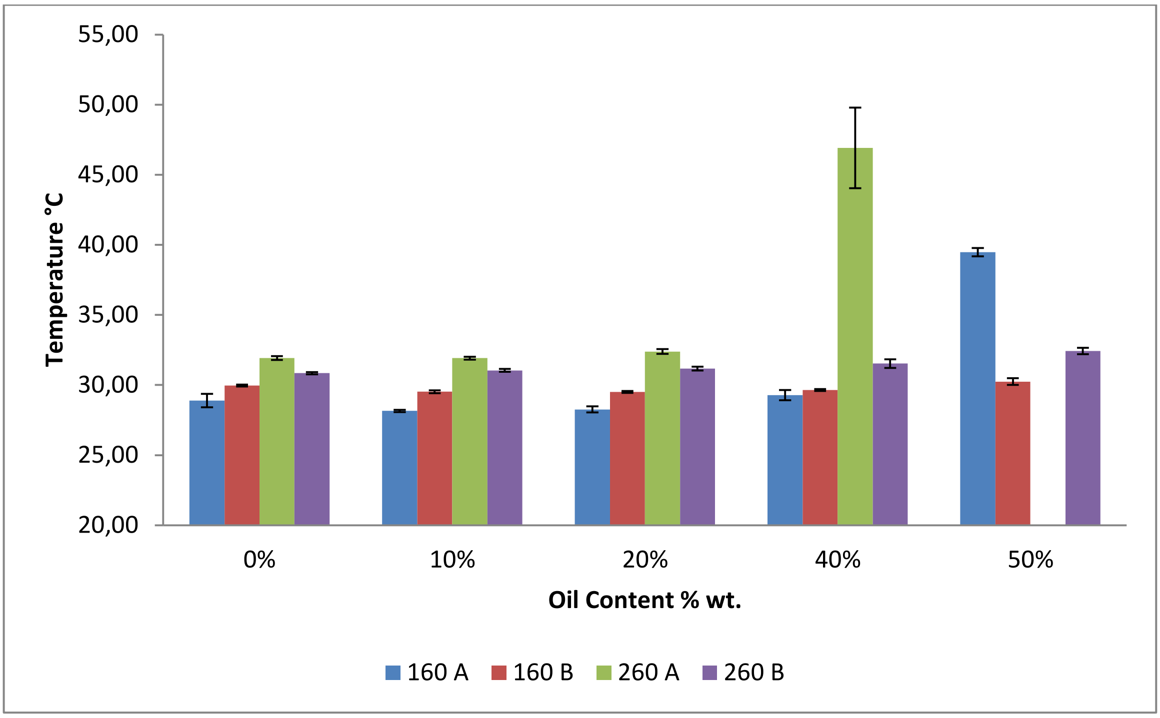

Figure 3.4.2: Gelling temperatures (T_g) for 160 g and 260 g gelatin type A and B filled gel emulsions, containing 0, 10, 20, 40 or 50 wt.% corn oil. (N = 5, average ±S.D. for 160 g Bloom type A and B, and 260 g Bloom type A with 40 wt.% oil. For the rest, N = 3, average ±S.D.).

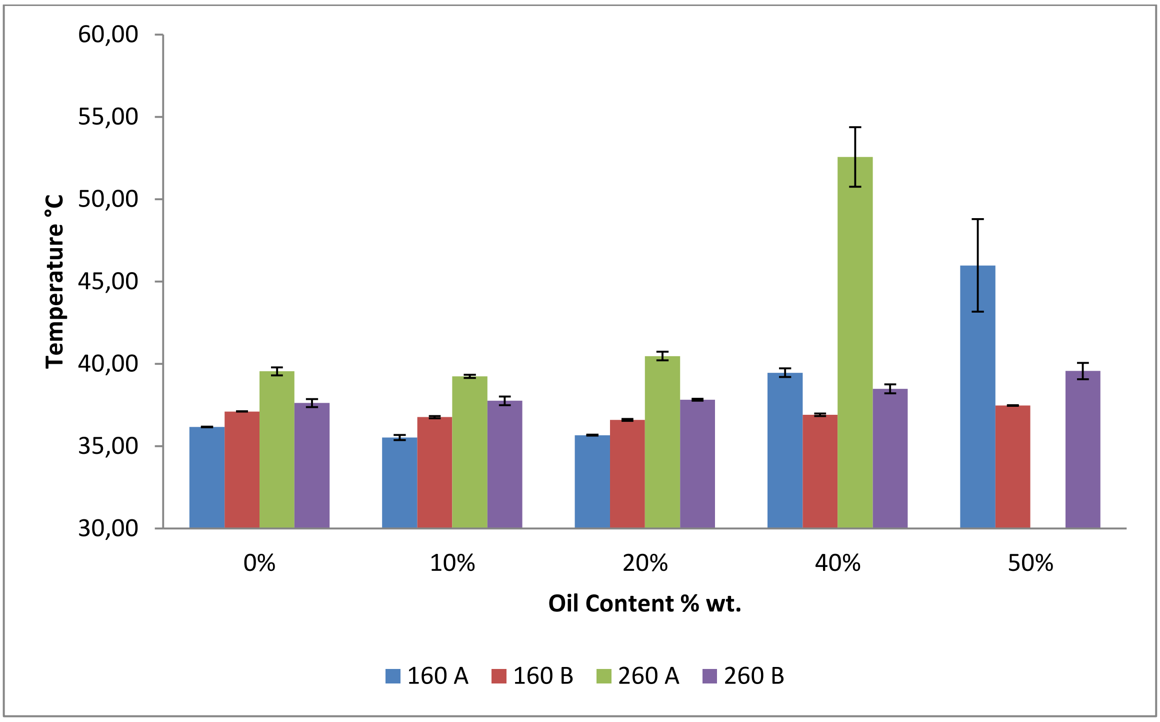

Figure 3.4.3: Melting temperatures (T_m) for 160 g and 260 g gelatin type A and B filled gel emulsions, containing 0, 10, 20, 40 or 50 wt.% corn oil. (N = 5, average ±S.D. for 160 g Bloom type A and B, and 260 g Bloom type A with 40 wt.% oil. For the rest, N = 3, average ±S.D.).

As can be observed from Figure 3.4.2 and Figure 3.4.3, the oil content did not have the same impact on the gelling and melting temperatures as on the storage modulus. There was not observed a clear correlation between the volume fraction of corn oil the and gelling/melting temperatures for the gelatins. Gelatin samples with no oil content showed differences in both gelling and melting temperature, this is because the gelling and melting temperature of a gelatin changes with increasing or decreasing Bloom value, as shown in the figures (Figure 3.4.2 and Figure 3.4.3). A higher Bloom value will give higher gelling and melting temperatures (Gelita, 2013). Gelling temperatures of gelatins are attributed to the pyrrolidine content of the gelatin in question. Pyrrolidine is the central part of the structures of the imino acids proline and hydroxyproline, and therefore, the amount of these imino acids is important for the thermostability of a gelatin. Other factors that may influence the gelling and melting temperatures of gelatins are the molecular weight distribution, the gelatin concentration, thermal history and the pH (Jones, 2004). However, filled gel emulsions with gelatin type A showed a significant increase in gelling and melting temperatures at high oil content. This behavior is seen at 50 wt.% oil in 160 g Bloom gelatin

A, and at 40 wt.% oil in 260 g Bloom gelatin type A. There was measured a small increase in gelling temperature with increasing oil content in 260 g Bloom gelatin type B, but not as pronounced as for gelatin type A. Again, this can be explained with the oil droplets working as active filler particles, and thus reinforces the gel structure.

It was not possible to obtain either gelling or melting temperature for 260 g Bloom gelatin type A with 50 wt.% oil because of a lack of a phase degree of 45°. The phase degree remained below 45° (solid state) throughout all the measurements, even at high temperatures, indicating that the presence of the corn oil droplets give the emulsions visco-elastic properties.

Changes in electrostatic interactions and influence on storage modulus, gelling and melting temperature

As described in the earlier sections, increasing the oil fraction had a larger effect on the rheological properties of gelatin type A compared to gelatin B, independent on Bloom value. It was suspected that the cause was some kind of flocculation effect between the dispersed oil droplets.

To investigate the effect of oil fraction and droplet interactions on the rheological properties of gelatin type A and B, filled gelatin emulsions were prepared with 260 g Bloom gelatin type A and B, containing 0 or 40 wt.% oil, and 0,1 M NaCl solution, pH buffers or MQ-water as the continuous phase. 0,5 wt.% polysorbate 80 was also added to samples with MQ-water as continuous phase. The different samples were investigated using small strain oscillatory measurements, to determine if changes in the electrostatic interactions in the emulsions had any influence on G’, T_g and T_m. Figure 3.4.4-9 shows changes in G’, T_g and T_m for gelatin A and B, respectively.

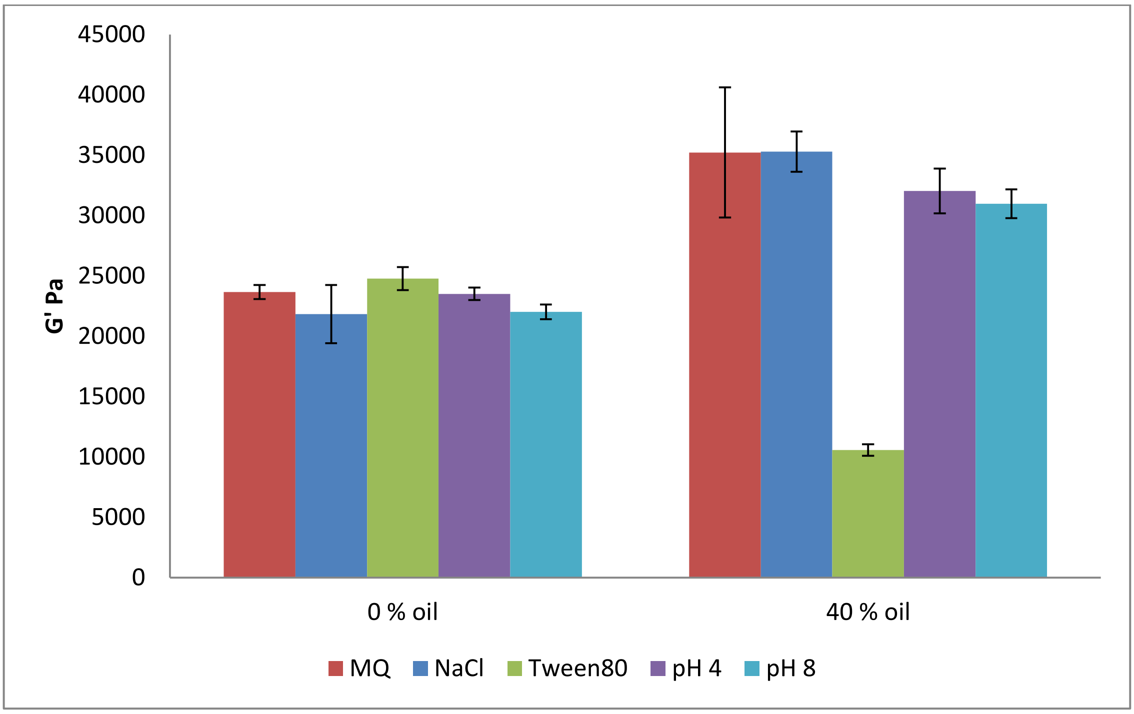

Figure 3.4.4: Storage modulus for 260 g Bloom gelatin type A, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

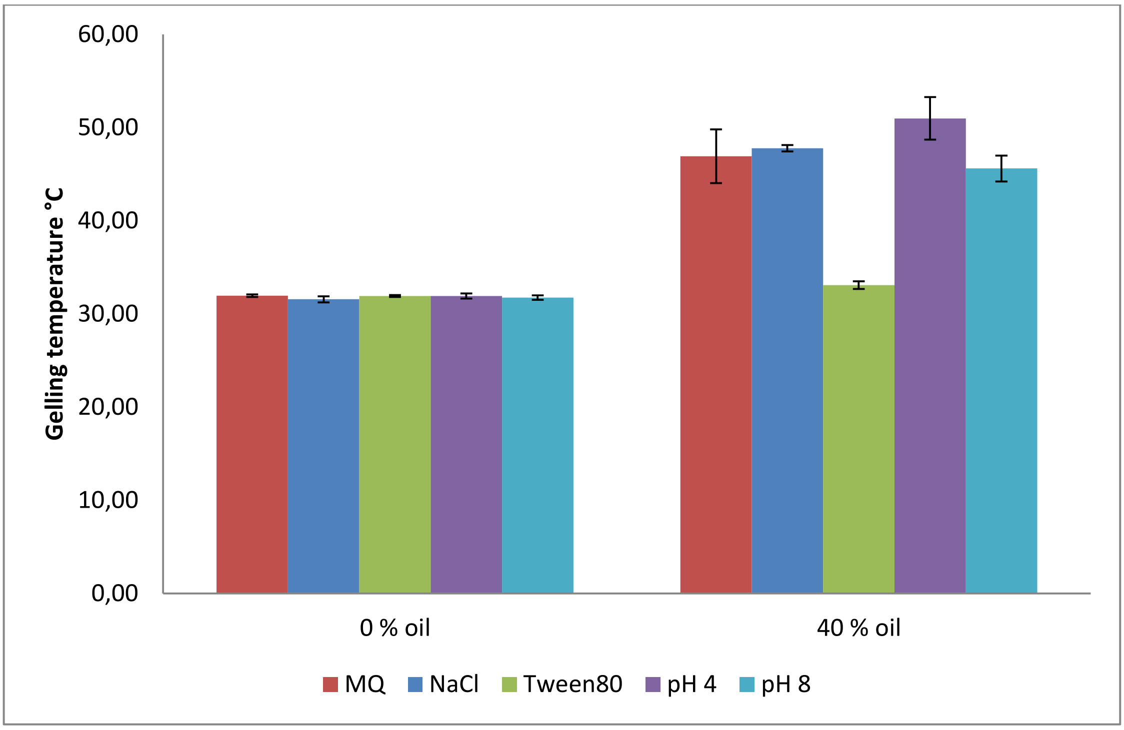

Figure 3.4.5: Gelling temperatures for 260 g Bloom gelatin type A, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

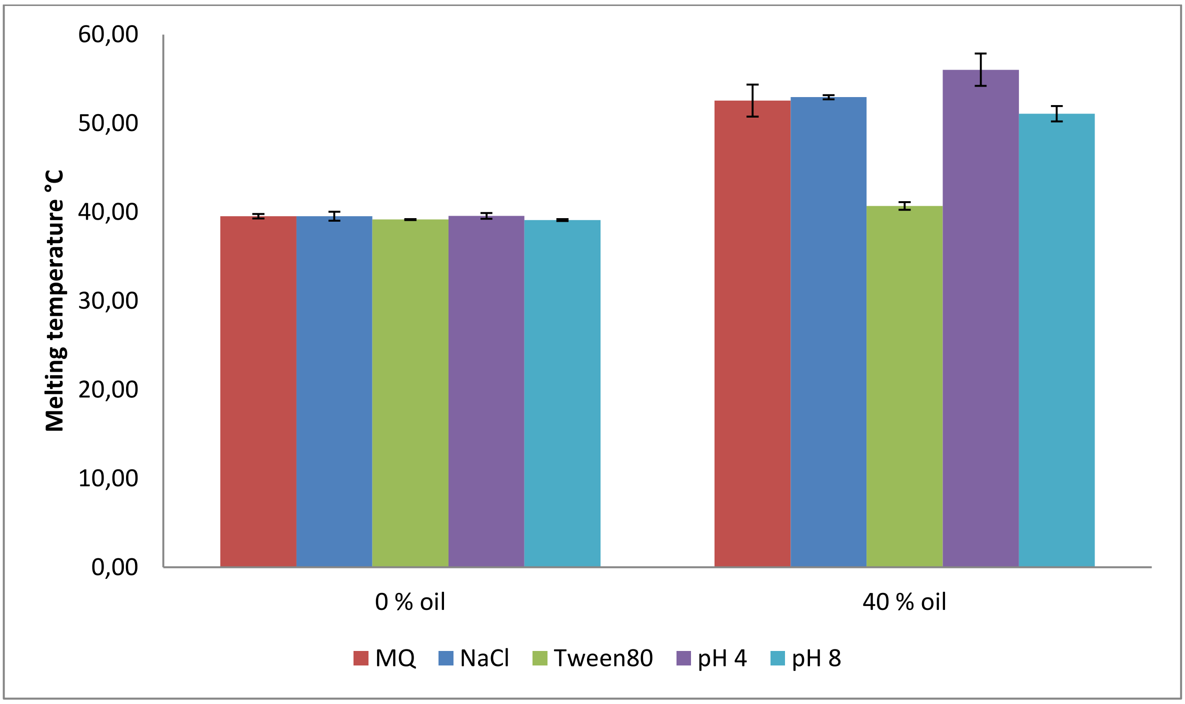

Figure 3.4.6: Melting temperatures for 260 g Bloom gelatin type A, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

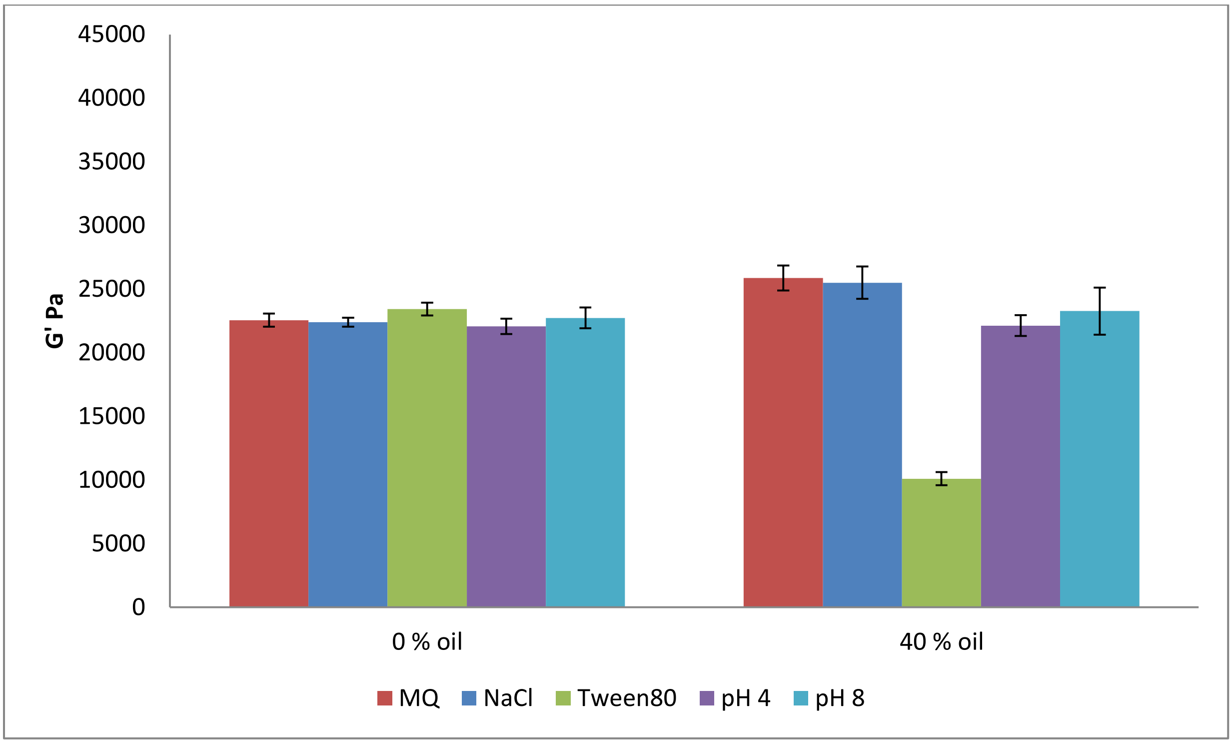

Figure 3.4.7: Storage modulus for 260 g Bloom gelatin type B, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

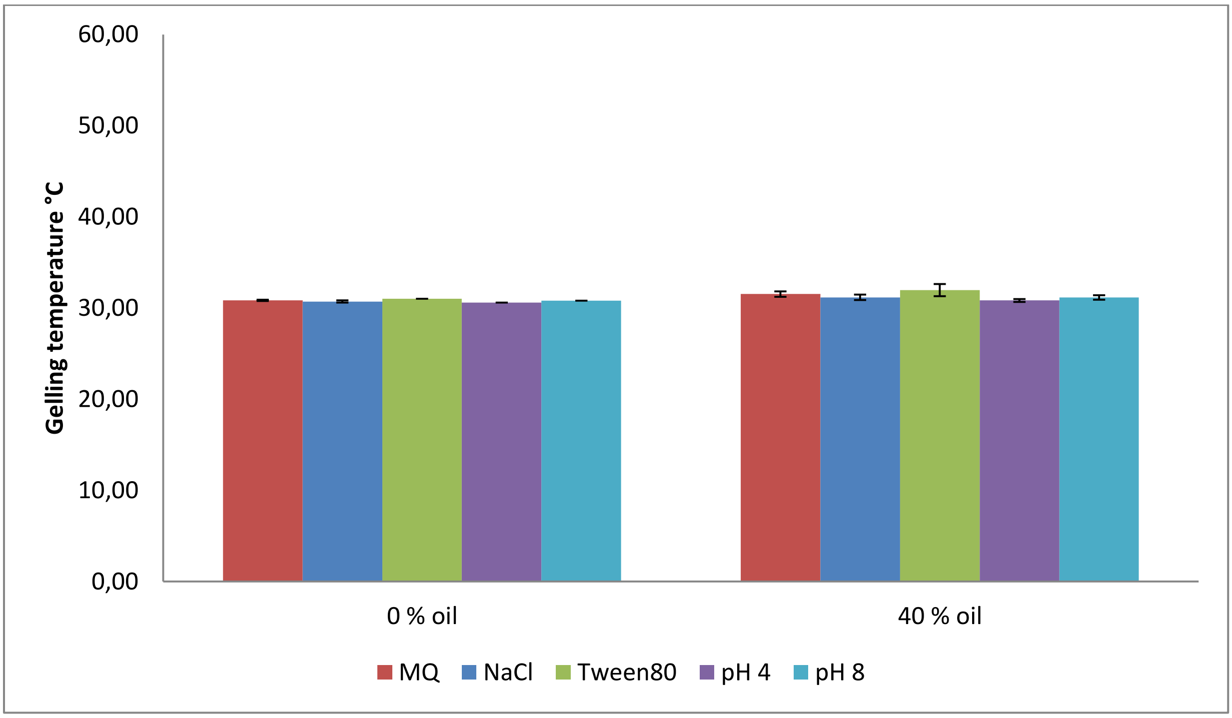

Figure 3.4.8: Gelling temperatures for 260 g Bloom gelatin type B, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

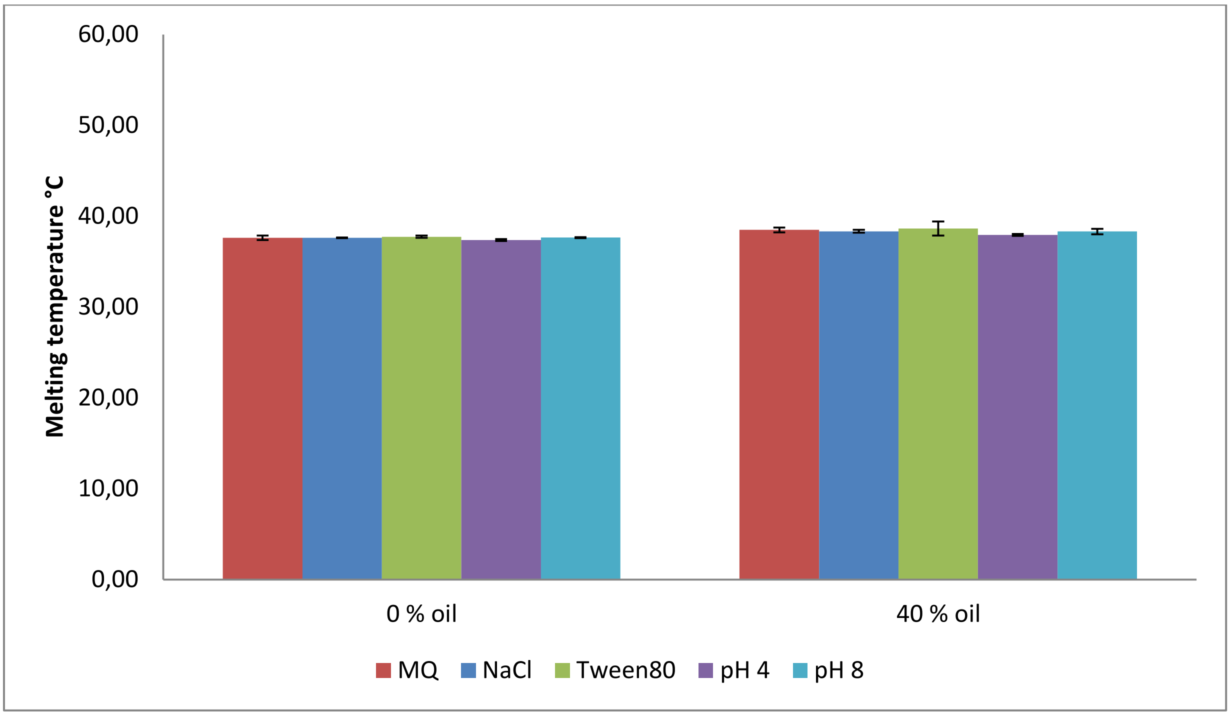

Figure 3.4.9: Melting temperatures for 260 g Bloom gelatin type B, with 0 and 40 wt.% corn oil. The standard emulsion was compared to emulsions containing 0,1 M NaCl, or with increased pH (pH 7,3), decreased pH (pH 4,6) or with 0,5 wt.% polysorbate 80 (Tween 80) added. (N = 3, average ±S.D.).

