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HIPE-TEMPLATED OLEOGELS STABILIZED BY PROTEIN-POLYSACCHARIDE: PREPARATION AND CHARACTERIZATION

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ABSTRACT

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Tiivistelmä/Referat – Abstract

The high intake of saturated and trans-fats could pose a chronic threat to human health. During the past decades, some fat alternatives in the form of oleogels have been developed to substitute the use of solid fats in food formulation. Generally, oleogels can be formed using chemically modified organogelators such as ethyl cellulose. In this work, we aimed to produce oleogels via high internal phase emulsion (HIPE, φoil=0.82) templates stabilized using food ingredients, i.e. protein and polysaccharide. The fabrication of HIPEs using a mixture of dispersions of two biopolymers, i.e. sodium caseinate (SC) and alginate (ALG), was optimized by varying the protein: polysaccharide ratios (i.e. 6:1, 8:1, 10:1, 12:1) and the pH (i.e. 5.5, 6.0, 7.0). Dry-heat treatment of the SC:ALG mixture at pH 7.0 was also employed for the preparation of the colloidal dispersions as a comparison to the HIPEs and oleogels stabilized by the mixtures adjusted at different pH values. HIPEs and oleogels exhibited better properties as shown by macrostructure, microstructure, physical stability against coalescence and oiling off, and rheological analysis when the pH value and the ratio of SC:ALG increased. The HIPEs and oleogels stabilized by dry-heat treated SC:ALG mixtures showed comparable results with those stabilized by SC:ALG mixed dispersions at pH 7.0. These results can provide a fundamental insight for future promising application of oleogels in fat-based product formulation.

Avainsanat – Nyckelord – Keywords

Oleogel; Structuring oil; HIPE; Emulsion-templated; Sodium Caseinate; Alginate;

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PREFACE

This is a Master's thesis carried out during the exchange study at the faculty of bioengineering, Ghent University from February 2017 to July 2017. The thesis research was supervised by Prof. dr. ir. Paul Van der Meeren and tutored by Wahyu Wijaya from Ghent University.

I would like to thank all the people who have helped and supported me during this intensive practical learning.

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Last but not least, I take this opportunity to thank my friends and my family for their continuous support, encouragement, and company.

LIST OF ABBREVIATIONS

Abbreviation	Definition
ALG	alginate
SC	sodium caseinate
HIPE	high internal phase emulsion
O/W	oil-in-water
IEP	Isoelectronic point
G′	elastic modulus
G″	viscous modulus
LVR	linear viscoelastic region
pfg-NMR	pulsed field gradient nuclear magnetic resonance
PGSTE	pulsed-gradient stimulated-echo

Contents

1. INTRODUCTION

With the increasing awareness of health risks (e.g. obesity, diabetes, and cardiovascular diseases) brought by high consumption of hydrogenated oils (trans-fats) and saturated fats, there is a growing need to substitute these solid lipids with alternatives that contain more monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) (Mozaffarian & Clarke 2009). Vegetable oils such as sunflower oil, rapeseed oil, and olive oil are rich in MUFAs and PUFAs, and thereby are promising candidates. However, the texture and sensory properties of lipid-based food products, such as baked goods, margarine, and fat spreads might be greatly sacrificed if the saturated and trans-fats are directly eliminated and replaced by unsaturated fats (liquid oils). Recently, structuring liquid oils into 3D systems has received a great deal of interest as it can possibly substitute or reduce the use of solid fats without significantly compromising the textural properties of food (Mezzenga 2011).

Oleogels are one of the novel structures that incorporate a high amount of liquid oil (>97 wt %) within a physical network and exhibit solid-like properties (Patel et al. 2014). Because of the wide availability of food-grade biopolymers, indirect oleogelation using hydrophilic polymers is preferred rather than direct oleogelation using chemically modified hydrophobic oleogelators (e.g. ethyl cellulose). In indirect oleogelation, emulsions are generally employed as a template for the oleogel formation. A solution of food polymers is initially dispersed in water to emulsify the liquid oil. Afterwards, the water is completely removed from the system, leaving a product with a polymeric network where the oil droplets are tightly packed. Then, this product can be further sheared into an oleogel (Patel et al. 2014).