In Figure 3.4.4 and Figure 3.4.7 one can observe that ionic strength did not have any significant impact on the storage modulus of the filled gel emulsions, either for gelatin type A or B. For the systems with altered pH, there were seen some slight changes in the storage moduli for both gelatin types. There was observed a decrease in G' for both gelatins at pH close to their respective isoelectric points. At IEP, the molecules have no/low netto charge. This might facilitate droplet flocculation and coalescence, as there is no repulsion between the peptide chains of the gelatins. However, there was also observed a decrease in gel strength for both gelatins when the pH was far from their IEP. As stated by Haug et al. (2004); at pH values far from a molecule's IEP, there will be an increased charge density. This will reduce the ability of the peptide chains to form junction zones, and as a consequence of this there will be a decrease in gel rigidity (Haug, Draget, & Smidsrød, 2004). The emulsions containing 0,5 wt.% polysorbate 80 had a large decrease in storage modulus, especially for the gels prepared with gelatin type A. This may be due to polysorbate adsorbing to the droplet interphase, displacing the gelatin molecules and thus reduce the affinity between the droplets and the gelatin network. This may lead to the droplets acting as inactive fillers, and hence cause a reduction in the gel strength. The same effect is seen for the gelling and melting temperature of gelatin type A, although this effect was not observed for type B gelatin.

The addition of polysorbate 80 did not have any effect on the systems with a zero oil fraction, indicating that the droplet interactions and size of oil fractions are responsible for changes in rheological properties.

Comparison of phase angles of gelatin emulsions

In Figure 3.4.10, the phase angles and of different gelatin emulsions are compared to determine if there is a shift in the phase angle at increasing oil fractions.

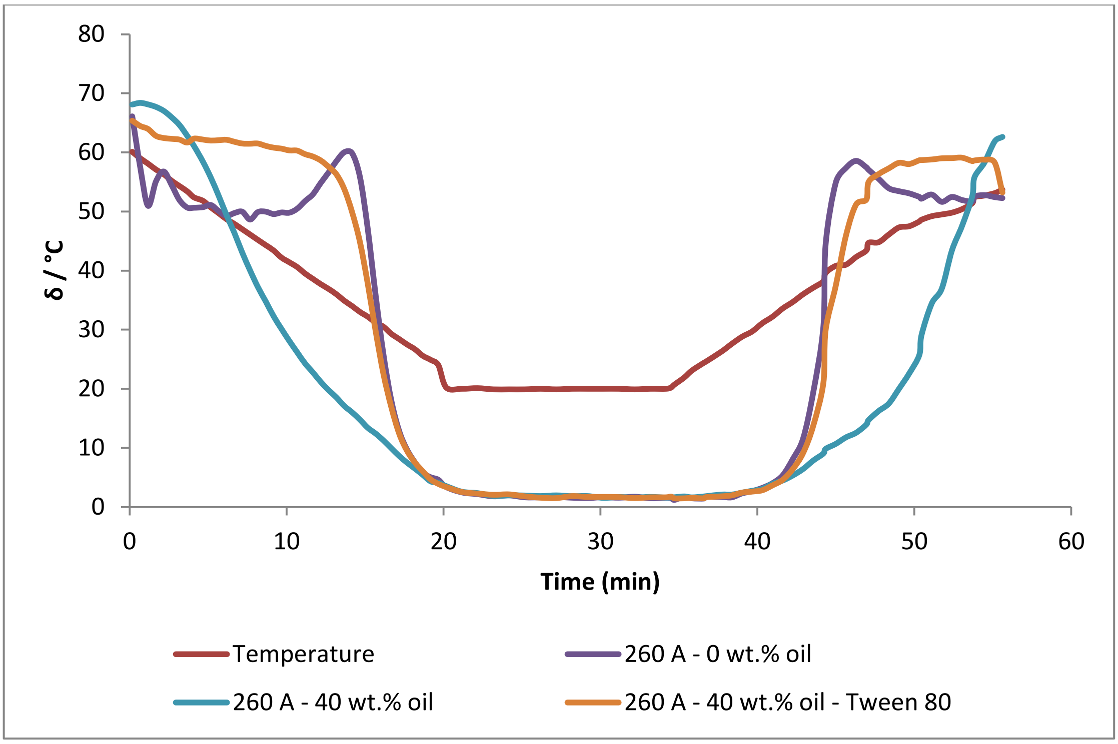

Figure 3.4.10: Phase angles for filled gel emulsions with 260 g Bloom gelatin type A, 0 and 40 wt.% corn oil. and 0,5 wt.% polysorbate 80 (Tween 80) as a function of time (min). The temperature interval is also shown.

It was suspected that there was a downward shift in the phase angle at increasing oil fractions, meaning that the emulsions would have a lower phase angle at the beginning of the measurements, which could prove the calculated gelling and melting temperatures faulty. Figure 3.4.10 shows that this is not the case, as the gelatin emulsions with high oil fraction had approximately the same phase angle at the beginning of the measurement as the system with zero oil fraction and the system with polysorbate 80. The only difference is that the phase angle drops rapidly after a few minutes of measurement for the system with large oil fraction, and thus it stays longer in the solid state than the other samples. The calculated gelling and melting temperatures are thus not faulty.

3.5 Longitudinal studies of filled gel emulsions

Filled gel emulsions containing 0, 10, 20, 40 and 50 wt.% corn oil, 160 g and 260 g Bloom gelatin (type A and B) were analyzed by performing longitudinal compression measurements. From these measurements, the Young's modulus (E) was determined, as well as force (N) and strain (%) at break. The filled gel emulsions with 50 wt.% corn oil and 260 g Bloom gelatin were excluded from the study, because of problems with the molding process, due to its highly viscous nature. Appendix F provides additional information on the instrument settings, data and calculations.

Young's Modulus

Figure 3.5.1 presents Young's modulus (E) for filled gel emulsions with 160 g and 260 g Bloom gelatin (type A and B) and varying amounts of corn oil. The respective values of E (kPa), together with information about the filled gel emulsion, and number of replicates (N) are listed in Table 3.5.1 for 160 g Bloom gelatins and in Table 3.5.2 for 260 g Bloom gelatins. Calculations are shown in appendix F (section F.3.1).

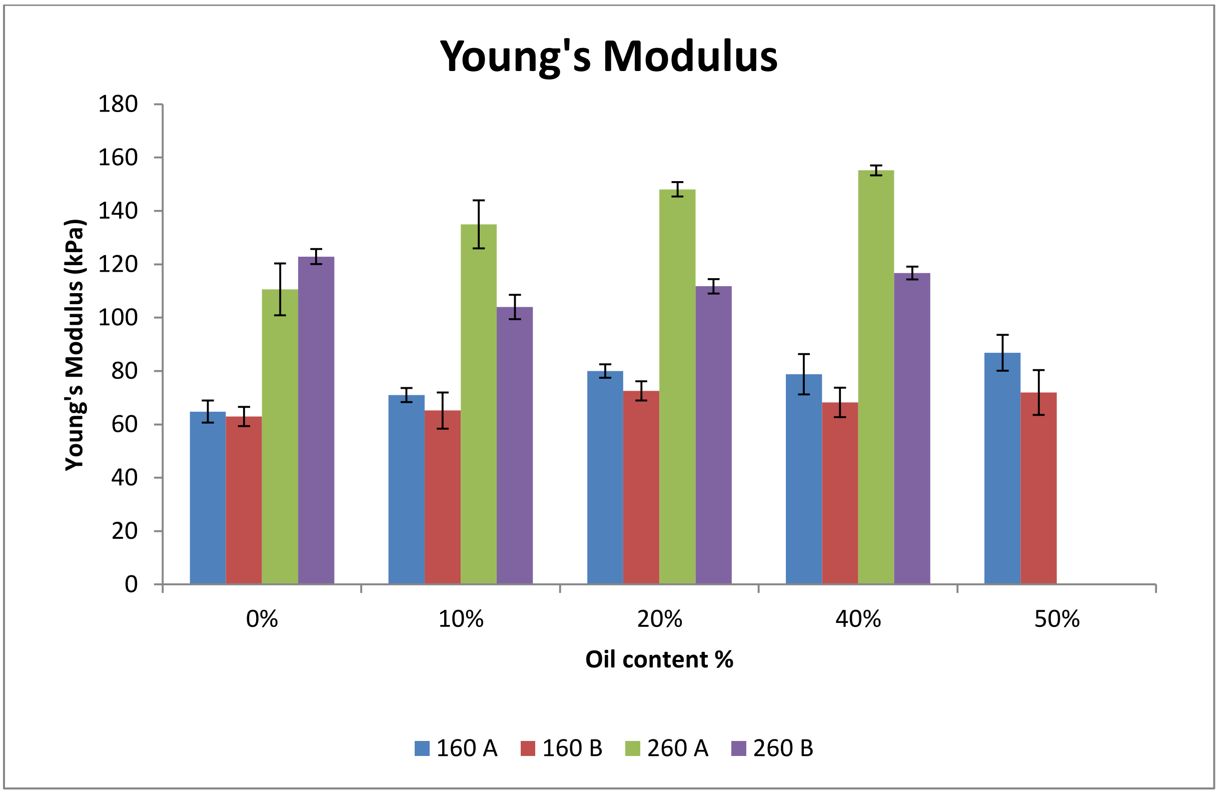

Figure 3.5.1: Young's modulus (E(kPa)) for filled gel emulsions containing 0, 10, 20, 40 and 50 wt.% corn oil and 160 g or 260 g Bloom gelatin type A and B. (Average ± S.D.) The number of replicates are found in Table 3.5.1 and Table 3.5.2.

Table 3.5.1: Young's modulus for filled gel emulsions with 160 g Bloom gelatin, type A and B and 0, 10, 20, 40 and 50 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average E (kPa)	± S.D.	N
A	0	64,8	4,1	13
A	10	71,0	2,6	6
A	20	80,0	2,5	6
A	40	78,8	7,6	6
A	50	86,8	6,7	6
B	0	62,9	3,6	12
B	10	65,2	6,8	6
B	20	72,5	3,6	6
B	40	68,2	5,5	6
B	50	71,9	8,4	6

Table 3.5.2: Young's modulus for filled gel emulsions with 260 g Bloom gelatin, type A and B and 0, 10, 20, and 40 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average E (kPa)	± S.D.	N
A	0	110,6	9,7	6
A	10	135,0	9,0	6
A	20	148,1	2,7	6
A	40	155,2	1,9	4
B	0	122,9	2,8	6
B	10	104,0	4,6	6
B	20	111,8	2,7	6
B	40	116,7	2,4	6

For gelatins with 160 g Bloom, a small increase in Young's modulus was observed with increasing oil content. A much more pronounced increase was observed for the 260 g Bloom gelatins, especially for type A. This implies that the oil content contribute to the rigidity of the gelatin gels. These results also correlates with the results obtained for storage modulus, which also increased with higher weight fraction of oil.

There is a correlation between Young's modulus and the storage modulus, and for ideal elastic solids, this correlation is $E = 3G'$, as described in the introduction.

Table 3.5.3 and Table 3.5.4 gives the values of E and G' for filled gel emulsions with 160 and 260 g Bloom, respectively, for type A or type B gelatin, with varying oil content.

Table 3.5.3: The correlation between storage modulus and Young's modulus for filled gel emulsions with 160 g Bloom gelatin, type A and B, and various amounts of corn oil (0, 10, 20, 40 and 50 wt.%).

Gelatin type and oil wt.%	Average G' (kPa)	Average E (kPa)	E/G'
A, 0 wt.%	11,7	64,8	5,6
A, 10 wt.%	13,1	71,0	5,4
A, 20 wt.%	13,8	80,0	5,8
A, 40 wt.%	17,2	78,8	4,6
A, 50 wt.%	18,8	86,8	4,6
B, 0 wt.%	13,5	62,9	4,7
B, 10 wt.%	13,4	65,2	4,9
B, 20 wt.%	13,1	72,5	5,5
B, 40 wt.%	14,8	68,2	4,6
B, 50 wt.%	15,3	71,9	4,7

Table 3.5.4: The correlation between storage modulus and Young's modulus for filled gel emulsions with 260 g Bloom gelatin, type A and B, and various amounts of corn oil (0, 10, 20 and 40 wt.%).

Gelatin type and oil wt.%	Average G' (kPa)	Average E (kPa)	E/G'
A, 0 wt.%	23,7	110,6	4,7
A, 10 wt.%	25,0	135,0	5,2
A, 20 wt.%	29,0	148,1	5,1
A, 40 wt.%	35,2	155,2	4,4
B, 0 wt.%	22,6	122,9	5,5
B, 10 wt.%	24,6	104,0	4,2
B, 20 wt.%	25,2	111,8	4,4
B, 40 wt.%	25,9	116,7	4,5

The tables show that the values of E was between 4,2 and 5,8 times the value of G' for all the investigated filled gel emulsions. These values are much higher than the claimed value of 3. The gels were stored for a total of 24 hours before measurements. This may have some impact on the measured Young's modulus, due to the fact that gelatin forms non-equilibrium gels, that becomes stronger with time. The shear modulus was measured after 15 minutes, while the Young's modulus was measured after 24 hours. Hence it is not surprising that the Poisson ratio is higher than 3. Other explanations may be that the gels were anisotropic, and probably contained heterotropic regions. There is also differences in how the values of E and G' were obtained; E was obtained by compression of the gel, while

G' was obtained by small strain oscillation measurements. This may have given variation in the Poisson ratio, which causes deviation from the theoretical value of 3.

Force at break

Figure 3.5.2 presents force at break for filled gel emulsions with 160 g and 260 g Bloom gelatin (type A and B) and varying amounts of corn oil. The respective values of force at break (N), together with information about the filled gel emulsion, and number of replicates (N) are listed in Table 3.5.5 for 160 g Bloom gelatins and in Table 3.5.6 for 260 g Bloom gelatins. The relevant data are presented in appendix F.4.

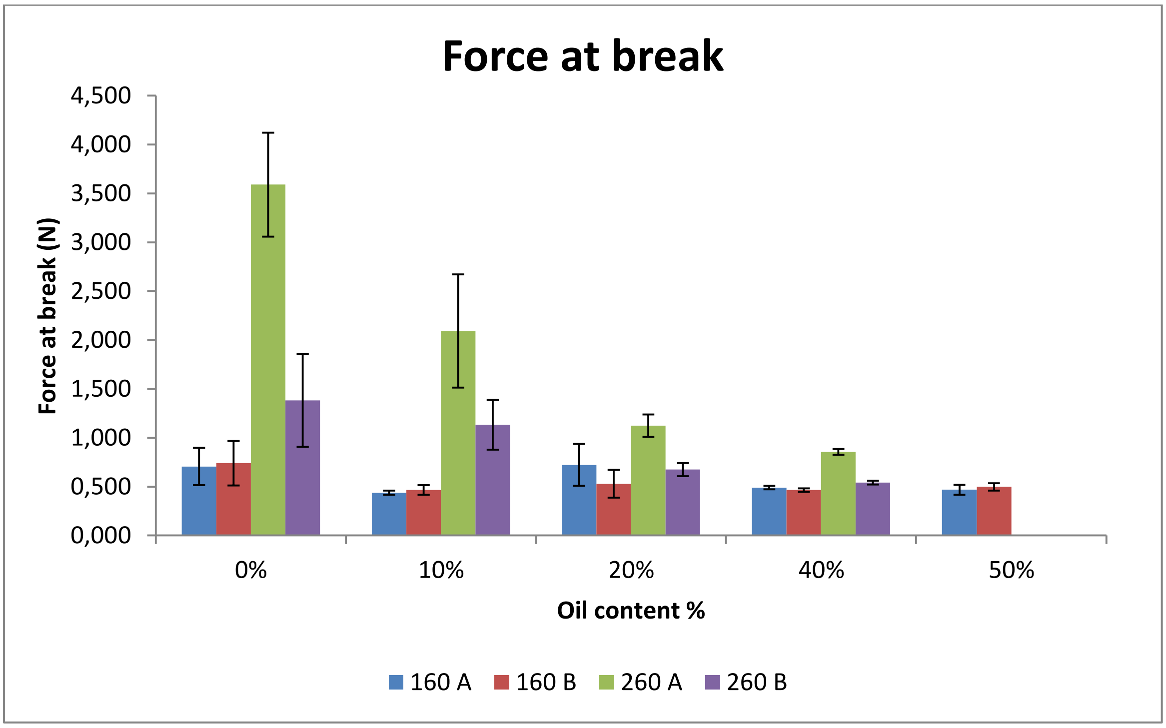

Figure 3.5.2: Force at break (N) for filled gelatin emulsions containing 0, 10, 20, 40 and 50 wt.% corn oil and 160 g or 260 g Bloom gelatin type A and B. (Average ± S.D.) The number of replicates are found in Table 3.5.5 and Table 3.5.6, respectively.

Table 3.5.5: Force at break (N) for filled gel emulsions with 160 g Bloom gelatin, type A and B and 0, 10, 20, 40 and 50 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average force (N)	± S.D.	N
A	0	0,705	0,191	13
A	10	0,437	0,021	6
A	20	0,707	0,237	6
A	40	0,490	0,017	6
A	50	0,468	0,051	6
B	0	0,739	0,227	12
B	10	0,465	0,050	6
B	20	0,529	0,142	6
B	40	0,465	0,018	6
B	50	0,498	0,038	6

Table 3.5.6: Force at break (N) for filled gel emulsions with 260 g Bloom gelatin, type A and B and 0, 10, 20, and 40 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average force (N)	± S.D.	N
A	0	3,590	0,532	6
A	10	2,092	0,578	6
A	20	1,124	0,115	5
A	40	0,854	0,030	4
B	0	1,382	0,475	6
B	10	1,133	0,254	6
B	20	0,674	0,067	6
B	40	0,540	0,020	6

From Figure 3.5.2, there was not observed any specific trends in force at break for the 160 g Bloom gelatins, although there was an overall decrease between the samples containing no corn oil to the samples containing 50 wt.% corn oil. The 260 g Bloom gelatins showed a clear inverse correlation between oil fraction and force at break. According to work by Sala et al. (2009), oil droplets working as active fillers in a gelatin emulsion gel represents structural defects. These defects induces stress concentration when the gel is exposed to deformation. Because of this effect, an increase in the oil fraction of an emulsions will also increase the number of structural defects, and thus the gel will be more susceptible to fracture (Sala, van Vliet, Cohen Stuart, Aken, & van de Velde, 2009). When visually examined, the cylindrical gels seemed brittle and flaky.

Strain at break

Figure 3.5.3 presents strain at break for filled gel emulsions with 160 g and 260 g Bloom gelatin (type A and B) and varying contents of corn oil. The respective values of strain at break (%), together with information about the filled gel emulsions, and number of replicates (N) are listed in Table 3.5.7 for 160 g Bloom gelatins and in Table 3.5.8 for 260 g Bloom gelatins. Calculations are shown in appendix F.3.3, and the relevant data are presented in appendix F.4.

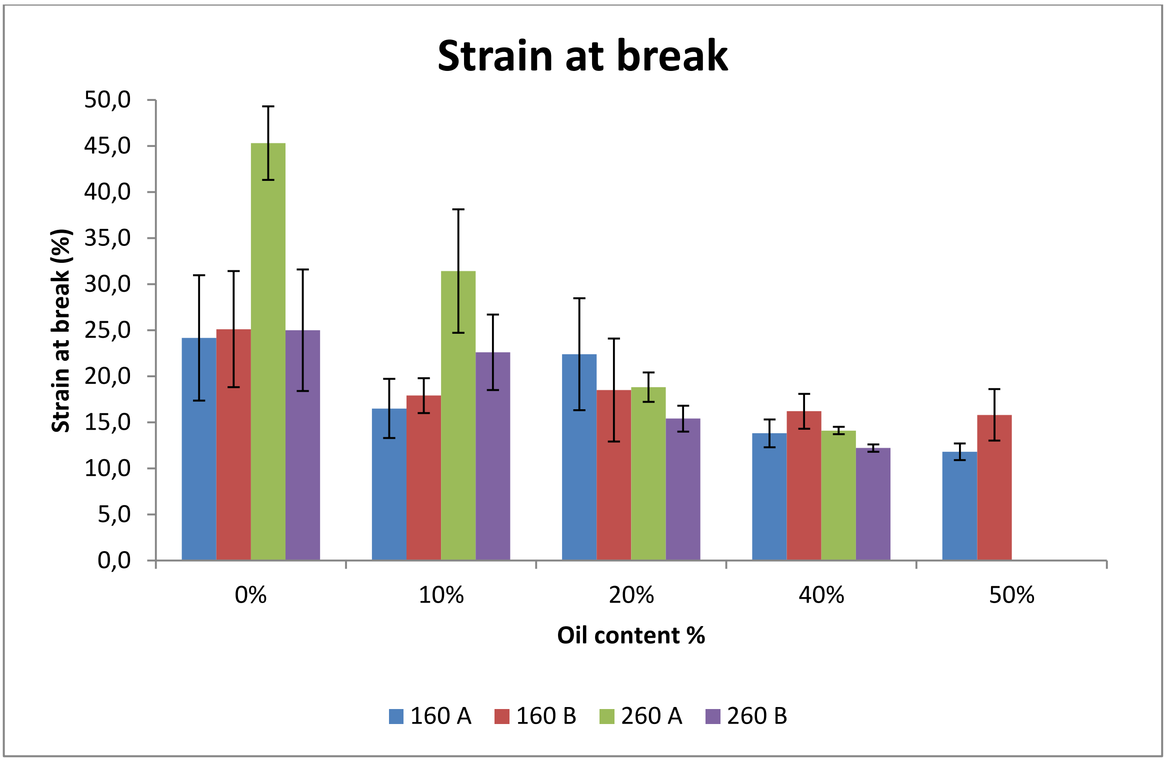

Figure 3.5.3: Strain at break (%) for filled gelatin emulsions containing 0, 10, 20, 40 and 50 wt.% corn oil and 160 g or 260 g Bloom gelatin type A and B. (Average ± S.D.) The number of replicates are found in Table 3.5.7 and Table 3.5.8, respectively.

Table 3.5.7: Strain at break (%) for filled gel emulsions with 160 g Bloom gelatin, type A and B and 0, 10, 20, 40 and 50 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average strain (%)	± S.D.	N
A	0	24,2	6,8	13
A	10	16,5	3,2	6
A	20	21,7	6,5	6
A	40	13,8	1,5	6
A	50	11,8	0,9	6
B	0	25,1	6,3	12
B	10	17,9	1,9	6
B	20	18,5	5,6	6
B	40	16,2	1,9	6
B	50	15,8	2,8	6

Table 3.5.8: Strain at break (%) for filled gel emulsions with 260 g Bloom gelatin, type A and B and 0, 10, 20, 40 and 50 wt.% corn oil (average ± S.D. and number of replicates).

Gelatin type	Oil content (wt.%)	Average strain (%)	± S.D.	N
A	0	45,3	4,0	6
A	10	31,4	6,7	6
A	20	18,8	1,6	6
A	40	14,1	0,4	4
B	0	25,0	6,6	6
B	10	22,6	4,1	6
B	20	15,4	1,4	6
B	40	12,2	0,4	6

As seen in Figure 3.5.3 and Table 3.5.7, the 160 g Bloom gelatins did not exhibit a clear trend for strain at break with varying oil fractions, but it showed that break was obtained at a substantially lower strain % when oil was added to the system, compared to the gels with no oil content. The 260 g Bloom gelatins (Table 3.5.8) showed a clear decrease in strain with increase of the oil fraction. The dispersed phase for the gelatin filled gel emulsion is considered to behave as active fillers, as they are bound to the continuous polymer matrix. When the gel is exposed to deformation, it will break due to a de-bonding between the dispersed droplets and the continuous gel matrix, making the droplets function as propagation zones. Thus, a higher oil fraction will lead to a lower strain at break. This is also dependent on the strength of the gel matrix itself. 260 g Bloom gelatin is considered a strong gelatin, while a 160 g Bloom gelatin is of mediate strength (Gelita, 2013). These results indicates that variation in oil fraction had a lower impact on force and strain at break for weaker gels.

3.6 *In vitro* dissolution studies of filled gel emulsions

In vitro dissolution studies of filled gel emulsions were performed in artificial gastric acid (0,1 M HCl). The studies were performed to determine how gelatin type, temperature and addition of surfactant influenced the dissolution profile of the gelled emulsions. Gels were prepared as described in section 2.3.2, with 260 g Bloom gelatin type A or B, 40 wt.% corn oil and acetaminophen as a marker of dissolution rate. Polysorbate 80 was added to a sample with 260 g Bloom gelatin type A and 40 wt.% corn oil. Measurements were performed at 20 and 37 °C, as described in section 2.3.7. Calculations are shown in appendix A.1, and raw data and standard curves are found in appendix G.1-3.

Figure 3.6.1 shows the dissolution profiles of the different gelled emulsions investigated.

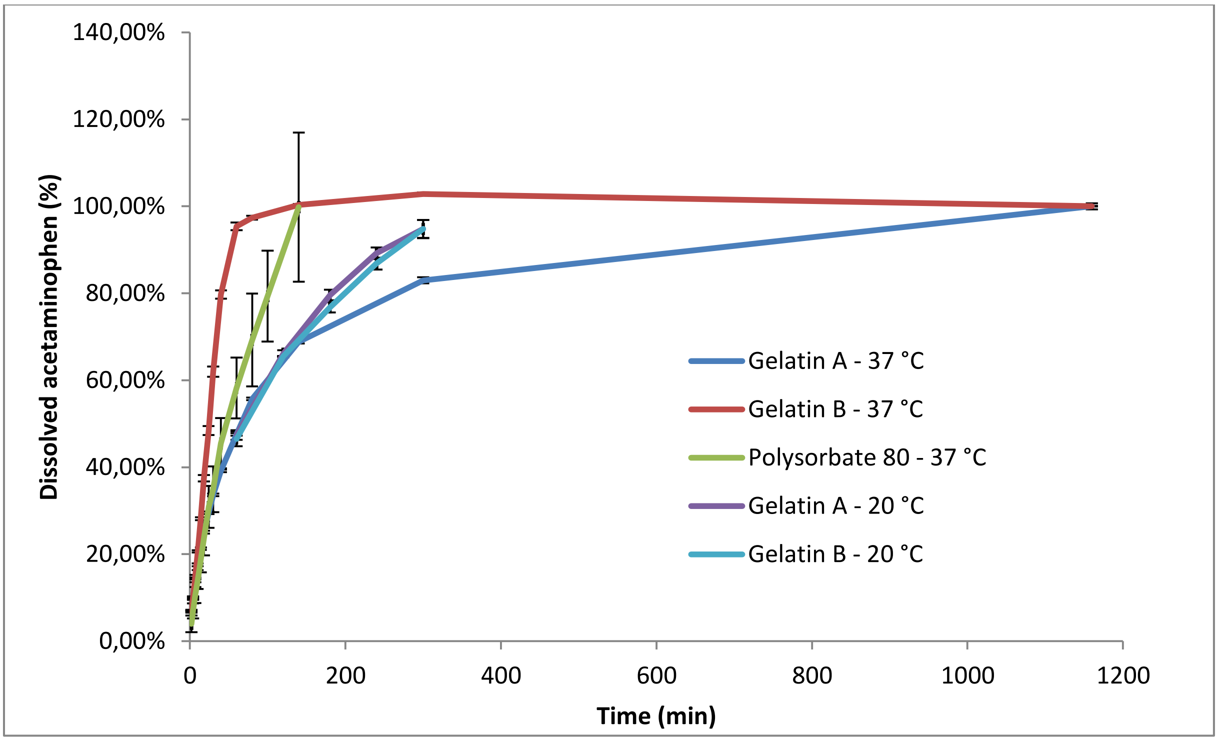

Figure 3.6.1: Dissolution profiles of gelled gelatin emulsions prepared with 260 g Bloom gelatin type A or B, 40 wt.% corn oil, with and without polysorbate 80. N = 6 for samples measured at 37 °C, and N = 2 for the samples measured at 20 °C, ± S.D.

In Figure 3.6.1, a slower dissolution rate is seen for gelatin type A at 37 °C, compared to gelatin type B at 37 °C. However, the dissolution rates are rather similar at the beginning of the measurements, meaning that similar amount of acetaminophen are released into the dissolution medium. This behavior did not correlate well to visual observations during the dissolution studies, in which a much slower dissolution rate was observed for the gels prepared with the acid pretreated gelatin. This may be due to the acetaminophen marker diffusing out of the emulsion gels and into the artificial gastric acid. To test this, similar filled gelled emulsions were prepared, and dissolution studies were performed at 20 °C. This is below the melting point of gelatin, and thus the emulsion matrices would not be dissolved during the measurements. Even though the gels were still intact after the final measurement (300 min), the dissolution profile showed that acetaminophen was released into the artificial gastric acid. These results indicate that there is some diffusion of marker into the dissolution medium at both 20 and 37 °C, as the dissolution profiles are rather similar in the beginning. It would be interesting to perform dissolution studies with the same parameters, but using a marker dissolved in the dispersed oil droplets in stead of the continuous phase, to see if the same effect would occur.

However, the dissolution profile suggested that 260 g Bloom gelatin type A, with an oil fraction of 40 wt.% is more stable in an acidic gastric environment than 260 g gelatin type B with 40 wt.% oil. This correlates with the other results obtained in this study, and indicates that there is some kind of droplet interaction at high oil fractions in gelatin type A, probably due to hydrogen bonding between the polar side groups in asparagine and glutamine and the peptide backbone of gelatin, which increases viscosity, storage modulus, and gelling and melting temperature. The experiments carried out on 260 g Bloom gelatin type A, with 40 wt.% and 0,5 wt.% polysorbate 80 showed that when a surfactant was added at 37 °C, the gelatin type A emulsion gels exhibited a small increase in dissolution rate. The gels were also totally dissolved at the end of the measurements. This effect is probably caused by the surfactant displacing the gelatin network and the gelatin adsorbed onto the dispersed oil droplets, thus making it more vulnerable to an acidic environment. This facilitates the release of acetaminophen, giving a steeper dissolution profile.

4 Concluding remarks

Studies of filled gel emulsion prepared with 160 g and 260 g Bloom gelatins and various amounts of corn oil, showed that Bloom value, gelatin type and oil content all influenced the rheological properties of the emulsion gels. The viscosity, storage modulus and Young's modulus of the emulsion gels were increased upon addition of corn oil, and the increase was more pronounced in gelatins of type A compared to type B, with the largest increase observed in the 260 g Bloom type A gelatin. The corn oil dispersed in the gelatin was considered to work as an active filler, which strengthened the emulsion gels. *In vitro* dissolution studies performed on the filled gel emulsions in an artificial gastric environment showed that gelatin type A had a longer dissolution time than gelatin type B.

The active filler effect obtained when the corn oil fraction of the emulsion gels was increased indicates that it is possible to tailor gelatin gels with varying rheological properties. These special properties of gelatin emulsions makes it possible to utilize them in different pharmaceutical applications for increased bioavailability of drugs and nutraceuticals and better patient compliance.

It would be interesting to perform studies on fish gelatins to investigate if increased oil fractions in a gelled emulsion system would improve its storage modulus, gelling and melting properties, especially in cold water fish gelatin.

Further research could include dissolution studies performed with a marker dissolved in the dispersed phase of the gelled emulsion instead of the continuous phase, which would require a suitable lipid-soluble drug or vitamin as a marker. It could also be interesting to measure the zeta potential of the dispersed oil droplets using a zeta sizer instrument, and to investigate if there are any hydrogen bonding between the gelatin molecules in the emulsion systems. This is possible to measure by using differential scanning calorimetry. It might also be relevant to include a patient questionnaire on the ConCordix system in future thesis work.

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List of Appendices

Appendix A: Various calculations

Appendix A: Various calculations

A.1 Filled gel emulsions containing acetaminophen for dissolution studies

Filled gel emulsions containing acetaminophen as a marker were prepared for the dissolution studies. 1,5 g filled gel emulsion was dissolved in 900 mL 0,1 M hydrochloric acid (HCl). The final concentration of acetaminophen in each gel was 0,1 mM:

$$0{,}1mM = 0{,}1 \cdot 10^{-3} \frac{mol}{L}$$

The acetaminophen concentration was multiplied with the volume of HCl to find the amount of moles:

$$0{,}1 \cdot 10^{-3} \frac{mol}{L} \cdot 0{,}9L = 9 \cdot 10^{-5} mol$$

The mass of acetaminophen in each 1,5 g gel was found by multiplying the number of moles with the molecular weight of acetaminophen:

$$9 \cdot 10^{-5} mol \cdot 151{,}17 \frac{g}{mol} \approx 0{,}01360g$$

20 g filled gel emulsion was prepared, and from this approximately 13 gel samples of 1,5 g could be obtained.

$$\frac{20g}{1{,}5g} = 13{,}33$$

The amount of acetaminophen needed in 20 g filled gel emulsion is calculated below:

$$\frac{20g}{1{,}5g} \cdot 0{,}01360g = 0{,}1813g$$

For a total of 20 g filled gel emulsion, $20{,}00g - 0{,}1813g = 19{,}82g$ emulsion was needed. The ingredients were weighed out and mixed using a spatula, to obtain a homogenous mixture of emulsion and acetaminophen.

When preparing 20 g emulsions containing 0,5 wt.% polysorbate 80, the amount polysorbate needed was:

$$20g \cdot 0,005 = 0,1g$$

For a total of 20 g, 19,82 g emulsion, 0,1813 g acetaminophen and 0,1 g polysorbate 80 was mixed.

A.2 Standard solution of acetaminophen

Acetaminophen (151,17 g/mol) was dissolved in 250 mL HCl to obtain a standard solution used to prepare a standard curve in the dissolution studies. The concentration of the final solution was 1 mM:

$$1mM = 1 \cdot 10^{-3} \frac{mol}{L}$$

The amount of acetaminophen needed was calculated by multiplying the final concentration with the molecular weight of acetaminophen:

$$1 \cdot 10^{-3} \frac{mol}{L} \cdot 151{,}17 \frac{g}{mol} \cdot 0,250L = 0,0378g$$

The standard solution of acetaminophen was then diluted with 0,1 M HCl according to table A.2.1, to create a standard curve with known acetaminophen concentrations for the dissolution studies.

Table A.2.1: Acetaminophen concentrations for standard curves in dissolution studies

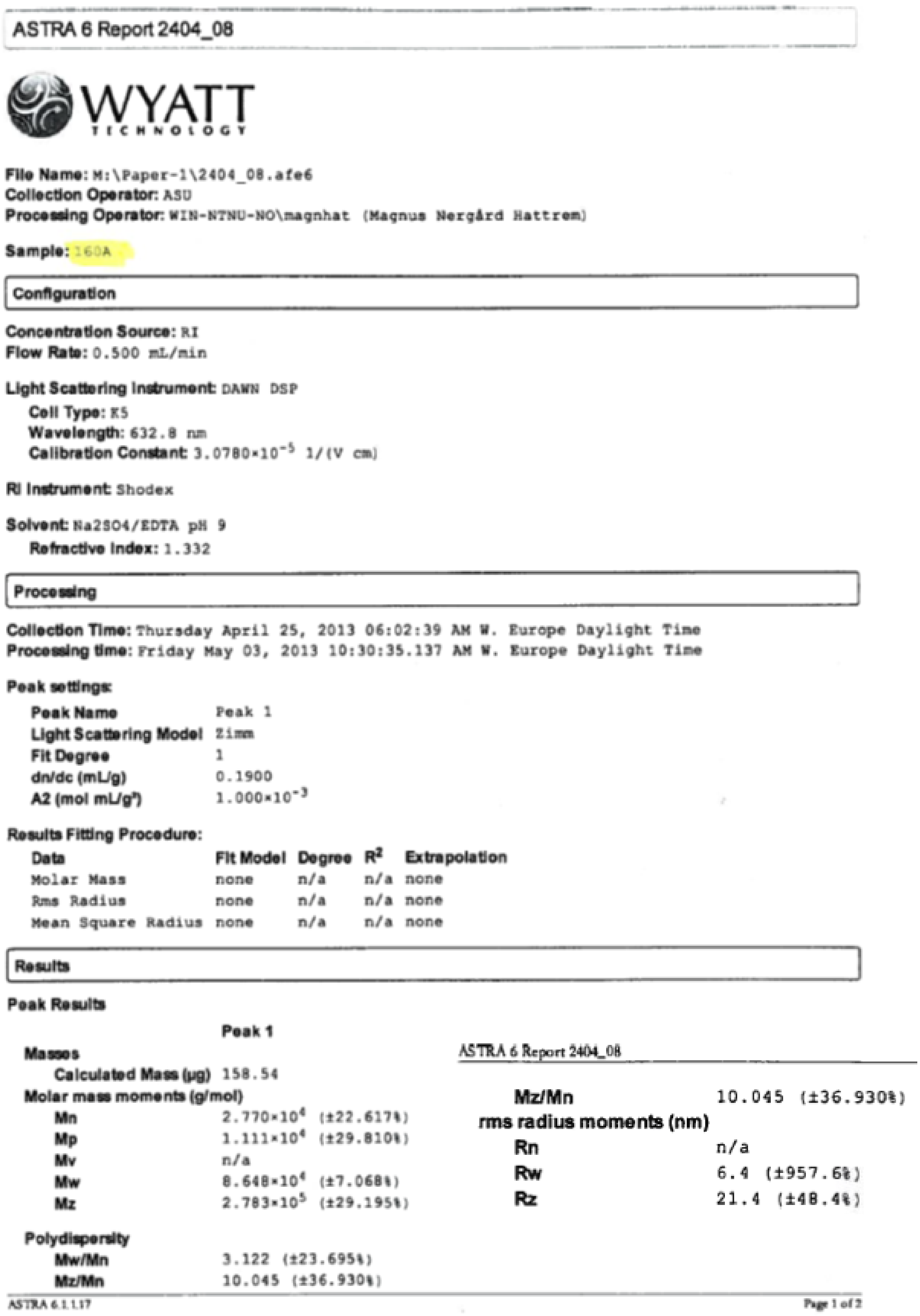
Amount of 1 mM acetaminophen (µL)	Amount of 0,1 M HCl (mL)	Acetaminophen concentration (mM)
50	2,95	0,015
100	2,90	0,030
150	2,85	0,050
200	2,80	0,070
250	2,75	0,080
300	2,70	0,100

Appendix B: SEC-MALLS

SEC-MALLS measurements were performed for 160 g and 260 g Bloom gelatin type A and type B.

Raw data are included on the attached CD.

The summary reports from Astra for the different gelatins are found below.



ASTRA 6 Report 2404_08

WYATT TECHNOLOGY

File Name: `M:\Paper-1\2404_08.afe6`
Collection Operator: ASU
Processing Operator: WIN-NTNU-NO\magnhat (Magnus Nergård Hattrem)

Sample: 160A

Configuration

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN DSP
Cell Type: K5
Wavelength: 632.8 nm
Calibration Constant: 3.0780×10^{-5} 1/(V cm)

RI Instrument: Shodex

Solvent: Na_2SO_4/EDTA pH 9
Refractive Index: 1.332

Processing

Collection Time: Thursday April 25, 2013 06:02:39 AM W. Europe Daylight Time
Processing time: Friday May 03, 2013 10:30:35.137 AM W. Europe Daylight Time

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1900
A2 (mol mL/g²)	1.000×10^{-3}

Results Fitting Procedure:

Data	Fit Model	Degree	R^2	Extrapolation
Molar Mass	none	n/a	n/a	none
Rms Radius	none	n/a	n/a	none
Mean Square Radius	none	n/a	n/a	none

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (µg)	158.54
Molar mass moments (g/mol)	
Mn	2.770×10^{4} (±22.617%)
Mp	1.111×10^{4} (±29.810%)
Mv	n/a
Mw	8.648×10^{4} (±7.068%)
Mz	2.783×10^{5} (±29.195%)
Polydispersity	
Mw/Mn	3.122 (±23.695%)
Mz/Mn	10.045 (±36.930%)

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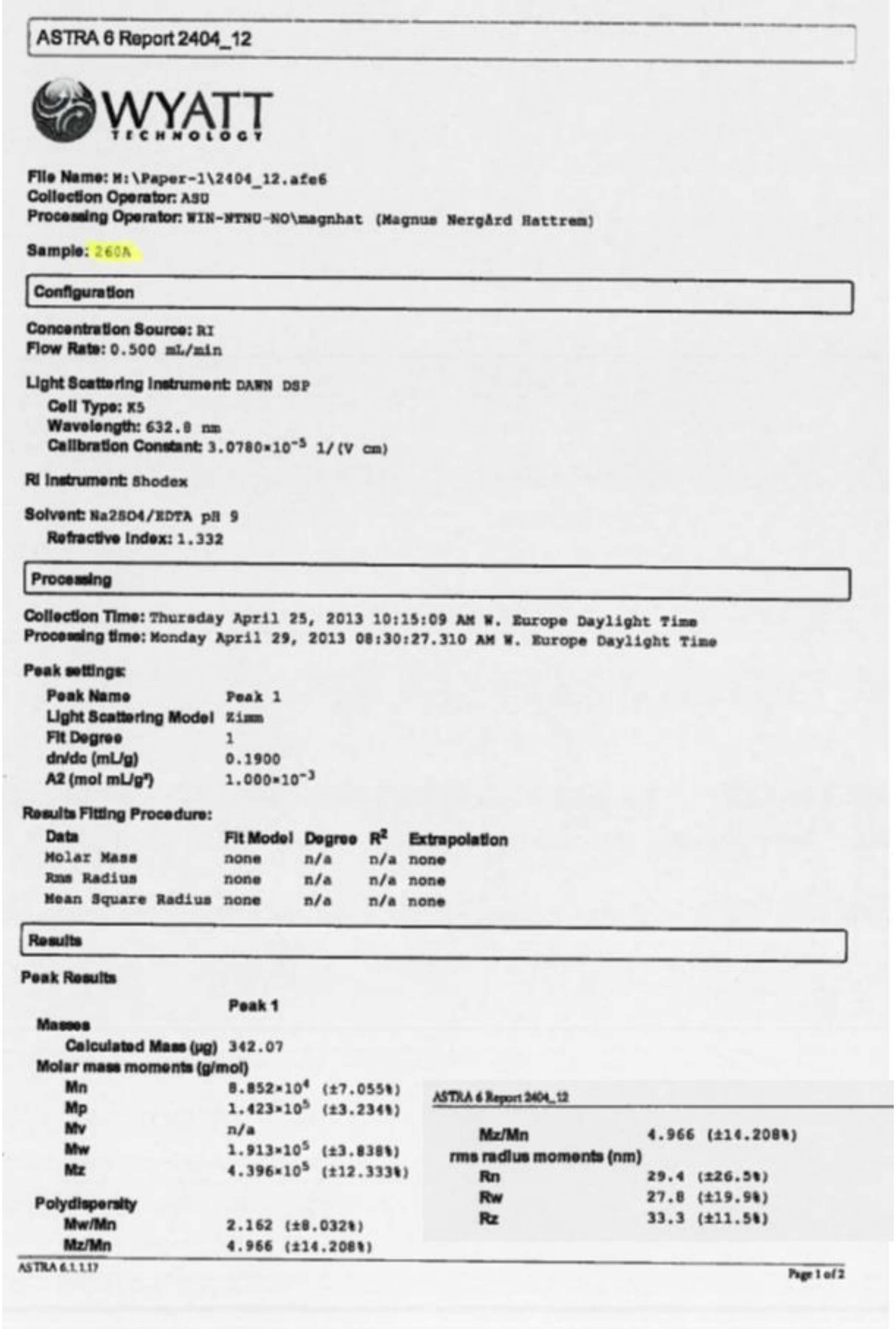
Mz/Mn	10.045 (±36.930%)
rms radius moments (nm)	
Rn	n/a
Rw	6.4 (±957.6%)
Rz	21.4 (±48.4%)



ASTRA 6 Report 2404_12

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Processing Operator: WIN-NTNU-NO\magnhat (Magnus Nergård Hattrem)

Sample: 260A

Configuration

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN DSP
Cell Type: K5
Wavelength: 632.8 nm
Calibration Constant: 3.0780×10^{-5} 1/(V cm)

RI Instrument: Shodex

Solvent: Na2SO4/EDTA pH 9
Refractive Index: 1.332

Processing

Collection Time: Thursday April 25, 2013 10:15:09 AM W. Europe Daylight Time
Processing time: Monday April 29, 2013 08:30:27.310 AM W. Europe Daylight Time

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1900
A2 (mol mL/g²)	1.000×10^{-3}

Results Fitting Procedure:

Data	**Fit Model**	**Degree**	**R²**	**Extrapolation**
Molar Mass	none	n/a	n/a	none
Rms Radius	none	n/a	n/a	none
Mean Square Radius	none	n/a	n/a	none

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (μg)	342.07
Molar mass moments (g/mol)	
Mn	8.852×10^{4} (±7.055%)
Mp	1.423×10^{5} (±3.234%)
Mv	n/a
Mw	1.913×10^{5} (±3.838%)
Mz	4.396×10^{5} (±12.333%)
Polydispersity	
Mw/Mn	2.162 (±8.032%)
Mz/Mn	4.966 (±14.208%)

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Mz/Mn	4.966 (±14.208%)
rms radius moments (nm)	
Rn	29.4 (±26.5%)
Rw	27.8 (±19.9%)
Rz	33.3 (±11.5%)

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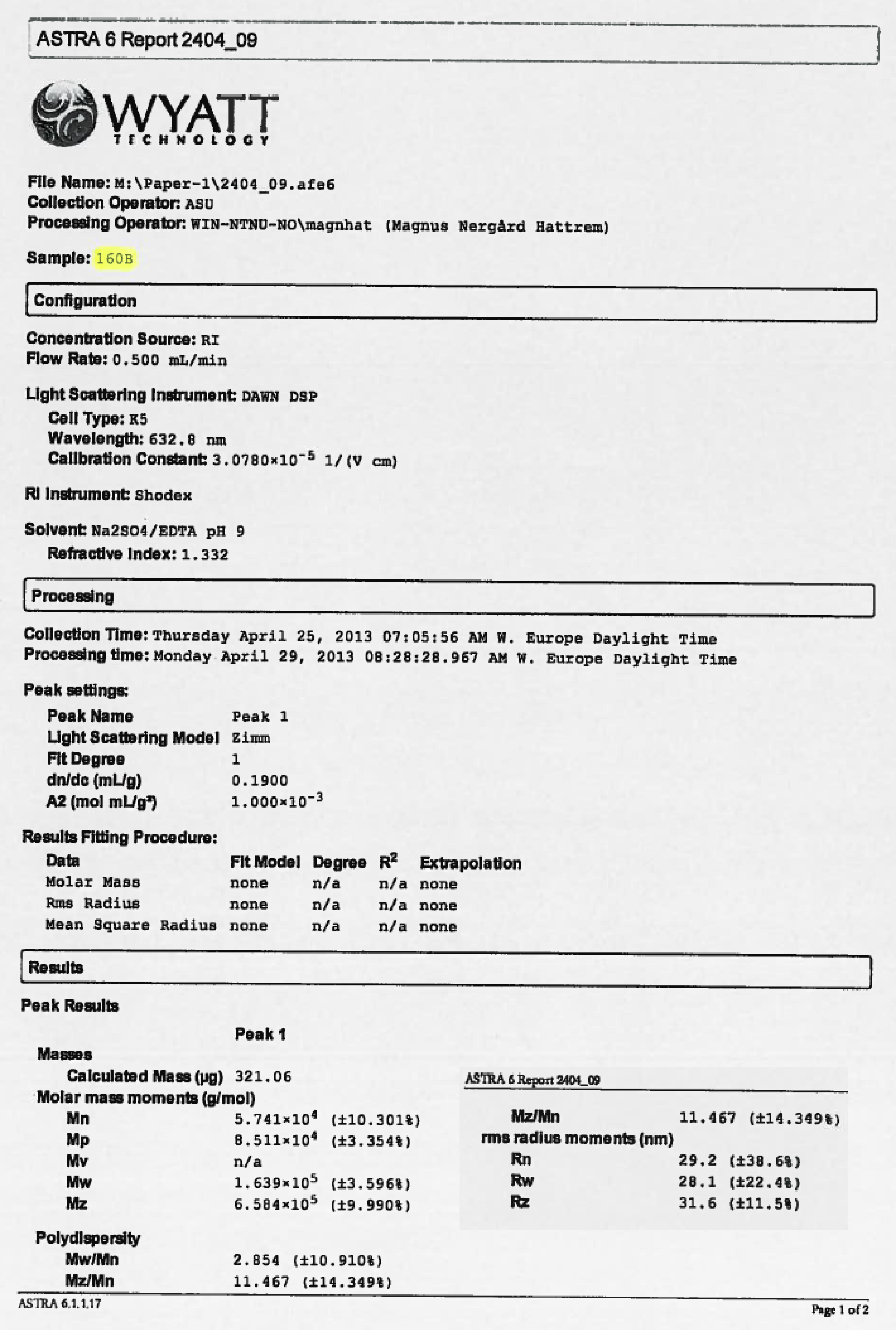

ASTRA 6 Report 2404_09

WYATT TECHNOLOGY

File Name: M:\Paper-1\2404_09.afe6
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Processing Operator: WIN-NTNU-NO\magnhat (Magnus Nergård Hattrem)

Sample: 160B

Configuration

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN DSP
Cell Type: K5
Wavelength: 632.8 nm
Calibration Constant: 3.0780×10^{-5} 1/(V cm)

RI Instrument: Shodex

Solvent: Na2SO4/EDTA pH 9
Refractive Index: 1.332

Processing

Collection Time: Thursday April 25, 2013 07:05:56 AM W. Europe Daylight Time
Processing time: Monday April 29, 2013 08:28:28.967 AM W. Europe Daylight Time

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1900
A2 (mol mL/g²)	1.000×10^{-3}

Results Fitting Procedure:

Data	Fit Model	Degree	R²	Extrapolation
Molar Mass	none	n/a	n/a	none
Rms Radius	none	n/a	n/a	none
Mean Square Radius	none	n/a	n/a	none

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (µg)	321.06
Molar mass moments (g/mol)	
Mn	5.741×10^{4} (±10.301%)
Mp	8.511×10^{4} (±3.354%)
Mv	n/a
Mw	1.639×10^{5} (±3.596%)
Mz	6.584×10^{5} (±9.990%)
Polydispersity	
Mw/Mn	2.854 (±10.910%)
Mz/Mn	11.467 (±14.349%)

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Mz/Mn	11.467 (±14.349%)
rms radius moments (nm)	
Rn	29.2 (±38.6%)
Rw	28.1 (±22.4%)
Rz	31.6 (±11.5%)

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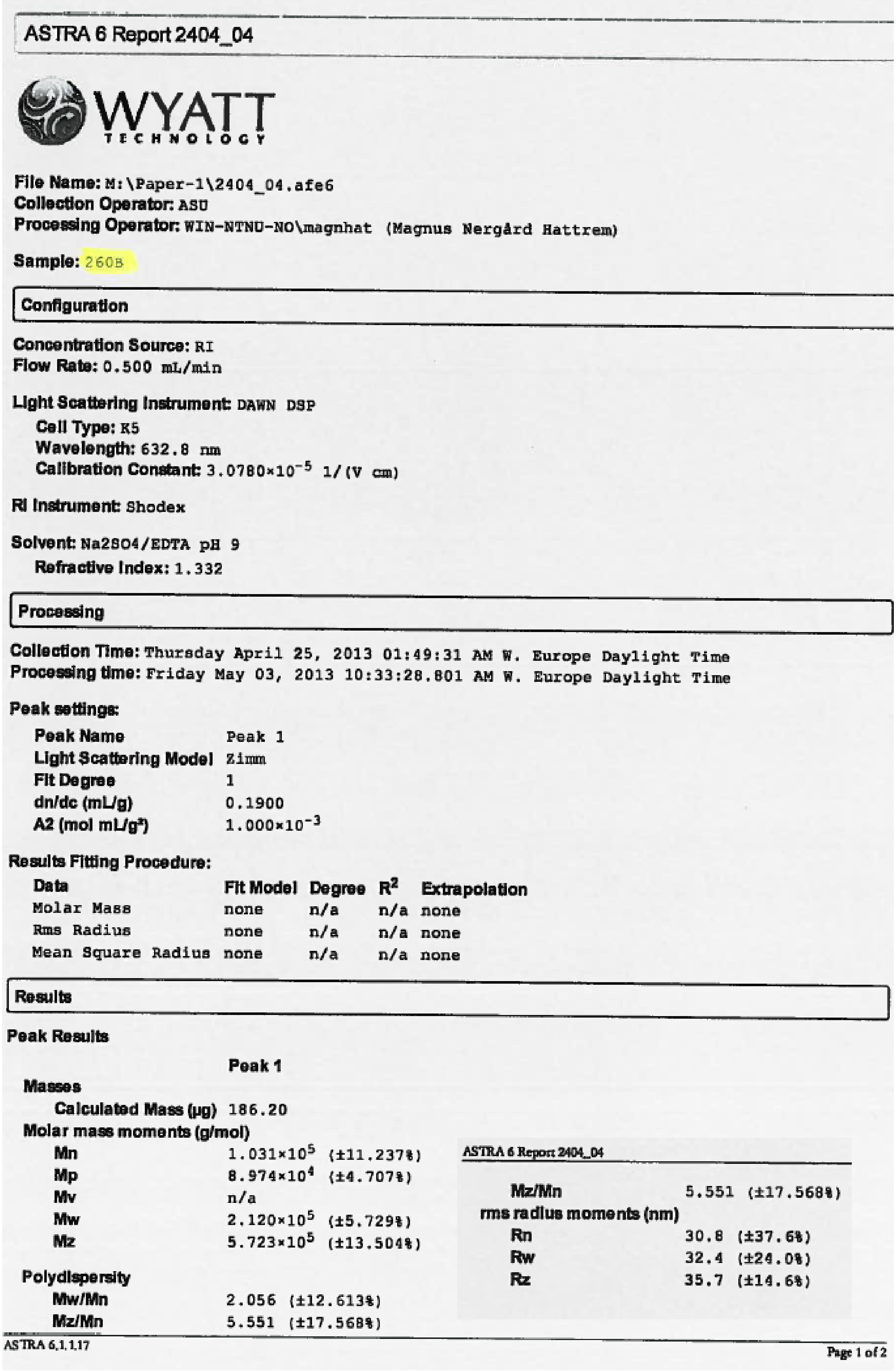

ASTRA 6 Report 2404_04

File Name: M:\Paper-1\2404_04.afe6
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Processing Operator: WIN-NTNU-NO\magnhat (Magnus Nergård Hattrem)

Sample: 260B

Configuration

Concentration Source: RI
Flow Rate: 0.500 mL/min

Light Scattering Instrument: DAWN DSP
Cell Type: K5
Wavelength: 632.8 nm
Calibration Constant: 3.0780×10^{-5} 1/(V cm)

RI Instrument: Shodex

Solvent: Na2SO4/EDTA pH 9
Refractive Index: 1.332

Processing

Collection Time: Thursday April 25, 2013 01:49:31 AM W. Europe Daylight Time
Processing time: Friday May 03, 2013 10:33:28.801 AM W. Europe Daylight Time

Peak settings:

Peak Name	Peak 1
Light Scattering Model	Zimm
Fit Degree	1
dn/dc (mL/g)	0.1900
A2 (mol mL/g²)	1.000×10^{-3}

Results Fitting Procedure:

Data	Fit Model	Degree	R^2	Extrapolation
Molar Mass	none	n/a	n/a	none
Rms Radius	none	n/a	n/a	none
Mean Square Radius	none	n/a	n/a	none

Results

Peak Results

	Peak 1
Masses	
Calculated Mass (µg)	186.20
Molar mass moments (g/mol)	
Mn	1.031×10^{5} (±11.237%)
Mp	8.974×10^{4} (±4.707%)
Mv	n/a
Mw	2.120×10^{5} (±5.729%)
Mz	5.723×10^{5} (±13.504%)
Polydispersity	
Mw/Mn	2.056 (±12.613%)
Mz/Mn	5.551 (±17.568%)

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Mz/Mn	5.551 (±17.568%)
rms radius moments (nm)	
Rn	30.8 (±37.6%)
Rw	32.4 (±24.0%)
Rz	35.7 (±14.6%)

Appendix C: Droplet size determination of filled gel emulsions

The raw data from analysis of filled gel emulsions by Mastersizer 3000 are provided in table C.1 – C.3.

Table C.1: Different droplet size classes (µm) obtained from analysis by Mastersizer 3000 of diluted filled gel emulsions with gelatin 160g Bloom (either type A or B) and different amounts of corn oil (10, 20, 40 and 50 wt.%). The amount in each size class is given as a percentage of the total amounts of droplets.

Size classes (µm)	**Results %**							
	10 wt.% Type A	10 wt.% Type B	20 wt.% Type A	20 wt.% Type B	40 wt.% Type A	40 wt.% Type B	50 wt.% Type A	50 wt.% Type B
0,0995	0,00	0,00	0,00	0,01	0,00	0,00	0,00	0,00
0,113	0,00	0,00	0,00	0,03	0,00	0,00	0,00	0,00
0,128	0,00	0,00	0,00	0,07	0,00	0,00	0,00	0,00
0,146	0,06	0,01	0,00	0,15	0,00	0,05	0,09	0,10
0,166	0,14	0,12	0,03	0,27	0,06	0,13	0,20	0,18
0,188	0,24	0,29	0,13	0,51	0,21	0,31	0,29	0,36
0,214	0,43	0,54	0,24	0,82	0,35	0,59	0,55	0,66
0,243	0,66	0,87	0,36	1,19	0,59	0,94	0,88	1,06
0,276	0,91	1,24	0,59	1,57	0,88	1,33	1,26	1,50
0,314	1,15	1,64	0,86	1,91	1,19	1,70	1,64	1,93
0,357	1,38	2,03	1,18	2,18	1,51	2,01	1,99	2,30
0,405	1,61	2,37	1,58	2,36	1,85	2,22	2,30	2,07
0,46	1,90	2,69	2,09	2,48	2,25	2,33	2,57	2,24
0,523	2,35	3,06	2,82	2,61	2,79	2,41	2,90	2,43
0,594	3,13	3,58	3,88	2,92	3,60	2,55	3,39	2,74
0,675	4,38	4,41	5,38	3,56	4,80	2,93	4,21	3,29
0,767	6,20	5,68	7,33	4,70	6,45	3,70	5,45	4,18
0,872	8,51	7,40	9,53	6,40	8,45	5,01	7,11	5,46
0,991	10,89	9,34	11,54	8,46	10,48	6,81	8,96	7,07
1,13	12,71	11,04	12,73	10,44	12,04	8,87	10,55	8,76
1,28	13,24	11,89	12,53	11,68	12,53	10,68	11,35	10,17
1,45	12,07	11,39	10,76	11,61	11,54	11,65	10,94	10,87
1,65	9,41	9,48	7,81	10,02	9,18	11,33	9,30	10,55
1,88	6,03	6,63	4,55	7,30	6,07	9,66	6,85	9,16
2,13	2,64	3,60	1,55	4,27	2,99	7,06	4,16	6,99
2,42	0,01	0,70	0,23	1,37	0,29	4,23	2,05	4,49
2,75	0,00	0,00	0,00	0,36	0,00	1,45	1,93	2,84
3,12	0,00	0,00	0,00	0,00	0,00	0,40	1,92	1,47
3,55	0,00	0,00	0,00	0,00	0,00	0,00	0,23	0,01

Table C.2: Different droplet size classes (µm) obtained from analysis by Mastersizer 3000 of diluted filled gel emulsions with gelatin 260g Bloom (either type A or B) and different amounts of corn oil (10, 20, 40 and 50 wt.%). The amount in each size class is given as a percentage of the total amounts of droplets.

Size classes (µm)	**Results %**							
	10 wt.% Type A	10 wt.% Type B	20 wt.% Type A	20 wt.% Type B	40 wt.% Type A	40 wt.% Type B	50 wt.% Type A	50 wt.% Type B
0,0995	0,00	0,00	0,00	0,06	0,00	0,00	0,00	0,00
0,113	0,00	0,08	0,08	0,10	0,08	0,08	0,10	0,00
0,128	0,11	0,22	0,23	0,26	0,25	0,19	0,26	0,13
0,146	0,30	0,44	0,49	0,49	0,55	0,41	0,57	0,35
0,166	0,59	0,75	0,85	0,80	0,96	0,73	1,01	0,68
0,188	0,99	1,12	1,31	1,17	1,49	1,15	1,57	1,13
0,214	1,46	1,53	1,81	1,56	2,08	1,62	2,22	1,67
0,243	1,97	1,93	2,33	1,95	2,69	2,12	2,90	2,27
0,276	2,47	2,28	2,82	2,28	3,27	2,59	3,56	2,87
0,314	2,89	2,55	3,21	2,53	3,76	3,01	4,12	3,44
0,357	3,19	2,69	3,45	2,65	4,11	3,31	4,54	3,90
0,405	3,33	2,69	3,54	2,65	4,30	3,49	4,77	4,23
0,46	3,33	2,60	3,49	2,55	4,36	3,56	4,82	4,41
0,523	3,25	2,50	3,41	2,48	4,37	3,59	4,76	4,48
0,594	3,25	2,55	3,43	2,57	4,46	3,70	4,69	4,50
0,675	3,49	2,95	3,74	3,03	4,80	4,06	4,75	4,60
0,767	4,19	3,88	4,53	4,05	5,47	4,78	5,06	4,86
0,872	5,42	5,46	5,85	5,68	6,45	5,90	5,64	5,34
0,991	7,09	7,54	7,54	7,80	7,54	7,29	6,40	6,00
1,13	8,84	9,70	9,15	9,91	8,33	8,60	7,07	6,67
1,28	10,07	11,25	10,07	11,33	8,42	9,40	7,37	7,15
1,45	10,21	11,52	9,78	11,42	7,61	9,31	7,07	7,22
1,65	9,00	10,20	8,18	9,95	6,01	8,21	6,16	6,78
1,88	6,70	7,59	5,70	7,28	4,05	6,34	4,77	5,83
2,13	4,01	4,52	3,10	4,25	2,05	4,15	3,21	4,53
2,42	1,44	1,46	0,55	1,22	1,26	1,99	1,80	3,13
2,75	0,49	0,00	0,04	0,00	1,57	0,71	0,72	1,89
3,12	0,00	0,00	0,00	0,00	0,65	0,00	0,28	0,97
3,55	0,00	0,00	0,00	0,00	0,17	0,00	0,08	0,40
4,03	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,40
4,58	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,07

Table C.3: Different droplet size classes (µm) obtained from analysis by Mastersizer 3000 of diluted filled gel emulsions with gelatin 260g Bloom (either type A or B), 40 wt.% corn oil and either 0,1 M NaCl, pH 4, pH 8 or 0,5 wt.% polysorbate 80 (Tween 80). The amount in each size class is given as a percentage of the total amounts of droplets.

Size classes (µm)	**Results %**							
	Type A NaCl	Type B NaCl	Type A Tween80	Type B Tween80	Type A pH 4	Type B pH 4	Type A pH 8	Type B pH 8
0,0995	0,00	0,00	0,00	0,00	0,00	0,00	0,05	0,08
0,113	0,00	0,00	0,00	0,00	0,02	0,05	0,22	0,23
0,128	0,12	0,00	0,00	0,00	0,18	0,23	0,49	0,44
0,146	0,34	0,16	0,00	0,00	0,44	0,48	0,86	0,72
0,166	0,71	0,40	0,00	0,00	0,83	0,83	1,34	1,05
0,188	1,22	0,76	0,00	0,00	1,35	1,27	1,90	1,45
0,214	1,84	1,22	0,13	0,00	1,98	1,77	2,50	1,87
0,243	2,52	1,74	0,37	0,11	2,67	2,30	3,11	2,32
0,276	3,20	2,29	0,74	0,31	3,39	2,82	3,70	2,77
0,314	3,80	2,78	1,19	0,61	4,08	3,28	4,20	3,20
0,357	4,25	3,19	1,65	0,94	4,70	3,66	4,60	3,61
0,405	4,53	3,47	2,03	1,25	5,19	3,93	4,87	3,96
0,46	4,66	3,65	2,27	1,47	5,58	4,14	5,03	4,30
0,523	4,72	3,80	2,34	1,56	5,88	4,34	5,16	4,65
0,594	4,85	4,05	2,26	1,53	6,17	4,66	5,36	5,11
0,675	5,23	4,55	2,10	1,43	6,52	5,20	5,73	5,73
0,767	5,97	5,43	1,96	1,34	6,95	6,05	6,32	6,53
0,872	7,05	6,68	1,90	1,32	7,41	7,13	7,04	7,44
0,991	8,23	8,12	1,96	1,40	7,71	8,23	7,66	8,24
1,13	9,05	9,34	2,06	1,55	7,61	8,95	7,86	8,61
1,28	9,02	9,90	2,12	1,69	6,95	8,94	7,35	8,31
1,45	7,92	9,45	2,05	1,80	5,74	8,01	6,13	7,26
1,65	5,92	7,99	1,87	1,88	4,20	6,32	4,44	5,64
1,88	3,58	5,85	1,67	2,02	2,62	4,27	2,68	3,77
2,13	1,25	3,58	1,65	2,38	1,19	2,33	1,22	2,05
2,42	0,00	1,60	2,04	3,11	0,47	0,70	0,18	0,64
2,75	0,00	0,00	2,95	4,23	0,15	0,11	0,00	0,01
3,12	0,00	0,00	4,31	5,65	0,00	0,00	0,00	0,00
3,55	0,00	0,00	5,84	7,09	0,00	0,00	0,00	0,00
4,03	0,00	0,00	7,21	8,23	0,00	0,00	0,00	0,00
4,58	0,00	0,00	8,10	8,85	0,00	0,00	0,00	0,00
5,21	0,00	0,00	8,34	8,85	0,00	0,00	0,00	0,00
5,92	0,00	0,00	7,91	8,22	0,00	0,00	0,00	0,00
6,72	0,00	0,00	6,89	7,06	0,00	0,00	0,00	0,00

7,64	0,00	0,00	5,43	5,53	0,00	0,00	0,00	0,00
8,68	0,00	0,00	3,79	3,86	0,00	0,00	0,00	0,00
9,86	0,00	0,00	2,19	2,32	0,00	0,00	0,00	0,00
11,2	0,00	0,00	0,89	1,12	0,00	0,00	0,00	0,00
12,7	0,00	0,00	0,26	0,06	0,00	0,00	0,00	0,00

Appendix D: Viscosity measurements

Information on the viscosity of the filled gel emulsions with 160 g and 260 g Bloom gelatin type A and B, containing 0, 10, 20, 40 or 50 wt. % corn oil, were obtained from continuous shear measurements at 60°C, performed with a rheometer (StressTech, Sweden). Table D1.1-D1.10 and D2.1-D2-10 gives the viscosity (Pa s) at various shear rates (1/s) for the different filled gel emulsion systems that were investigated.

D.1. Shear viscosity for filled gel emulsions and gels with 160 g Bloom gelatin

Table D1.1: Shear viscosity for gels with gelatin 160 g Bloom, type A and 0 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,1813	0,2440	0,2665	0,2601	0,2491	0,2402	0,0341
0,80	0,1746	0,2473	0,2206	0,2284	0,2314	0,2205	0,0274
1,00	0,1667	0,2358	0,2022	0,1999	0,2119	0,2033	0,0249
2,00	0,1242	0,1815	0,1686	0,1711	0,1773	0,1645	0,0231
4,00	0,1075	0,1615	0,1457	0,1518	0,1598	0,1453	0,0220
7,00	0,1018	0,1514	0,1334	0,1386	0,1482	0,1347	0,0198
10,00	0,0958	0,1467	0,1283	0,1331	0,1436	0,1295	0,0203
20,00	0,0912	0,1424	0,1224	0,1253	0,1371	0,1237	0,0200
40,00	0,0883	0,1356	0,1169	0,1226	0,1346	0,1196	0,0192
60,00	0,0872	0,1312	0,1144	0,1239	0,1347	0,1183	0,0190
80,00	0,0867	0,1276	0,1122	0,1284	0,1377	0,1185	0,0200
100,00	0,0865	0,1253	0,1112	0,1293	0,1384	0,1181	0,0202

Table D1.2: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type A and 10 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,2520	0,2340	0,1875	0,1549	0,1932	0,2043	0,0387
0,80	0,2361	0,2481	0,1921	0,1748	0,2014	0,2105	0,0307
1,00	0,2319	0,2199	0,1926	0,1757	0,1887	0,2018	0,0233
2,00	0,2203	0,2139	0,1891	0,1525	0,1922	0,1936	0,0266
4,00	0,2166	0,2108	0,1821	0,1500	0,1867	0,1892	0,0265
7,00	0,2110	0,2033	0,1730	0,1491	0,1837	0,1840	0,0247
10,00	0,2075	0,2051	0,1687	0,1459	0,1818	0,1818	0,0258
20,00	0,1954	0,1956	0,1605	0,1418	0,1714	0,1729	0,0232
40,00	0,1816	0,1811	0,1553	0,1381	0,1622	0,1637	0,0184
60,00	0,1729	0,1723	0,1530	0,1364	0,1558	0,1581	0,0152
80,00	0,1664	0,1661	0,1523	0,1349	0,1528	0,1545	0,0129
100,00	0,1612	0,1598	0,1509	0,1339	0,1498	0,1511	0,0109

Table D1.3: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type A and 20 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3906	0,3341	0,2866	0,2333	0,2926	0,3074	0,0587
0,80	0,4117	0,3167	0,2802	0,2390	0,2993	0,3094	0,0641
1,00	0,3939	0,3099	0,2598	0,2262	0,2875	0,2955	0,0633
2,00	0,3858	0,3100	0,2592	0,2083	0,2754	0,2877	0,0659
4,00	0,3777	0,3073	0,2503	0,2037	0,2669	0,2812	0,0655
7,00	0,3652	0,2978	0,2402	0,2028	0,2650	0,2742	0,0616
10,00	0,3489	0,2964	0,2392	0,2019	0,2647	0,2702	0,0560
20,00	0,3089	0,2880	0,2288	0,1961	0,2500	0,2544	0,0452
40,00	0,2744	0,2738	0,2204	0,1916	0,2334	0,2387	0,0357
60,00	0,2602	0,2672	0,2153	0,1893	0,2248	0,2314	0,0324
80,00	0,2522	0,2626	0,2124	0,1879	0,2194	0,2269	0,0304
100,00	0,2508	0,2598	0,2103	0,1874	0,2163	0,2249	0,0299

Table D1.4: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type A and 40 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	1,0950	0,8895	1,3820	1,5340	1,4150	1,2631	0,2638
0,80	1,1210	0,9021	1,3820	1,5470	1,4320	1,2768	0,2612
1,00	1,1300	0,8976	1,3700	1,5400	1,4430	1,2761	0,2603
2,00	1,0950	0,8926	1,3240	1,5060	1,4190	1,2473	0,2506
4,00	1,0530	0,8728	1,2410	1,4130	1,3560	1,1872	0,2232
7,00	0,9975	0,8342	1,1550	1,2980	1,2640	1,1097	0,1935
10,00	0,9564	0,8120	1,1000	1,2360	1,1980	1,0605	0,1760
20,00	0,8912	0,7689	1,0220	1,1330	1,1130	0,9856	0,1542
40,00	0,8287	0,7261	0,9425	1,0470	1,0380	0,9165	0,1382
60,00	0,7935	0,7061	0,9127	1,0050	1,0030	0,8841	0,1318
80,00	0,7779	0,6919	0,8893	0,9829	0,9781	0,8640	0,1273
100,00	0,7651	0,6824	0,8725	0,9616	0,9571	0,8477	0,1222

Table D1.5: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type A and 50 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	3,4040	6,5730	4,2140	2,7090	4,8000	4,3400	1,4790
0,80	3,3680	6,3200	4,1560	2,7000	4,7450	4,2578	1,3891
1,00	3,2830	6,0630	4,0700	2,6760	4,6110	4,1406	1,3041
2,00	2,9030	5,1020	3,5460	2,4510	4,0050	3,6014	1,0282
4,00	2,5360	4,3170	3,0780	2,2350	3,4550	3,1242	0,8172
7,00	2,2850	3,7600	2,7570	2,0740	3,1090	2,7970	0,6729
10,00	2,1600	3,4930	2,5740	1,9790	2,9200	2,6252	0,6076
20,00	1,9260	3,0010	2,2810	1,8130	2,6010	2,3244	0,4890
40,00	1,7090	2,6400	2,0260	1,6510	2,3120	2,0676	0,4157
60,00	1,5970	2,4410	1,8940	1,5640	2,1630	1,9318	0,3747
80,00	1,5230	2,3000	1,8050	1,5050	2,0550	1,8376	0,3434
100,00	1,4640	2,1900	1,7370	1,4620	1,9750	1,7656	0,3193

Table D1.6: Shear viscosity for gels with gelatin 160 g Bloom, type B and 0 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,2981	0,2448	0,2986	0,3189	0,3070	0,2935	0,0285
0,80	0,2939	0,2446	0,2735	0,3046	0,2913	0,2816	0,0235
1,00	0,2848	0,2389	0,2449	0,2791	0,2705	0,2636	0,0206
2,00	0,2545	0,1981	0,2249	0,2449	0,2386	0,2322	0,0219
4,00	0,2390	0,1827	0,2072	0,2245	0,2210	0,2149	0,0213
7,00	0,2338	0,1764	0,1964	0,2147	0,2109	0,2064	0,0214
10,00	0,2280	0,1723	0,1938	0,2121	0,2081	0,2029	0,0210
20,00	0,2242	0,1676	0,1882	0,2048	0,2011	0,1972	0,0210
40,00	0,2206	0,1648	0,1845	0,1981	0,1959	0,1928	0,0204
60,00	0,2192	0,1635	0,1820	0,1952	0,1930	0,1906	0,0203
80,00	0,2199	0,1629	0,1806	0,1937	0,1909	0,1896	0,0208
100,00	0,2204	0,1626	0,1790	0,1927	0,1896	0,1889	0,0212

Table D1.7: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type B and 10 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,2674	0,2097	0,2830	0,2092	0,2797	0,2498	0,0373
0,80	0,2584	0,2118	0,2786	0,2195	0,2736	0,2484	0,0309
1,00	0,2384	0,2080	0,2717	0,2317	0,2663	0,2432	0,0262
2,00	0,2443	0,1949	0,2689	0,2140	0,2626	0,2369	0,0317
4,00	0,2429	0,1948	0,2632	0,2144	0,2556	0,2342	0,0288
7,00	0,2396	0,1947	0,2578	0,2148	0,2523	0,2318	0,0266
10,00	0,2413	0,1956	0,2554	0,2118	0,2453	0,2299	0,0251
20,00	0,2402	0,1930	0,2507	0,2089	0,2349	0,2255	0,0238
40,00	0,2389	0,1911	0,2485	0,2061	0,2277	0,2225	0,0236
60,00	0,2386	0,1904	0,2477	0,2046	0,2232	0,2209	0,0236
80,00	0,2390	0,1902	0,2475	0,2039	0,2198	0,2201	0,0238
100,00	0,2396	0,1903	0,2483	0,2037	0,2202	0,2204	0,0241

Table D1.8: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type B and 20 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3499	0,3720	0,4602	0,3313	0,4092	0,3845	0,0513
0,80	0,3639	0,3626	0,4707	0,3289	0,3879	0,3828	0,0534
1,00	0,3643	0,3617	0,4792	0,3278	0,3744	0,3815	0,0574
2,00	0,3449	0,3566	0,4662	0,3112	0,3781	0,3714	0,0583
4,00	0,3414	0,3545	0,4643	0,3072	0,3696	0,3674	0,0589
7,00	0,3404	0,3532	0,4621	0,3059	0,3597	0,3643	0,0585
10,00	0,3368	0,3502	0,4575	0,3074	0,3540	0,3612	0,0569
20,00	0,3299	0,3439	0,4538	0,3027	0,3472	0,3555	0,0577
40,00	0,3245	0,3384	0,4494	0,3004	0,3393	0,3504	0,0575
60,00	0,3206	0,3346	0,4474	0,2986	0,3347	0,3472	0,0579
80,00	0,3179	0,3329	0,4448	0,2977	0,3320	0,3451	0,0575
100,00	0,3158	0,3299	0,4435	0,2966	0,3313	0,3434	0,0577

Table D1.9: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type B and 40 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	1,5100	1,6310	1,4780	1,4730	1,2680	1,4720	0,1307
0,80	1,4940	1,6170	1,4460	1,4610	1,2470	1,4530	0,1333
1,00	1,4710	1,6170	1,4220	1,4210	1,2210	1,4304	0,1418
2,00	1,4200	1,5560	1,3790	1,3410	1,1870	1,3766	0,1335
4,00	1,3740	1,5160	1,3420	1,2860	1,1520	1,3340	0,1325
7,00	1,3420	1,4890	1,3160	1,2330	1,1340	1,3028	0,1320
10,00	1,3240	1,4670	1,3050	1,2120	1,1240	1,2864	0,1287
20,00	1,2700	1,4210	1,2610	1,1790	1,0880	1,2438	0,1233
40,00	1,1970	1,3610	1,2040	1,1380	1,0420	1,1884	0,1163
60,00	1,1600	1,3150	1,1730	1,1090	1,0190	1,1552	0,1079
80,00	1,1330	1,2820	1,1460	1,0890	0,9993	1,1299	0,1026
100,00	1,1090	1,2550	1,1260	1,0720	0,9826	1,1089	0,0987

Table D1.10: Shear viscosity for filled gel emulsions with gelatin 160 g Bloom, type B and 50 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	3,5870	4,3790	3,8600	3,1370	5,9880	4,1902	1,1011
0,80	3,5310	4,3080	3,7200	3,0600	5,7440	4,0726	1,0359
1,00	3,4970	4,2510	3,6210	2,9800	5,5600	3,9818	0,9915
2,00	3,2880	4,0090	3,3420	2,7620	5,0340	3,6870	0,8735
4,00	3,1350	3,8150	3,1150	2,5880	4,6230	3,4552	0,7848
7,00	3,0100	3,6550	2,9310	2,4570	4,3180	3,2742	0,7228
10,00	2,9130	3,5200	2,8480	2,3830	4,1390	3,1606	0,6801
20,00	2,7280	3,2260	2,6360	2,2140	3,7760	2,9160	0,6005
40,00	2,5010	2,9160	2,3950	2,0510	3,3490	2,6424	0,5013
60,00	2,3460	2,7150	2,2520	1,9510	3,0890	2,4706	0,4403
80,00	2,2200	2,5700	2,1460	1,8810	2,9400	2,3514	0,4108
100,00	2,1260	2,4460	2,0610	1,8240	2,8050	2,2524	0,3805

D.2. Shear viscosity for filled gel emulsions and gels with gelatin 260 g Bloom

Table D2.1: Shear viscosity for gels with gelatin 260 g Bloom, type A and 0 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,8734	0,8212	0,7156	0,8034	0,0804
0,80	0,8054	0,7748	0,6663	0,7488	0,0731
1,00	0,7751	0,7374	0,6228	0,7118	0,0793
2,00	0,6814	0,6604	0,5669	0,6362	0,0610
4,00	0,6346	0,6180	0,5250	0,5925	0,0591
7,00	0,6020	0,5910	0,5032	0,5654	0,0541
10,00	0,5839	0,5804	0,4965	0,5536	0,0495
20,00	0,5432	0,5543	0,4832	0,5269	0,0383
40,00	0,4981	0,5251	0,4737	0,4990	0,0257
60,00	0,4722	0,5077	0,4681	0,4827	0,0218
80,00	0,4597	0,4939	0,4622	0,4719	0,0191
100,00	0,4462	0,4781	0,4547	0,4597	0,0165

Table D2.2: Shear viscosity for filled gel emulsions with gelatin 260 g Bloom, type A and 10 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,7673	0,7612	0,8330	0,7872	0,0398
0,80	0,7647	0,7556	0,8170	0,7791	0,0331
1,00	0,7561	0,7484	0,8036	0,7694	0,0299
2,00	0,7511	0,7507	0,7881	0,7633	0,0215
4,00	0,7371	0,7432	0,7750	0,7518	0,0204
7,00	0,7235	0,7291	0,7645	0,7390	0,0222
10,00	0,7150	0,7246	0,7605	0,7334	0,0240
20,00	0,6936	0,7114	0,7514	0,7188	0,0296
40,00	0,6842	0,7031	0,7435	0,7103	0,0303
60,00	0,6783	0,6989	0,7393	0,7055	0,0310
80,00	0,6745	0,6952	0,7368	0,7022	0,0317
100,00	0,6711	0,6921	0,7354	0,6995	0,0328

Table D2.3: Shear viscosity for filled gel emulsions with gelatin 260 g Bloom, type A and 20 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	1,5860	1,7140	1,5140	1,6047	0,1013
0,80	1,5890	1,7150	1,4920	1,5987	0,1118
1,00	1,5720	1,7180	1,4720	1,5873	0,1237
2,00	1,5550	1,6700	1,4490	1,5580	0,1105
4,00	1,5270	1,6260	1,4170	1,5233	0,1045
7,00	1,5060	1,5930	1,3890	1,4960	0,1024
10,00	1,4930	1,5680	1,3750	1,4787	0,0973
20,00	1,4550	1,5160	1,3420	1,4377	0,0883
40,00	1,4120	1,4540	1,3020	1,3893	0,0785
60,00	1,3820	1,4180	1,2740	1,3580	0,0749
80,00	1,3620	1,3900	1,2540	1,3353	0,0718
100,00	1,3440	1,3690	1,2380	1,3170	0,0695

Table D2.4: Shear viscosity for filled gel emulsions with gelatin 260 g Bloom, type A and 40 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	21,6800	21,6800	17,3400	18,3300	14,5900	18,7240	3,0264
0,80	21,0300	21,0300	16,7700	17,7300	14,1600	18,1440	2,9406
1,00	20,3000	20,3000	16,2000	17,1100	13,8100	17,5440	2,7896
2,00	17,7000	17,7000	14,5500	15,2600	12,4600	15,5340	2,2291
4,00	15,3000	15,3000	12,7900	13,3300	11,1600	13,5760	1,7649
7,00	13,3100	13,3100	11,2500	11,6700	10,0300	11,9140	1,4096
10,00	12,1100	12,1100	10,3300	10,6400	9,3020	10,8984	1,2118
20,00	9,8770	9,8770	8,6140	8,7460	7,9740	9,0176	0,8371
40,00	8,0160	8,0160	7,0180	7,2500	6,5640	7,3728	0,6369
60,00	7,0130	7,0130	6,1310	6,4700	5,8280	6,4910	0,5279
80,00	6,4030	6,4030	5,0080	5,9740	5,2540	5,8084	0,6486
100,00	5,6940	5,6940	3,4010	5,4910	4,2050	4,8970	1,0416

Table D2.5: Shear viscosity for filled gel emulsions with gelatin 260 g Bloom, type A and 50 wt.% corn oil (N = 5, ± standard deviation)

Replications	1	2	3	4	5	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	127,4000	69,5700	89,9200	51,5300	44,9700	75,7060	33,3032
0,80	114,8000	63,5600	81,3400	46,7800	40,1100	69,3460	30,0307
1,00	105,2000	58,2800	74,8600	45,3200	40,2500	63,1180	26,2585
2,00	78,8900	45,4000	56,9700	35,8400	31,9300	48,3920	18,9157
4,00	58,2700	35,1200	43,2500	28,2000	24,8600	36,9480	13,3736
7,00	45,1900	28,1300	34,3700	22,2800	19,9000	29,0880	10,1850
10,00	36,9700	24,2300	29,3000	19,7000	15,4700	24,3480	8,3843
20,00	22,7500	17,8400	21,2300	15,0000	11,5400	16,7900	4,5611
40,00	12,3200	10,9700	13,6800	10,5200	7,1300	10,3290	2,4548
60,00	6,6390	6,8790	9,1050	6,0840	4,1550	6,0196	1,7745
80,00	4,4720	3,7230	5,2590	2,9260	1,3910	3,4674	1,4876
100,00	1,2770	2,7220	1,3530	1,5220	0,9572	1,3748	0,6778

Table D2.6: Shear viscosity for gels with gelatin 260 g Bloom, type B and 0 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,4227	0,4667	0,7156	0,5350	0,1579
0,80	0,4178	0,4882	0,6663	0,5241	0,1281
1,00	0,4110	0,4720	0,6228	0,5019	0,1090
2,00	0,3927	0,4342	0,5669	0,4646	0,0910
4,00	0,3867	0,4139	0,5250	0,4419	0,0733
7,00	0,3773	0,3880	0,5032	0,4228	0,0698
10,00	0,3689	0,3797	0,4965	0,4150	0,0708
20,00	0,3534	0,3438	0,4832	0,3935	0,0779
40,00	0,3297	0,3142	0,4737	0,3725	0,0880
60,00	0,3141	0,3047	0,4681	0,3623	0,0917
80,00	0,3031	0,2979	0,4622	0,3544	0,0934
100,00	0,2961	0,2951	0,4547	0,3486	0,0919

Table D2.7: Shear viscosity for gels with gelatin 260 g Bloom, type B and 10 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,4757	0,5400	0,5480	0,5212	0,0396
0,80	0,5021	0,5625	0,5629	0,5425	0,0350
1,00	0,5051	0,5609	0,5635	0,5432	0,0330
2,00	0,4847	0,5393	0,5460	0,5233	0,0336
4,00	0,4840	0,5344	0,5424	0,5203	0,0317
7,00	0,4822	0,5254	0,5393	0,5156	0,0298
10,00	0,4779	0,5184	0,5358	0,5107	0,0297
20,00	0,4716	0,5079	0,5285	0,5027	0,0288
40,00	0,4663	0,4968	0,5220	0,4950	0,0279
60,00	0,4636	0,4911	0,5183	0,4910	0,0274
80,00	0,4614	0,4870	0,5161	0,4882	0,0274
100,00	0,4605	0,4850	0,5152	0,4869	0,0274

Table D2.8: Shear viscosity for gels with gelatin 260 g Bloom, type B and 20 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	1,0410	0,8206	0,9721	0,9446	0,1128
0,80	1,0460	0,8039	0,9650	0,9383	0,1232
1,00	1,0370	0,7878	0,9612	0,9287	0,1277
2,00	0,9908	0,7915	0,9416	0,9080	0,1038
4,00	0,9621	0,7846	0,9317	0,8928	0,0949
7,00	0,9407	0,7759	0,9186	0,8784	0,0895
10,00	0,9247	0,7765	0,9138	0,8717	0,0826
20,00	0,9046	0,7691	0,8990	0,8576	0,0767
40,00	0,8836	0,7597	0,8823	0,8419	0,0712
60,00	0,8720	0,7553	0,8738	0,8337	0,0679
80,00	0,8641	0,7517	0,8659	0,8272	0,0654
100,00	0,8570	0,7483	0,8601	0,8218	0,0637

Table D2.9: Shear viscosity for gels with gelatin 260 g Bloom, type B and 40 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	5,3750	4,1660	4,1170	4,5527	0,7126
0,80	5,0390	4,1090	3,9350	4,3610	0,5936
1,00	5,0440	4,0390	3,8020	4,2950	0,6594
2,00	4,2500	3,7310	3,5650	3,8487	0,3573
4,00	3,9230	3,4440	3,3180	3,5617	0,3192
7,00	3,5430	3,2430	3,1520	3,3127	0,2046
10,00	3,3740	3,1200	3,0390	3,1777	0,1748
20,00	3,1090	2,8890	2,8490	2,9490	0,1400
40,00	2,8690	2,6550	2,6410	2,7217	0,1278
60,00	2,7270	2,5190	2,5130	2,5863	0,1219
80,00	2,6220	2,4250	2,4150	2,4873	0,1167
100,00	2,5350	2,3550	2,3350	2,4083	0,1102

Table D2.10: Shear viscosity for gels with gelatin 260 g Bloom, type B and 50 wt.% corn oil (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	12,8700	15,2600	19,2500	15,7933	3,2233
0,80	12,0400	14,2000	17,7500	14,6633	2,8831
1,00	11,3600	13,3800	16,6100	13,7833	2,6481
2,00	9,5900	11,2500	13,6600	11,5000	2,0465
4,00	8,1420	9,5840	11,3000	9,6753	1,5810
7,00	7,1020	8,4620	9,6750	8,4130	1,2872
10,00	6,5000	7,8370	8,7780	7,7050	1,1447
20,00	5,4970	6,7170	7,2950	6,5030	0,9179
40,00	4,6720	5,7350	6,1540	5,5203	0,7640
60,00	4,2370	5,2050	5,5720	5,0047	0,6897
80,00	3,9350	4,8120	5,0610	4,6027	0,5915
100,00	3,6580	4,4660	4,6740	4,2660	0,5367

D.3. Shear viscosity for filled gel emulsions with 260 g Bloom gelatin, 0,1 M NaCl, pH 4, pH 8 or polysorbate 80

Information on the shear viscosity filled gel emulsions with 40 wt. % corn oil, gelatin 260 g Bloom, type A and B, and with either NaCl (0,1 M), pH 4, pH or 0,5 wt.% polysorbate 80 was obtained as described for the original filled gel emulsion systems in D1 and D2. Viscosity (Pa s) at various shear rates (1/s) is presented in table D3.1-D2.16.

Table D3.1: Shear viscosity for gels with gelatin 260 g Bloom, type A, 0 wt.% corn oil and 0,1 M NaCl (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,4239	0,4538	0,6545	0,5107	0,1254
0,80	0,3908	0,4150	0,6111	0,4723	0,1208
1,00	0,3759	0,3923	0,6005	0,4562	0,1252
2,00	0,3529	0,3546	0,5320	0,4132	0,1029
4,00	0,3391	0,3353	0,4991	0,3912	0,0935
7,00	0,3316	0,3263	0,4829	0,3803	0,0889
10,00	0,3300	0,3204	0,4746	0,3750	0,0864
20,00	0,3254	0,3155	0,4658	0,3689	0,0841
40,00	0,3230	0,3126	0,4620	0,3659	0,0834
60,00	0,3209	0,3120	0,4613	0,3647	0,0837

Table D3.2: Shear viscosity for gels with gelatin 260 g Bloom, type A, 40 wt.% corn oil and 0,1 M NaCl (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	22,8800	14,3100	16,0900	17,7600	4,5225
0,80	22,0500	13,9300	15,2500	17,0767	4,3573
1,00	21,5200	13,6100	14,6700	16,6000	4,2937
2,00	18,7800	12,0400	12,8700	14,5633	3,6752
4,00	16,4900	11,1200	11,4900	13,0333	2,9993
7,00	14,6900	10,1800	10,4400	11,7700	2,5321
10,00	13,5100	9,5540	9,5740	10,8793	2,2782
20,00	11,3600	8,2500	7,9750	9,1950	1,8800
40,00	9,5260	6,8870	6,6040	7,6723	1,6115
60,00	8,4680	6,1600	5,9970	6,8750	1,3820

Table D3.3: Shear viscosity for gels with gelatin 260 g Bloom, type B, 0 wt.% corn oil and 0,1 M NaCl (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3161	0,2992	0,2967	0,3040	0,0106
0,80	0,2971	0,2883	0,2913	0,2922	0,0045
1,00	0,3075	0,2694	0,3017	0,2929	0,0205
2,00	0,2933	0,2691	0,2829	0,2818	0,0121
4,00	0,2876	0,2610	0,2789	0,2758	0,0136
7,00	0,2839	0,2544	0,2773	0,2719	0,0155
10,00	0,2815	0,2567	0,2754	0,2712	0,0129
20,00	0,2793	0,2537	0,2740	0,2690	0,0135
40,00	0,2795	0,2531	0,2741	0,2689	0,0139
60,00	0,2803	0,2528	0,2747	0,2693	0,0145

Table D3.4: Shear viscosity for gels with gelatin 260 g Bloom, type B, 40 wt.% corn oil and 0,1 M NaCl (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	3,9260	2,9210	3,6840	3,5103	0,5245
0,80	3,8850	2,8990	3,5950	3,4597	0,5067
1,00	3,8580	2,8830	3,5310	3,4240	0,4962
2,00	3,6030	2,7380	3,3250	3,2220	0,4416
4,00	3,4160	2,6410	3,1390	3,0653	0,3927
7,00	3,2900	2,5770	3,0140	2,9603	0,3595
10,00	3,2000	2,5190	2,9400	2,8863	0,3437
20,00	3,0170	2,4040	2,7730	2,7313	0,3086
40,00	2,8000	2,2670	2,5910	2,5527	0,2686
60,00	2,6640	2,1810	2,4840	2,4430	0,2441

Table D3.5: Shear viscosity for gels with gelatin 260 g Bloom, type A, 0 wt.% corn oil and pH 4 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,7333	0,7815	0,7712	0,7620	0,0254
0,80	0,6814	0,7099	0,6913	0,6942	0,0145
1,00	0,6436	0,6662	0,6849	0,6649	0,0207
2,00	0,6056	0,5998	0,5897	0,5984	0,0080
4,00	0,5812	0,5529	0,5326	0,5556	0,0244
7,00	0,5636	0,5306	0,5116	0,5353	0,0263
10,00	0,5628	0,5218	0,5026	0,5291	0,0308
20,00	0,5586	0,5104	0,4922	0,5204	0,0343
40,00	0,5560	0,5047	0,4857	0,5155	0,0364
60,00	0,5555	0,5033	0,4841	0,5143	0,0369

Table D3.6: Shear viscosity for gels with gelatin 260 g Bloom, type A, 40 wt.% corn oil and pH 4 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	29,4100	20,8900	59,3300	36,5433	20,1884
0,80	26,4400	17,8600	51,0200	31,7733	17,2113
1,00	24,5300	16,6000	46,6500	29,2600	15,5734
2,00	19,5400	13,6400	31,8800	21,6867	9,3076
4,00	15,4600	10,9400	22,9500	16,4500	6,0659
7,00	12,5600	9,3780	17,5600	13,1660	4,1245
10,00	10,9100	8,2790	14,6000	11,2630	3,1753
20,00	8,3010	6,4480	10,6000	8,4497	2,0800
40,00	6,6120	5,0960	7,8930	6,5337	1,4001
60,00	5,6640	4,5090	6,6650	5,6127	1,0789

Table D3.7: Shear viscosity for gels with gelatin 260 g Bloom, type B, 0 wt.% corn oil and pH 4 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3053	0,2715	0,2967	0,2912	0,0176
0,80	0,2974	0,2872	0,2968	0,2938	0,0057
1,00	0,2829	0,2861	0,2869	0,2853	0,0021
2,00	0,2810	0,2630	0,2811	0,2750	0,0104
4,00	0,2743	0,2597	0,2744	0,2695	0,0085
7,00	0,2672	0,2605	0,2703	0,2660	0,0050
10,00	0,2695	0,2579	0,2724	0,2666	0,0077
20,00	0,2669	0,2562	0,2696	0,2642	0,0071
40,00	0,2669	0,2559	0,2690	0,2639	0,0070
60,00	0,2679	0,2563	0,2691	0,2644	0,0071

Table D3.8: Shear viscosity for gels with gelatin 260 g Bloom, type B, 40 wt.% corn oil and pH 4 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	2,2770	2,4000	2,1280	2,2683	0,1362
0,80	2,2150	2,3040	2,0580	2,1923	0,1246
1,00	2,1670	2,1870	2,0020	2,1187	0,1015
2,00	2,0760	2,0180	1,8900	1,9947	0,0952
4,00	1,9830	1,9150	1,8220	1,9067	0,0808
7,00	1,9270	1,8370	1,7750	1,8463	0,0764
10,00	1,8980	1,7960	1,7510	1,8150	0,0753
20,00	1,8260	1,7050	1,6860	1,7390	0,0759
40,00	1,7330	1,6070	1,6120	1,6507	0,0713
60,00	1,6800	1,5400	1,5610	1,5937	0,0755

Table D3.9: Shear viscosity for gels with gelatin 260 g Bloom, type A, 0 wt.% corn oil and pH 8 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,5612	0,5136	0,4661	0,5136	0,0476
0,80	0,5242	0,4860	0,4708	0,4937	0,0275
1,00	0,5065	0,4823	0,4615	0,4834	0,0225
2,00	0,4772	0,4591	0,4220	0,4528	0,0281
4,00	0,4579	0,4431	0,4095	0,4368	0,0248
7,00	0,4487	0,4341	0,4073	0,4300	0,0210
10,00	0,4470	0,4300	0,4030	0,4267	0,0222
20,00	0,4422	0,4262	0,4001	0,4228	0,0213
40,00	0,4384	0,4244	0,3980	0,4203	0,0205
60,00	0,4381	0,4247	0,3980	0,4203	0,0204

Table D3.10: Shear viscosity for gels with gelatin 260 g Bloom, type A, 40 wt.% corn oil and pH 8 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	16,4100	16,8700	12,0800	15,1200	2,6427
0,80	15,0400	15,7000	11,5500	14,0967	2,2300
1,00	14,2600	14,9500	11,1300	13,4467	2,0357
2,00	12,3500	12,8600	10,0000	11,7367	1,5255
4,00	10,7200	11,2100	8,9810	10,3037	1,1714
7,00	9,4530	9,9550	8,1490	9,1857	0,9322
10,00	8,6210	8,9210	7,5970	8,3797	0,6942
20,00	7,0110	7,0990	6,3290	6,8130	0,4215
40,00	5,6450	5,7690	5,2410	5,5517	0,2761
60,00	5,0620	5,2110	4,8370	5,0367	0,1883

Table D3.11: Shear viscosity for gels with gelatin 260 g Bloom, type B, 0 wt.% corn oil and pH 8 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3414	0,2908	0,3143	0,3155	0,0253
0,80	0,3314	0,2943	0,2958	0,3072	0,0210
1,00	0,3183	0,2981	0,2967	0,3044	0,0121
2,00	0,3205	0,2870	0,3020	0,3032	0,0168
4,00	0,3165	0,2850	0,2973	0,2996	0,0159
7,00	0,3115	0,2853	0,2947	0,2972	0,0133
10,00	0,3134	0,2839	0,2933	0,2969	0,0151
20,00	0,3120	0,2828	0,2930	0,2959	0,0148
40,00	0,3121	0,2836	0,2938	0,2965	0,0144
60,00	0,3128	0,2840	0,2951	0,2973	0,0145

Table D3.12: Shear viscosity for gels with gelatin 260 g Bloom, type B, 40 wt.% corn oil and pH 8 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	5,0450	6,3390	9,3150	6,8997	2,1895
0,80	4,7820	6,1370	8,6100	6,5097	1,9410
1,00	4,5560	5,8500	7,9280	6,1113	1,7011
2,00	3,8380	4,7910	6,2570	4,9620	1,2185
4,00	3,2680	4,0370	5,0960	4,1337	0,9178
7,00	2,9010	3,5960	4,4110	3,6360	0,7558
10,00	2,7130	3,3730	4,0520	3,3793	0,6695
20,00	2,3840	2,9860	3,4700	2,9467	0,5441
40,00	2,0610	2,5650	2,9150	2,5137	0,4293
60,00	1,9110	2,3290	2,6730	2,3043	0,3816

Table D3.13: Shear viscosity for gels with gelatin 260 g Bloom, type A, 0 wt.% corn oil and polysorbate 80 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,5377	0,4816	0,4790	0,4994	0,0332
0,80	0,5244	0,4843	0,4644	0,4910	0,0306
1,00	0,5402	0,4794	0,4588	0,4928	0,0423
2,00	0,5304	0,4755	0,4645	0,4901	0,0353
4,00	0,5242	0,4727	0,4570	0,4846	0,0352
7,00	0,5228	0,4703	0,4587	0,4839	0,0342
10,00	0,5220	0,4717	0,4583	0,4840	0,0336
20,00	0,5199	0,4700	0,4584	0,4828	0,0327
40,00	0,5181	0,4685	0,4570	0,4812	0,0325
60,00	0,5190	0,4682	0,4560	0,4811	0,0334

Table D3.14: Shear viscosity for gels with gelatin 260 g Bloom, type A, 40 wt.% corn oil and polysorbate 80 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	12,9400	8,4210	12,5900	10,4737	2,5141
0,80	10,6400	7,3550	10,5500	8,9720	1,8712
1,00	9,2630	6,6510	9,2270	8,0163	1,4978
2,00	6,7360	5,2380	6,7130	6,1260	0,8583
4,00	4,9650	4,1560	5,1090	4,8087	0,5137
7,00	4,0030	3,4900	4,1650	3,9977	0,3524
10,00	3,5540	3,1360	3,6550	3,5600	0,2752
20,00	2,9280	2,6260	2,9900	2,9283	0,1947
40,00	2,3380	2,1650	2,3690	2,3317	0,1099
60,00	1,6960	1,8730	1,9410	1,9120	0,1265

Table D3.15: Shear viscosity for gels with gelatin 260 g Bloom, type B, 0 wt.% corn oil and polysorbate 80 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	0,3074	0,3248	0,3228	0,3183	0,0095
0,80	0,3141	0,3357	0,3372	0,3290	0,0129
1,00	0,3106	0,3265	0,3324	0,3232	0,0113
2,00	0,2953	0,3136	0,3112	0,3067	0,0099
4,00	0,2924	0,3101	0,3094	0,3040	0,0100
7,00	0,2938	0,3106	0,3104	0,3049	0,0096
10,00	0,2914	0,3102	0,3115	0,3044	0,0112
20,00	0,2910	0,3081	0,3104	0,3032	0,0106
40,00	0,2911	0,3086	0,3102	0,3033	0,0106
60,00	0,2911	0,3091	0,3109	0,3037	0,0109

Table D3.16: Shear viscosity for gels with gelatin 260 g Bloom, type B, 40 wt.% corn oil and polysorbate 80 (N = 3, ± standard deviation)

Replications	1	2	3	Average	S.D.
Shear rate (1/s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	Viscosity (Pa s)	±
0,60	8,7590	4,1350	5,2170	6,0370	2,4186
0,80	7,4920	3,9830	4,8810	5,4520	1,8229
1,00	6,6810	3,8560	4,6180	5,0517	1,4616
2,00	4,8430	3,2710	3,7860	3,9667	0,8014
4,00	3,7080	2,7940	3,1330	3,2117	0,4621
7,00	3,0420	2,4610	2,7100	2,7377	0,2915
10,00	2,6750	2,2520	2,4650	2,4640	0,2115
20,00	2,1380	1,8980	2,0600	2,0320	0,1224
40,00	1,7120	1,5590	1,6820	1,6510	0,0811
60,00	1,4840	1,3670	1,4570	1,4360	0,0613

Appendix E: Small strain oscillatory measurements

Small strain oscillatory measurements were performed by using a Rheometer (StressTech, Sweden). The parameters examined were storage modulus, (G'), gelling (T_g) and melting (T_m) temperature. The water bath held a temperature of 25 °C for measurements at 60 °C, and 30 °C for the measurements performed at 70 °C. To analyse the data obtained, the software "RheoExplorer", connected to the rheometer was used. In figure E.1 a typical measurement plot is shown. The first 20 minutes of the measurement is a cooling period, where the sample was cooled from 60 °C to 20 °C. Then there was a holding period of 15 minutes at 20 °C, followed by a heating period, where the sample is heated up to 60 °C. The storage modulus was found at the transition from cooling to heating, at the end of the holding period. The gelling temperature was found during the cooling period, at the intersection between the temperature and the phase angle, while the melting temperature was the temperature at a phase angle of 45° during the heating period.

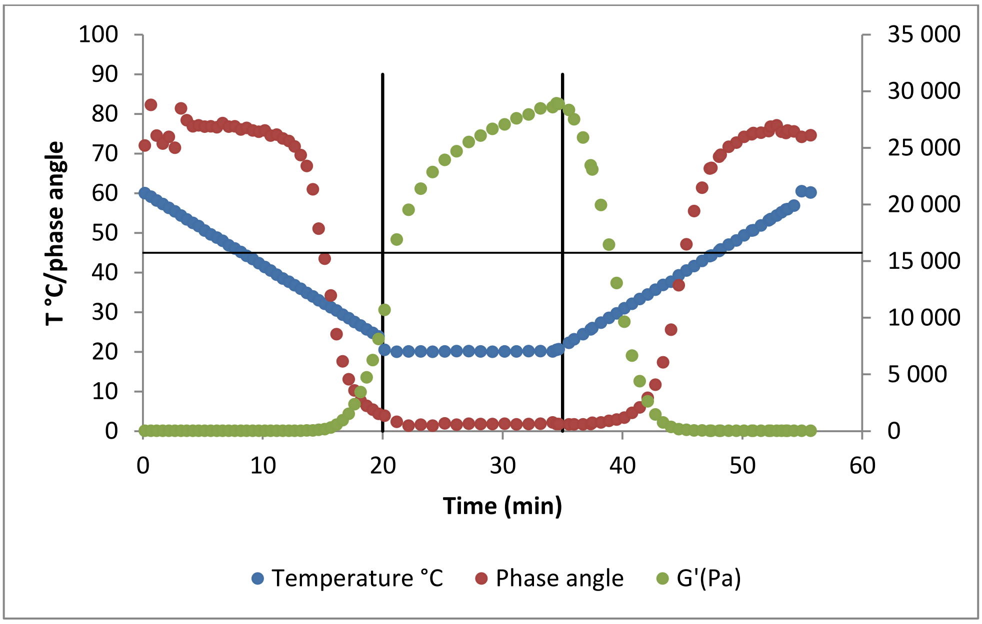

Figure E.1: Example plot from a small strain oscillatory measurement of a filled gel emulsion made from 260 g Bloom gelatin type A, with 20 wt. % corn oil. The temperature (T) and phase angle is given as a function of time on the primary y-axis, G' is given as a function of time at the secondary y-axis. The vertical black lines are marking the transitions between the cooling, holding and heating periods (from left to right), while the horizontal line is marking where the phase angle is 45°.

E.1. Storage modulus

The storage modulus (G’) is the peak value at 20°C, as shown above in figure E1 The value of G’ was obtained from the table created by RheoExplorer during measurements. Table E1.1-2 gives the values of G’ for filled gel emulsions with gelatin 160 g and 260 g Bloom, type A and B, and different weight percentages (wt. %) of corn oil. Table E.1.3 gives the G’ values for the emulsion systems with changed ionic strength, pH and added surfactant.

Table E.1.1: Storage modulus (G’) for filled gel emulsions with 160 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil (N = 5), average G’ and standard deviation (S.D.)

Gelatin type and oil wt.%	G’ (Pa) 1	G’ (Pa) 2	G’ (Pa) 3	G’ (Pa) 4	G’ (Pa) 5	Average G’ (Pa)	S.D. (Pa)
A – 0 wt.%	10900	11200	12200	11500	12500	11660	673
B – 0 wt.%	15200	10600	13500	13300	13800	13533	252
A – 10 wt.%	13600	12400	13800	12700	13200	13140	590
B – 10 wt.%	14500	12000	14100	13100	13000	13400	608
A – 20 wt.%	14700	14600	12000	13400	14200	13780	1119
B – 20 wt.%	12500	13100	14700	11700	13700	13100	600
A – 40 wt.%	16500	14800	17200	18400	19000	17180	1653
B – 40 wt.%	14200	16100	15500	14700	14300	14833	611
A – 50 wt.%	17300	20000	19500	16700	20300	18760	1646
B – 50 wt.%	14800	15600	14400	15500	18800	15300	436

Table E.1.2: Storage modulus (G’) for filled gel emulsions with 260 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil, (N = 3) average G’ and standard deviation (S.D.).

Gelatin type and oil wt.%	G’ (Pa) 1	G’ (Pa) 2	G’ (Pa) 3	Average G’ (Pa)	S.D. (Pa)
A – 0 wt.%	23460	23210	24310	23660	577
B – 0 wt.%	22190	22330	23150	22557	519
A – 10 wt.%	25290	25610	27020	25973	920
B – 10 wt.%	24090	24330	25220	24547	595
A – 20 wt.%	29560	28910	28560	29010	507
B – 20 wt.%	24430	24840	26390	25220	1034
A – 40 wt.%	36150	34830	34680	35220	809
B – 40 wt.%	26890	25800	24930	25873	982
A – 50 wt.%	34370	35590	32250	34070	1690
B – 50 wt.%	24630	27190	28210	26677	1844

Table E.1.3: Storage modulus (G') for filled gel emulsions with 260 g Bloom gelatin type A or B, 0 and 40 wt.% corn oil, and 0,1 M NaCl, 0,5 wt.% polysorbate 80 (Tween 80), pH 4 or pH 8. (N = 3) average G' and standard deviation (S.D.).

Gelatin type and oil wt.%	G' (Pa) 1	G' (Pa) 2	G' (Pa) 3	Average G' (Pa)	S.D. (Pa)
A 0%, NaCl	20200	20700	24600	21833	2409
B 0%, NaCl	22700	22000	22500	22400	361
A 40%, NaCl	37100	35000	33800	35300	1670
B 40%, NaCl	26600	24100	25800	25500	1277
A 0%,Tween80	25700	24800	23810	24770	945
B 0%,Tween80	22900	23900	23500	23433	503
A 40%,Tween80	11100	10400	10200	10567	473
B 40%,Tween80	10400	9520	10400	10107	508
A 0%, pH 4	24100	23200	23200	23500	520
B 0%, pH 4	22700	21500	22000	22067	603
A 40%, pH 4	33100	29900	33100	32033	1848
B 40%, pH 4	22700	21200	22500	22133	814
A 0%, pH 8	21300	22300	22400	22000	608
B 0%, pH 8	23300	21800	23100	22733	814
A 40%, pH 8	32300	30600	30000	30967	1193
B 40%, pH 8	22200	22200	25400	23267	1848

E.2. Gelling temperature (T_g)

The gelling temperature (T_g) was found during the cooling period, by interpolation of the phase angles and corresponding temperatures at the two measured points above and below phase angle of 45°. Table E2.1-2 gives the values of T_g for filled gel emulsions with gelatin 160 g and 260 g Bloom, type A and B, and different weight percentages of corn oil. Table E.2.3 provides T_g for the emulsion systems with changed ionic strength, pH and added surfactant.

Table E.2.1: Gelling temperatures (T_g) for filled gel emulsions with 160 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil, (N = 5) average T_g and standard deviation (S.D.).

Gelatin type and oil wt.%	T_g 1 (°C)	T_g 2 (°C)	T_g 3 (°C)	T_g 4 (°C)	T_g 5 (°C)	Average T_g (°C)	S.D. (°C)
A – 0 wt.%	28,20	-	28,88	29,19	28,92	28,90	0,48
B – 0 wt.%	29,89	29,85	30,08	30,03	29,95	29,96	0,07
A – 10 wt.%	27,91	28,39	28,16	28,22	28,07	28,15	0,08
B – 10 wt.%	29,62	29,29	29,43	29,64	29,51	29,52	0,10
A – 20 wt.%	28,50	28,12	27,77	28,88	28,17	28,26	0,21
B – 20 wt.%	29,42	29,56	29,54	29,15	29,65	29,51	0,08
A – 40 wt.%	29,20	28,27	28,95	29,82	29,68	29,28	0,37
B – 40 wt.%	29,73	29,87	29,57	29,58	29,62	29,64	0,08
A – 50 wt.%	39,69	-	40,30	31,76	39,26	39,48	0,30
B – 50 wt.%	30,05	30,50	30,19	30,04	30,90	30,25	0,23

Table E.2.2: Gelling temperatures (T_g) for filled gel emulsions with 260 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil, (N = 3) average T_g and standard deviation (S.D.).

Gelatin type and oil wt.%	T_g 1 (°C)	T_g 2 (°C)	T_g 3 (°C)	Average T_g (°C)	S.D. (°C)
A – 0 wt.%	31,98	32,03	31,77	31,93	0,14
B – 0 wt.%	30,8	30,94	30,79	30,84	0,08
A – 10 wt.%	32,04	31,83	31,88	31,92	0,11
B – 10 wt.%	30,97	31,01	31,16	31,05	0,10
A – 20 wt.%	32,51	32,47	32,19	32,39	0,17
B – 20 wt.%	31,29	31,02	31,18	31,16	0,14
A – 40 wt.%	43,63	48,98	48,11	46,91	2,87
B – 40 wt.%	31,61	31,78	31,19	31,53	0,30
A – 50 wt.%	-	-	-	-	-
B – 50 wt.%	32,19	32,65	32,46	32,43	0,23

Table E.2.3: Gelling temperatures (T_g) for filled gel emulsions with 260 g Bloom gelatin type A or B, 0 and 40 wt.% corn oil, and 0,1 M NaCl, 0,5 wt.% polysorbate 80 (Tween 80), pH 4 or pH 8. (N = 3) average T_g and standard deviation (S.D.).

Gelatin type and oil wt.%	T_g (°C) 1	T_g (°C) 2	T_g (°C) 3	Average G' (Pa)	S.D. (Pa)
A 0%, NaCl	31,37	31,36	31,94	31,56	0,33
B 0%, NaCl	30,85	30,62	30,66	30,71	0,12
A 40%, NaCl	48,10	47,40	47,80	47,77	0,35
B 40%, NaCl	31,50	31,00	31,00	31,17	0,29
A 0%,Tween80	32,00	31,90	31,80	31,90	0,10
B 0%,Tween80	31,00	31,00	31,00	31,00	0,00
A 40%,Tween80	33,30	32,60	33,30	33,07	0,40
B 40%,Tween80	32,70	31,40	31,80	31,97	0,67
A 0%, pH 4	31,60	32,00	32,10	31,90	0,26
B 0%, pH 4	30,60	30,60	30,60	30,60	0,00
A 40%, pH 4	49,10	50,30	53,50	50,97	2,27
B 40%, pH 4	30,80	31,00	30,70	30,83	0,15
A 0%, pH 8	32,00	31,70	31,50	31,73	0,25
B 0%, pH 8	30,80	30,80	30,80	30,80	0,00
A 40%, pH 8	46,50	46,30	44,00	45,60	1,39
B 40%, pH 8	30,90	31,20	31,40	31,17	0,25

E.3. Melting temperature (T_m)

The melting temperature (T_m) was found during the heating period using the same method as described in section E.2. Table E3.1-2 gives the values of T_m for filled gel emulsions with gelatin 160 g and 260 g Bloom, type A and B, and different weight percentages of corn oil. Table E.3.3 provides T_m for the emulsion systems with changed ionic strength, pH and added surfactant.

Table E.3.1: Melting temperatures (T_m) for filled gel emulsions with 160 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil, (N = 5), average T_m and standard deviation (S.D.).

Gelatin type and oil wt.%	T_m 1 (°C)	T_m 2 (°C)	T_m 3 (°C)	T_m 4 (°C)	T_m 5 (°C)	Average T_m (°C)	S.D. (°C)
A – 0 wt.%	35,75	36,05	36,68	-	36,07	36,16	0,01
B – 0 wt.%	37,11	-	37,09	36,75	37,17	37,10	0,01
A – 10 wt.%	35,38	35,69	35,46	-	35,68	35,51	0,16
B – 10 wt.%	36,91	36,41	-	36,85	36,66	36,76	0,06
A – 20 wt.%	35,84	35,70	35,45	-	35,74	35,66	0,03
B – 20 wt.%	36,72	36,63	-	36,22	36,54	36,59	0,06
A – 40 wt.%	40,38	36,67	39,95	40,83	40,43	39,46	0,26
B – 40 wt.%	36,96	37,16	36,93	37,07	36,80	36,90	0,07
A – 50 wt.%	50,30	37,79	50,47	45,33	50,08	45,97	2,81
B – 50 wt.%	37,38	-	37,45	37,48	38,32	37,47	0,02

Table E.3.2: Melting temperatures (T_m) for filled gel emulsions with 260 g Bloom gelatin type A or B and 0, 10, 20, 40 or 50 wt.% corn oil, (N = 3), average T_g and standard deviation (S.D.).

Gelatin type and oil wt.%	T_m 1 (°C)	T_m 2 (°C)	T_m 3 (°C)	Average T_m (°C)	S.D. (°C)
A – 0 wt.%	39,60	39,74	39,27	39,54	0,24
B – 0 wt.%	37,57	37,88	37,38	37,61	0,25
A – 10 wt.%	39,34	39,21	39,15	39,23	0,10
B – 10 wt.%	37,45	37,82	37,97	37,75	0,27
A – 20 wt.%	40,66	40,57	40,17	40,47	0,26
B – 20 wt.%	37,75	37,85	37,83	37,81	0,05
A – 40 wt.%	50,50	53,91	53,26	52,56	1,81
B – 40 wt.%	38,7	38,55	38,18	38,48	0,27
A – 50 wt.%	-	-	-	-	-
B – 50 wt.%	38,99	39,92	39,77	39,56	0,50

Table E.3.3: Melting temperatures (T_m) for filled gel emulsions with 260 g Bloom gelatin type A or B, 0 and 40 wt.% corn oil, and 0,1 M NaCl, 0,5 wt.% polysorbate 80 (Tween 80), pH 4 or pH 8. (N = 3), average T_m and standard deviation (S.D.).

Gelatin type and oil wt.%	T_m (°C) 1	T_m (°C) 2	T_m (°C) 3	Average G' (Pa)	S.D. (Pa)
A 0%, NaCl	38,87	38,86	39,73	39,15	0,50
B 0%, NaCl	37,66	37,64	37,61	37,64	0,03
A 40%, NaCl	53,20	52,70	52,90	52,93	0,25
B 40%, NaCl	38,50	38,20	38,30	38,33	0,15
A 0%,Tween80	39,20	39,20	39,10	39,17	0,06
B 0%,Tween80	37,80	37,80	37,60	37,73	0,12
A 40%,Tween80	40,90	40,20	41,00	40,70	0,44
B 40%,Tween80	39,50	38,00	38,40	38,63	0,78
A 0%, pH 4	39,20	39,70	39,80	39,57	0,32
B 0%, pH 4	37,50	37,30	37,30	37,37	0,12
A 40%, pH 4	54,60	55,40	58,10	56,03	1,83
B 40%, pH 4	37,80	38,00	38,00	37,93	0,12
A 0%, pH 8	39,20	39,10	39,00	39,10	0,10
B 0%, pH 8	37,60	37,60	37,70	37,63	0,06
A 40%, pH 8	51,80	51,30	50,10	51,07	0,87
B 40%, pH 8	38,00	38,30	38,60	38,30	0,30

Appendix F: Longitudinal deformation

F.1. Instrument information

A TA.XT.-Plus Texture Analyzer (Stable Micro Systems, UK) with serial number 11085 was used to determine Young's modulus, force and strain at break for filled gel emulsions. The instrument was connected to the "Exponent" software, which was used for acquisition of data and analysis of these. A 5 kg load cell was used in all the experiments.

F.2. Settings

F.2.1 Young's Modulus

Young's modulus was found by determining the gradient (N/m) by compression of filled gel emulsions using a cylindrical aluminium probe with diameter = 35 mm (p/35). The other parameter settings are found in table F.2.1.1.

Table F.2.1.1: Settings used for measurements of Young's modulus

Parameter	Value
Test mode	Compression
Load cell	5 kg
Test speed	0,1 mm/sec
Target mode	Distance
Distance	2 mm
Trigger type	Auto (force)
Trigger force	1,5 g

F.2.2 Break

Force and strain was measured using a stainless steel probe with diameter = 2 mm (p/2). The parameter settings are found in table F.2.2.1.

Table F.2.2.1: Settings used for determination of force and strain at break

Parameter	Value
Test mode	Compression
Load cell	5 kg
Test speed	0,1 mm/sec
Target mode	Distance
Distance	18 mm
Trigger type	Auto (force)
Trigger force	1,5 g

F.3. Example calculations

F.3.1 Young's modulus

Young's modulus was determined by using equation for Young's modulus in Table 1.5.2 from the main report. Force (N) as a function of distance was recorded from compression of the emulsion gels, and the Exponent software provided a force-distance curve. A macro was programmed to determine the gradient F/ΔL (N/m) from the curve, by defining the slope in the 0,10 - 0,15 mm region. The height (L_0) and the diameter (d) of the gels was measured using a digital caliper and used in in the calculation of Young's modulus. An example calculation for sample 1 in table F.4.1.1 is presented below.

$$Height: 0{,}01957m$$

$$Diameter: 0{,}01620m$$

$$Area = A_0 = \pi\left(\frac{d}{2}\right)^2 = \pi\left(\frac{0{,}01620}{2}\right)^2 = 0{,}0002060m^2$$

$$Gradient = 759{,}2\frac{N}{m}$$

$$E = 759{,}2\frac{N}{m}\left(\frac{0{,}01957m}{0{,}0002060m^2}\right) = 72124\frac{N}{m^2} = 72{,}12kPa$$

F.3.2 Force at break

The software defined the force at break as the first peak in the force-distance curve, which corresponded to the first break in the emulsion gel.

F.3.3 Strain at break

Strain is the deformation of the gel at break. A percentage deformation was calculated by first dividing the distance at break (mm) with the original height of the gel (mm) and then multiplied by a 100. An example calculation for sample 2 in table F.4.2.4 is presented below.

$$Height: 19,36mm$$

$$Compression: 3,508mm$$

$$Strain(\%) = \frac{3,508mm}{19,36mm} \cdot 100\% = 18,1\%$$

F.4. Data

F.4.1 Young's Modulus

The height (L_0), diameter (d), area (A), gradient (N/m) and the calculated Young's modulus are presented in table F.4.1.1 – 18.

Table F.4.1.1: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 160 g Bloom, type A (N = 13).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01957	0,01620	0,0002060	759,2	72,12
2	0,01971	0,01651	0,0002140	712,9	65,67
3	0,01954	0,01689	0,0002239	738,9	64,48
4	0,01973	0,01681	0,0002218	682,4	60,69
5	0,01946	0,01662	0,0002168	658,2	59,07
6	0,01958	0,01670	0,0002189	717,7	64,19
7	0,01938	0,01630	0,0002086	645,5	59,98
10	0,01938	0,01649	0,0002135	765,0	69,46
11	0,01939	0,01664	0,0002174	735,0	65,57
12	0,01952	0,01665	0,0002176	753,0	67,54
13	0,01964	0,01656	0,0002153	765,0	69,79
14	0,01932	0,01664	0,0002174	678,0	60,26
15	0,01944	0,01664	0,0002174	710,0	63,50
Average					**64,8**
± S.D.					**4,1**

Table F.4.1.2: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 160 g Bloom, type B (N = 12).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01971	0,01666	0,0002179	657,1	59,45
2	0,01941	0,01651	0,0002140	699,3	63,43
3	0,01976	0,01667	0,0002181	751,9	68,11
4	0,01944	0,01648	0,0002132	710,6	64,80
6	0,02001	0,01688	0,0002237	656,0	58,69
8	0,01956	0,01666	0,0002179	754,3	67,72
9	0,01935	0,01677	0,0002208	733,0	64,25
10	0,01943	0,01702	0,0002274	678,0	57,93
11	0,01932	0,01689	0,0002239	703,0	60,65
12	0,01957	0,01695	0,0002255	738,0	64,04
13	0,01953	0,01664	0,0002174	742,0	66,67
14	0,01906	0,01693	0,0002250	702,0	59,47
Average					**62,9**
± S.D.					**3,6**

Table F.4.1.3: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01954	0,01693	0,0002250	845,0	73,38
6	0,01960	0,01662	0,0002168	796,0	71,95
7	0,01963	0,01666	0,0002179	782,0	70,45
8	0,01960	0,01646	0,0002127	718,0	66,17
12	0,01931	0,01711	0,0002298	849,0	71,34
16	0,01954	0,01680	0,0002216	823,0	72,58
Average					**70,98**
± S.D.					**2,56**

Table F.4.1.4: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01885	0,01673	0,0002197	682,0	58,51
2	0,01936	0,01695	0,0002255	713,0	61,20
3	0,01850	0,01698	0,0002263	703,0	57,46
12	0,01946	0,01694	0,0002253	834,0	72,05
13	0,01943	0,01664	0,0002174	795,0	71,07
15	0,01953	0,01678	0,0002210	802,0	70,86
Average					**65,2**
± S.D.					**6,8**

Table F.4.1.5: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01932	0,01702	0,0002274	920,0	78,16
3	0,01948	0,01658	0,0002158	915,0	82,60
4	0,01932	0,01650	0,0002137	892,0	80,64
6	0,01958	0,01674	0,0002200	857,0	76,28
7	0,01937	0,01669	0,0002187	897,0	79,46
8	0,01956	0,01665	0,0002176	921,0	82,78
Average					**80,0**
± S.D.					**2,5**

Table F.4.1.6: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01950	0,01661	0,0002166	838,0	75,45
3	0,01964	0,01652	0,0002142	835,0	76,55
4	0,01930	0,01681	0,0002218	770,0	67,00
5	0,01944	0,01662	0,0002168	823,0	73,78
7	0,01963	0,01712	0,0002301	821,0	70,05
8	0,01939	0,01660	0,0002163	805,0	72,16
Average					**72,5**
± S.D.					**3,6**

Table F.4.1.7: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	**L_0 (m)**	**d (m)**	**A (m^2)**	**N/m**	**E (kPa)**
1	0,01928	0,01675	0,0002202	895,0	78,35
2	0,01895	0,01688	0,0002237	755,0	63,96
4	0,01935	0,01677	0,0002208	926,0	81,16
5	0,01915	0,01681	0,0002218	961,0	82,96
6	0,01939	0,01644	0,0002122	897,0	81,98
7	0,01955	0,01680	0,0002216	958,0	84,53
Average					**78,8**
± S.D.					**7,6**

Table F.4.1.8: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	**L_0 (m)**	**d (m)**	**A (m^2)**	**N/m**	**E (kPa)**
1	0,01934	0,01655	0,0002150	807,0	72,59
2	0,01968	0,01667	0,0002181	761,0	68,65
3	0,01962	0,01638	0,0002106	762,0	70,98
4	0,01932	0,01671	0,0002192	810,0	71,40
6	0,01948	0,01644	0,0002122	742,0	68,13
8	0,01945	0,01655	0,0002150	637,0	57,62
Average					**68,2**
± S.D.					**5,5**

Table F.4.1.9: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 50 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	**L_0 (m)**	**d (m)**	**A (m^2)**	**N/m**	**E (kPa)**
1	0,01907	0,01682	0,0002221	917,0	78,74
2	0,01953	0,01699	0,0002266	1016,0	87,57
4	0,01942	0,01680	0,0002216	940,0	82,39
6	0,01932	0,01674	0,0002200	1104,0	96,96
7	0,01920	0,01678	0,0002210	1053,0	91,47
8	0,01899	0,01677	0,0002208	970,0	83,44
Average					**86,8**
± S.D.					**6,7**

Table F.4.1.10: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 50 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01902	0,01683	0,0002224	668,0	57,14
2	0,01895	0,01641	0,0002114	742,0	66,52
3	0,01907	0,01678	0,0002210	899,0	77,56
5	0,01917	0,01694	0,0002253	898,0	76,42
7	0,01908	0,01667	0,0002181	889,0	77,76
8	0,01896	0,01665	0,0002176	871,0	75,89
Average					**71,9**
± S.D.					**8,4**

Table F.4.1.11: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01948	0,01671	0,0002192	1423,0	126,47
3	0,01936	0,01669	0,0002187	1176,0	104,12
4	0,01935	0,01684	0,0002226	1275,0	110,82
6	0,01947	0,01684	0,0002226	1136,0	99,36
7	0,01958	0,01679	0,0002213	1317,0	116,53
8	0,01973	0,01644	0,0002122	1146,0	106,57
Average					**110,6**
± S.D.					**9,7**

Table F.4.1.12: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01983	0,01679	0,0002213	1387,0	124,29
3	0,01953	0,01671	0,0002192	1412,0	125,81
4	0,01966	0,01688	0,0002237	1406,0	123,58
5	0,01944	0,01672	0,0002195	1398,0	123,84
6	0,01977	0,01692	0,0002247	1390,0	122,28
7	0,01962	0,01689	0,0002239	1343,0	117,66
Average					**122,9**
± S.D.					**2,8**

Table F.4.1.13: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01951	0,01673	0,0002197	1661,0	147,49
3	0,01932	0,01692	0,0002247	1435,0	123,36
4	0,01948	0,01691	0,0002245	1503,0	130,43
6	0,01954	0,01692	0,0002247	1551,0	134,85
8	0,01943	0,01666	0,0002179	1557,0	138,85
Average					**135,0**
± S.D.					**9,0**

Table F.4.1.14: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01874	0,01688	0,0002237	1235,0	103,47
3	0,01924	0,01690	0,0002242	1283,0	110,10
4	0,01915	0,01697	0,0002261	1213,0	102,75
5	0,01943	0,01688	0,0002237	1208,0	104,94
6	0,01955	0,01693	0,0002250	1108,0	96,27
8	0,01904	0,01697	0,0002261	1265,0	106,54
Average					**104,0**
± S.D.					**4,6**

Table F.4.1.15: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01955	0,01665	0,0002176	1664,0	149,49
3	0,01974	0,01688	0,0002237	1667,0	147,12
4	0,01945	0,01682	0,0002221	1695,0	148,45
6	0,01968	0,01687	0,0002234	1680,0	147,99
7	0,01945	0,01665	0,0002176	1609,0	143,81
8	0,01973	0,01669	0,0002187	1683,0	151,85
Average					**148,1**
± S.D.					**2,7**

Table F.4.1.16: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
1	0,01937	0,01688	0,0002237	1292,0	111,89
3	0,01937	0,01684	0,0002226	1230,0	107,02
4	0,01941	0,01678	0,0002210	1297,0	113,90
5	0,01925	0,01689	0,0002239	1293,0	111,15
7	0,01956	0,01680	0,0002216	1274,0	112,47
8	0,01944	0,01673	0,0002197	1295,0	114,58
Average					**111,8**
± S.D.					**2,7**

Table F.4.1.17: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 260 g Bloom, type A (N = 4).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01986	0,01684	0,0002226	1750,0	156,12
3	0,01971	0,01690	0,0002242	1781,0	156,57
4	0,01961	0,01688	0,0002237	1739,0	152,46
5	0,01962	0,01692	0,0002247	1783,0	155,66
Average					**155,2**
± S.D.					**1,9**

Table F.4.1.18: Calculation of Young's modulus (E) from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	L_0 (m)	d (m)	A (m^2)	N/m	E (kPa)
2	0,01946	0,01670	0,0002189	1312,0	116,62
3	0,01976	0,01679	0,0002213	1349,0	120,46
4	0,01944	0,01685	0,0002229	1296,0	113,04
6	0,01944	0,01672	0,0002195	1307,0	115,78
7	0,01941	0,01674	0,0002200	1323,0	116,74
8	0,01947	0,01681	0,0002218	1338,0	117,44
Average					**116,7**
± S.D.					**2,4**

F.4.2 Force and strain at break

The height (L_0), distance, strain and break are presented in table F.4.2.1 – 18.

Table F.4.2.1: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 160 g Bloom, type A (N = 13).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,57	3,223	16,5	0,452
2	19,71	4,438	22,5	0,661
3	19,54	5,170	26,5	0,806
4	19,73	6,036	30,6	0,962
5	19,46	5,219	26,8	0,790
6	19,58	5,492	28,0	0,843
7	19,38	5,739	29,6	0,883
8	19,67	3,123	15,9	0,398
10	19,38	5,647	29,1	0,927
11	19,39	3,623	18,7	0,509
12	19,52	5,266	27,0	0,817
14	19,32	4,3	22,3	0,576
15	19,44	3,989	20,5	0,544
Average			**24,2**	**0,705**
± S.D.			**6,8**	**0,191**

Table F.4.2.2: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 160 g Bloom, type B (N = 12).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,71	2,633	13,4	0,340
2	19,41	5,389	27,8	0,833
3	19,76	5,981	30,3	0,957
4	19,44	3,846	19,8	0,533
5	19,63	5,724	29,2	0,930
6	20,01	6,241	31,2	0,962
7	19,33	3,019	15,6	0,389
8	19,56	4,626	23,7	0,689
9	19,35	5,324	27,5	0,792
10	19,43	4,107	21,1	0,588
13	19,53	5,986	30,7	0,947
14	19,06	6,014	31,6	0,913
Average			**25,1**	**0,739**
± S.D.			**6,3**	**0,227**

Table F.4.2.3: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,54	2,859	14,6	0,413
2	18,84	4,276	22,7	0,419
5	19,57	3,342	17,1	0,441
10	19,72	2,926	14,8	0,462
12	19,31	2,964	15,3	0,46
16	19,54	2,815	14,4	0,427
Average			**16,5**	**0,437**
± S.D.			**3,2**	**0,021**

Table F.4.2.4: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
2	19,36	3,508	18,1	0,481
3	18,50	3,953	21,4	0,41
5	19,27	3,451	17,9	0,426
9	19,62	3,455	17,6	0,541
12	19,46	3,278	16,8	0,498
16	19,26	3,023	15,7	0,433
Average			**17,9**	**0,465**
± S.D.			**1,9**	**0,050**

Table F.4.2.5: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,32	5,114	26,5	0,864
3	19,48	4,995	25,6	0,8
5	19,53	3,025	15,5	0,528
6	19,58	3,099	15,8	0,535
7	19,37	3,983	20,6	0,558
8	19,56	5,928	30,3	1,049
Average			**21,7**	**0,707**
± S.D.			**6,5**	**0,237**

Table F.4.2.6: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,48	2,836	14,6	0,421
2	19,50	3,562	18,3	0,558
4	19,30	2,856	14,8	0,416
5	19,44	2,962	15,2	0,427
7	19,63	3,708	18,9	0,572
8	19,39	5,693	29,4	0,781
Average			**18,5**	**0,529**
± S.D.			**5,6**	**0,142**

Table F.4.2.7: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
2	18,95	2,489	13,1	0,484
3	19,58	2,53	12,9	0,477
4	19,35	2,672	13,8	0,521
5	19,15	3,211	16,8	0,488
6	19,39	2,609	13,5	0,496
7	19,55	2,446	12,5	0,476
Average			**13,8**	**0,490**
± S.D.			**1,5**	**0,017**

Table F.4.2.8: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,34	3,853	19,9	0,459
4	19,32	2,911	15,1	0,449
5	19,20	2,96	15,4	0,454
6	19,48	2,915	15,0	0,45
7	19,50	3,098	15,9	0,489
8	19,45	3,148	16,2	0,487
Average			**16,2**	**0,465**
± S.D.			**1,9**	**0,018**

Table F.4.2.9: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 50 wt. % corn oil, gelatin 160 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,07	2,048	10,7	0,403
3	18,90	2,136	11,3	0,428
4	19,42	2,149	11,1	0,45
5	19,31	2,418	12,5	0,494
6	19,32	2,338	12,1	0,486
8	18,99	2,482	13,1	0,544
Average			**11,8**	**0,468**
± S.D.			**0,9**	**0,051**

Table F.4.2.10: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 50 wt. % corn oil, gelatin 160 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,02	2,854	15,0	0,487
3	19,07	2,728	14,3	0,473
4	19,38	2,712	14,0	0,475
5	19,17	2,755	14,4	0,477
6	18,91	2,996	15,8	0,506
8	18,96	4,03	21,3	0,572
Average			**15,8**	**0,498**
± S.D.			**2,8**	**0,038**

Table F.4.2.11: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,46	7,512	38,6	2,809
3	19,36	9,268	47,9	3,921
5	19,73	8,761	44,4	3,261
7	19,58	9,327	47,6	3,952
8	19,73	9,426	47,8	4,007
Average			**45,3**	**3,590**
± S.D.			**4,0**	**0,532**

Table F.4.2.12: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 0 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,38	4,912	25,3	1,373
2	19,83	3,555	17,9	0,915
4	19,66	5,597	28,5	1,583
5	19,44	5,666	29,1	1,568
7	19,62	3,158	16,1	0,783
8	19,67	6,468	32,9	2,072
Average			**25,0**	**1,382**
± S.D.			**6,6**	**0,475**

Table F.4.2.13: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,57	4,838	24,7	1,622
3	19,32	7,927	41,0	2,949
4	19,48	7,354	37,8	2,626
5	19,24	5,579	29,0	1,624
6	19,54	4,916	25,2	1,631
8	19,43	6,007	30,9	2,102
Average			**31,4**	**2,092**
± S.D.			**6,7**	**0,578**

Table F.4.2.14: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 10 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,38	5,365	27,7	1,52
2	18,74	4,711	25,1	1,193
3	19,24	3,95	20,5	1,01
4	19,15	3,298	17,2	0,817
5	19,43	4,947	25,5	1,291
6	19,55	3,778	19,3	0,964
Average			**22,6**	**1,133**
± S.D.			**4,1**	**0,254**

Table F.4.2.15: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 260 g Bloom, type A (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
2	19,55	3,894	19,9	1,22
3	19,74	4,003	20,3	1,232
4	19,45	3,118	16,0	0,931
5	19,51	3,781	19,4	1,118
7	19,45	3,476	17,9	1,057
8	19,73	3,849	19,5	1,185
Average			**18,8**	**1,124**
± S.D.			**1,6**	**0,115**

Table F.4.2.16: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 20 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,37	2,855	14,7	0,645
2	19,51	2,936	15,0	0,667
4	19,41	2,684	13,8	0,604
5	19,25	2,894	15,0	0,64
6	19,4	3,467	17,9	0,796
7	19,56	3,079	15,7	0,692
Average			**15,4**	**0,674**
± S.D.			**1,4**	**0,067**

Table F.4.2.17: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 260 g Bloom, type A (N = 4).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
1	19,71	2,871	14,6	0,879
4	19,61	2,78	14,2	0,88
5	19,62	2,683	13,7	0,83
6	19,56	2,72	13,9	0,825
Average			**14,1**	**0,854**
± S.D.			**0,4**	**0,030**

Table F.4.2.18: Calculation of force and strain at break from texture analyzing of filled gel emulsions containing 40 wt. % corn oil, gelatin 260 g Bloom, type B (N = 6).

Sample	Height (mm)	Distance (mm)	Strain %	Force (N)
2	19,46	2,27	11,7	0,521
4	19,44	2,496	12,8	0,573
5	19,58	2,349	12,0	0,542
6	19,44	2,405	12,4	0,543
7	19,41	2,335	12,0	0,516
8	19,47	2,393	12,3	0,544
Average			**12,2**	**0,540**
± S.D.			**0,4**	**0,020**

Appendix G: *In vitro* dissolution studies of filled gel emulsions

G.1. Dissolution studies of 260 g Bloom gelatin type A and B with 40 wt.% corn oil at 37 °C

The dissolution studies for filled gel emulsions with 260 g Bloom gelatin type A and type B and 40 wt.% corn oil were performed as two independent experiments, each with three replications for gelatin type A and three for gelatin type B (N = 3). Three replications for gelatin A and three replications for gelatin B were studied at the same time in the dissolution unit. A total of six replications (N = 6) for each gelatin type were obtained from the experiments.

Table G.1.1-2 presents average percentage dissolved acetaminophen, average concentration of acetaminophen and the average absorbance from the two independent experiments. The standard curves for both experiments are included. Raw data can be found on the attached DVD.

Table G.1.1: Dissolution study of 260 g Bloom gelatin type A, with 40 wt.% corn oil and acetaminophen (N = 6)

Time (min)	Average absorbance (nm)	Concentration dissolved acetaminophen (mM/L)	Percentage dissolved acetaminophen	Standard deviation
2	0,0731	0,0076	7,03 %	0,20 %
4	0,1016	0,0109	9,77 %	0,29 %
7	0,1445	0,0160	13,89 %	0,34 %
10	0,1827	0,0205	17,57 %	0,32 %
14	0,2211	0,0251	21,26 %	0,31 %
18	0,2608	0,0298	25,07 %	0,31 %
24	0,3070	0,0353	29,52 %	0,31 %
30	0,3499	0,0404	33,64 %	0,31 %
40	0,4075	0,0472	39,18 %	0,31 %
60	0,4947	0,0575	47,55 %	0,27 %
80	0,5794	0,0676	55,70 %	0,30 %
140	0,7163	0,0838	68,86 %	0,37 %
300	0,8631	0,1013	82,97 %	0,72 %
1160	1,0402	0,1227	100,00 %	0,68 %

Table G.1.2: Dissolution study of 260 g Bloom gelatin type B, with 40 wt.% corn oil and acetaminophen (N = 6)

Time (min)	Average absorbance (nm)	Concentration dissolved acetaminophen (mM/L)	Percentage dissolved acetaminophen	Standard deviation
2	0,0616	0,0062	6,83 %	0,26 %
4	0,0904	0,0096	10,02 %	0,29 %
7	0,1346	0,0149	14,92 %	0,28 %
10	0,1863	0,0210	20,65 %	0,23 %
14	0,2539	0,0291	28,15 %	0,35 %
18	0,3380	0,0392	37,47 %	0,70 %
24	0,4372	0,0506	48,47 %	1,03 %
30	0,5592	0,0657	61,99 %	1,19 %
40	0,7194	0,0843	79,74 %	0,96 %
60	0,8604	0,1014	95,37 %	0,87 %
80	0,8783	0,1053	97,36 %	0,43 %
140	0,9052	0,1086	100,34 %	0,21 %
300	0,9278	0,1113	102,85 %	0,34 %
1160	0,9021	0,1082	100,00 %	0,07 %

The samples were normalized to the maximal absorbance measured

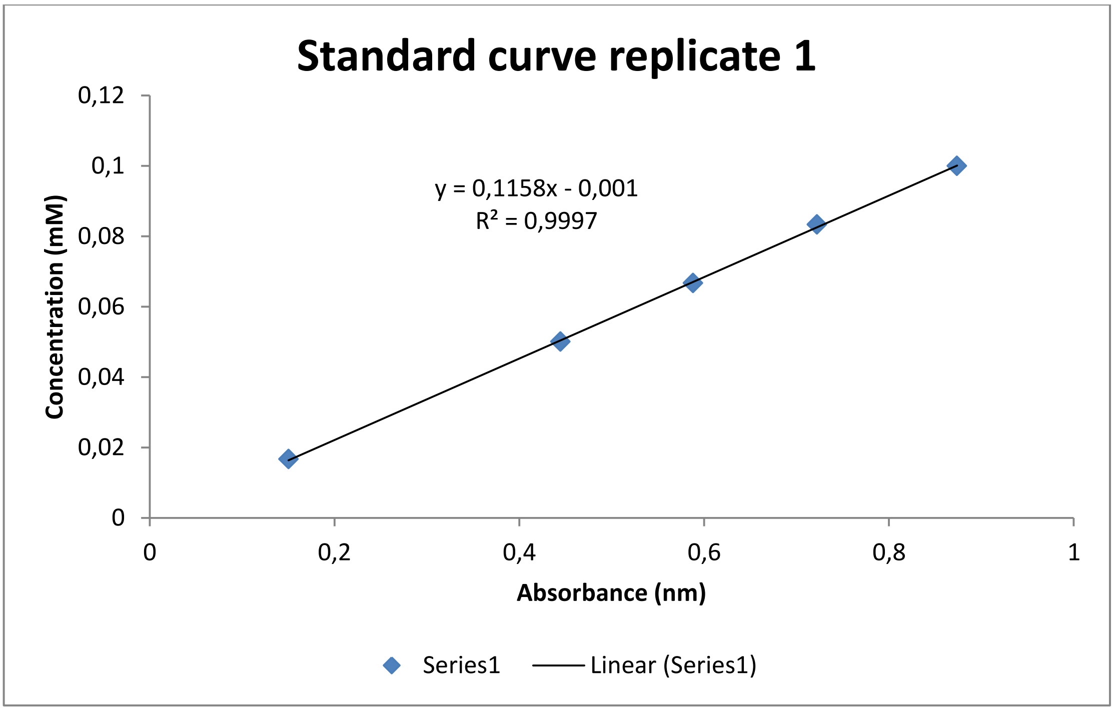

Figure G.1.1: Concentration (mM) of acetaminophen as a function of absorbance (nm), given as a standard curve

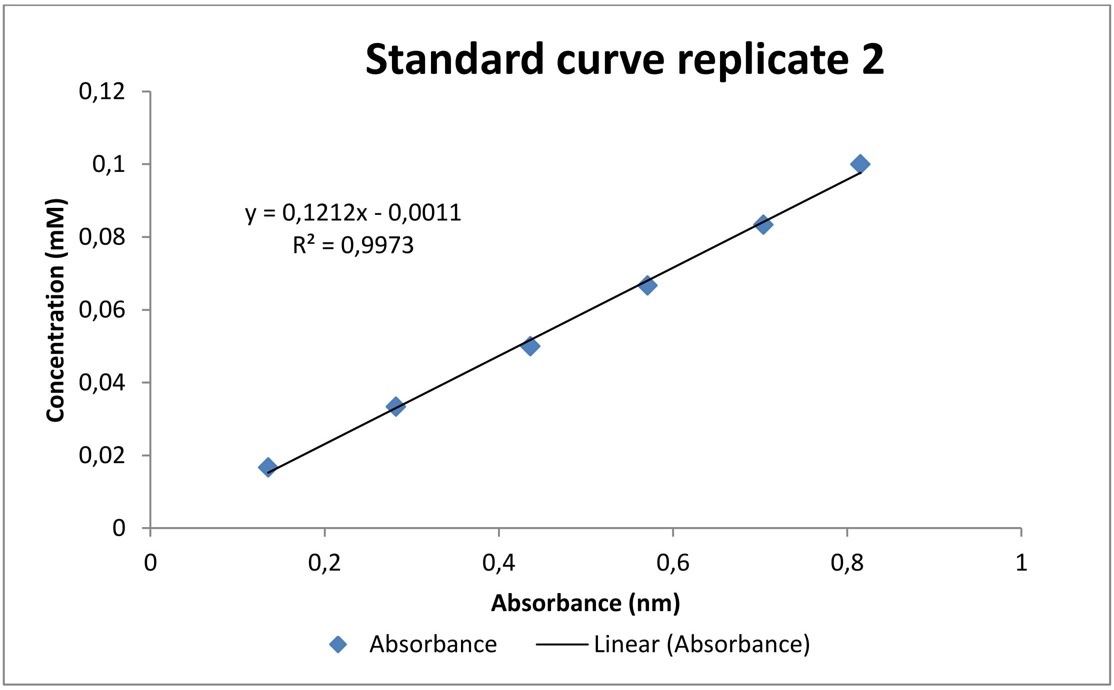

Figure G.1.2: Concentration (mM) of acetaminophen as a function of absorbance (nm), given as a standard curve

G.2. Dissolution study of 260 g Bloom gelatin type A with 40 wt.% corn oil and 0,5 wt.% polysorbate 80 at 37 °C

The dissolution study of filled gel emulsions with 260 g Bloom gelatin type A, 40 wt.% corn oil an 0,5 wt.% polysorbate 80 was performed as one experiment with 6 replicates (N = 6).

Table G.2.1 presents average percentage dissolved acetaminophen, average concentration of acetaminophen and the average absorbance from the experiment. The standard curve is included. Raw data can be found on the attached CD.

Table G.2.1: Dissolution study of 260 g Bloom gelatin type A, with 40 wt.% corn oil, 0,5 wt.% polysorbate 80 and acetaminophen (N = 6)

Time (min)	Average absorbance (nm)	Concentration dissolved acetaminophen (mM/L)	Percentage dissolved acetaminophen	Standard deviation
2	0,0322	0,0028	3,95 %	1,91 %
4	0,0570	0,0058	6,99 %	1,72 %
7	0,0867	0,0094	10,63 %	1,85 %
10	0,1154	0,0129	14,15 %	2,17 %
14	0,1521	0,0173	18,66 %	2,83 %
18	0,1927	0,0223	23,63 %	3,92 %
24	0,2517	0,0294	30,86 %	4,82 %
30	0,2849	0,0334	34,94 %	5,26 %
40	0,3710	0,0439	45,50 %	5,78 %
60	0,4748	0,0564	58,23 %	6,96 %
80	0,5647	0,0673	69,26 %	10,64 %
100	0,6468	0,0773	79,32 %	10,44 %
140	0,8138	0,0975	99,80 %	17,14 %

The samples were normalized to the maximal absorbance on the standard curve

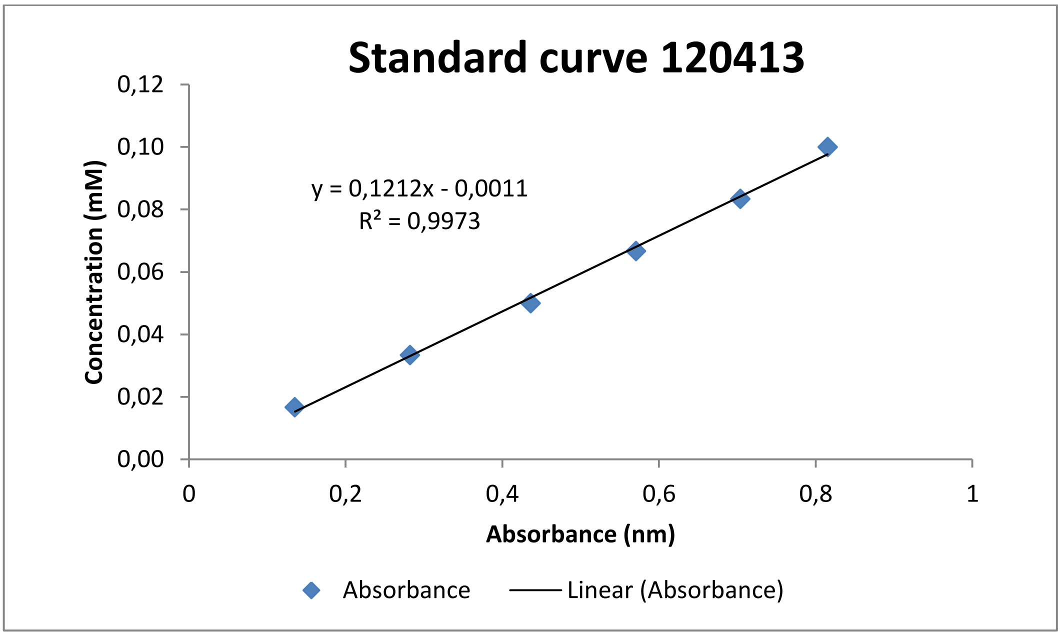

Figure G.2.1: Concentration (mM) of acetaminophen as a function of absorbance (nm), given as a standard curve

G.3. Control study of 260 g Bloom gelatin type A and B with 40 wt.% corn oil at 20°C

A control study with 260 g Bloom gelatin type A and B with 40 wt.% corn oil was performed at 20 °C. Each type of gelatin had two replicates (N = 2).

Table G.3.1-2 presents average percentage dissolved acetaminophen, average concentration of acetaminophen and the average absorbance from the experiment. The standard curve is included. Raw data can be found on the attached CD.

Table G.3.1: Dissolution study of 260 g Bloom gelatin type A, with 40 wt.% corn oil and acetaminophen at 20 °C (control) (N = 2)

Time (min)	Average absorbance (nm)	Concentration dissolved acetaminophen (mM/L)	Percentage dissolved acetaminophen	Standard deviation
60	0,3898	0,0482	47,38 %	1,10 %
120	0,5437	0,0668	66,09 %	0,81 %
180	0,6548	0,0802	79,60 %	1,19 %
240	0,7339	0,0898	89,22 %	1,34 %
300	0,7796	0,0953	94,77 %	2,09 %

Table G.3.2: Dissolution study of 260 g Bloom gelatin type B, with 40 wt.% corn oil and acetaminophen at 20 °C (control) (N = 2)

Time (min)	Average absorbance (nm)	Concentration acetaminophen (mM/L)	Percentage dissolved acetaminophen	Standard deviation
60	0,3830	0,0474	46,55 %	1,76 %
120	0,5390	0,0662	65,52 %	0,04 %
180	0,6317	0,0774	76,79 %	1,19 %
240	0,7143	0,0874	86,83 %	1,34 %
300	0,7799	0,0953	94,81 %	2,09 %

The samples were normalized to the maximal absorbance of the standard curve.

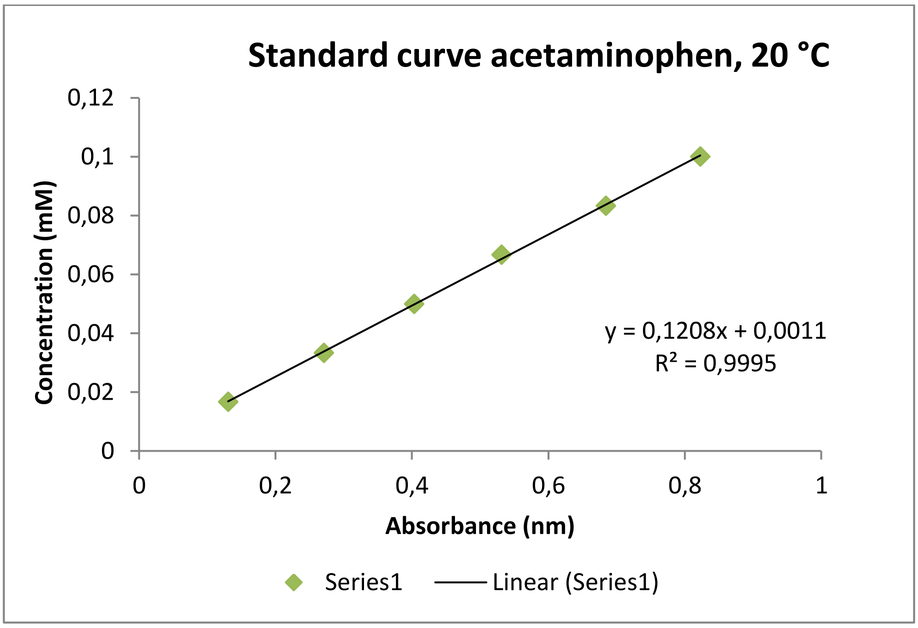

Figure G.3.1: Concentration (mM) of acetaminophen as a function of absorbance (nm), given as a standard curve