Proteins and polysaccharides are the two groups of biopolymers that can stabilize oleogels via indirect oleogelation (Stone and Nickerson 2012). Proteins have surface activity due to the coexistence of amino and carboxyl moieties in their molecules, and hence are able to stabilize oil-in-water emulsions (Morr 1981). Polysaccharides, because of their inherently hydrophilic nature, can play a role as thickening agents in food and can further improve the

stabilizing performance of proteins via complexation (Stone and Nickerson 2012). Recently, Patel et al. (2014) and Tavernier et al. (2017) studied two different methods using proteins and polysaccharides to obtain oleogels. The former used a layer-by-layer technique where the oil was first dispersed into a solution of protein, followed by an addition of polysaccharide during the emulsification. Phase separation may occur when homogenizing the primary emulsion with the addition of another viscous water phase. The latter authors designed a method to fabricate oleogels by templating of concentrated emulsions using preformed mixtures of the two biopolymers. Meanwhile, Tavernier et al. (2017) also concluded that the ratio and concentration of the two biopolymers and the pH are the most important factors that influence the mixture formation and thereby the stability of the emulsions and the oleogels.

The use of a low pH and a relatively high amount of water (40%) in the emulsions were the main limitations of the previous work. A low pH is less applicable in food formulation, while a high fraction of water requires a high temperature and a prolonged time during the drying process, which might cause oil deterioration. In our study, we produced high internal phase emulsions (HIPEs) by increasing the fraction of oil up to 80% which was beyond the maximum close packing of equal sphere particles (φ=0.74) (Capron and Cathala 2013). With these HIPEs as templates, the drying behavior of the samples was prominently improved as the drying method could be operated at low temperature within shorter time.

In this work, we aimed to produce oleogels via the HIPE-templating approach. We investigated the effect of various SC:ALG ratios at different pH values on the production of stable oleogels. The characterization of the mixed dispersions was performed by dynamic light scattering and ζ-potential measurement, whereas the emulsions were characterized by microscopy, static light scattering, analytical photo-centrifugation, rheology, and pfg-NMR. Last but not least, experiments on oleogels by centrifugation, rheology and pfg-NMR were carried out.

2. LITERATURE REVIEW

2.1. Structured liquid oil as solid fat alternatives

Nowadays, finding healthier fat alternatives is a great challenge for food manufacturers. Fat in the form of partially hydrogenated oils (PHOs) or known as trans-fat is suspected to have harmful effects on human health (Jebb et al. 2010). In 2013, the claim was pronounced by the decision of US Food and Drug Administration (FDA) for removing the GRAS (generally recognized as safe) status of PHOs as these artificial fats are currently being the most significant dietary source of fat (Wang, Gravelle, Blake, & Marangoni, 2016). Other fats high in saturated fatty acids, such as palm fat and animal fat, are considered as high calorie foods which can increase the risk of cardiovascular diseases (Siri-Tarino et al. 2015). In response to this, dietary guidelines released by world-wide institutions recommend to raise the consumption of unsaturated fatty acids from vegetable sources.

However, direct replacement of saturated and trans-fats with unsaturated fats will significantly affect the textural (e.g. plasticity, solidity, hardness) and organoleptic properties (e.g. taste, flavor and mouthfeel) (Stortz et al. 2012). Therefore, the manufacturers are now faced with the technical challenge to produce fat alternatives based on unsaturated fats (liquid oils) which mimic the functionality of solid fats. In addition, novel oil structuring is developed to match the solid-like properties of saturated fats. Thus, some technical problems such as oil-leakage or poor texture during fat-based products formulation can be avoided.

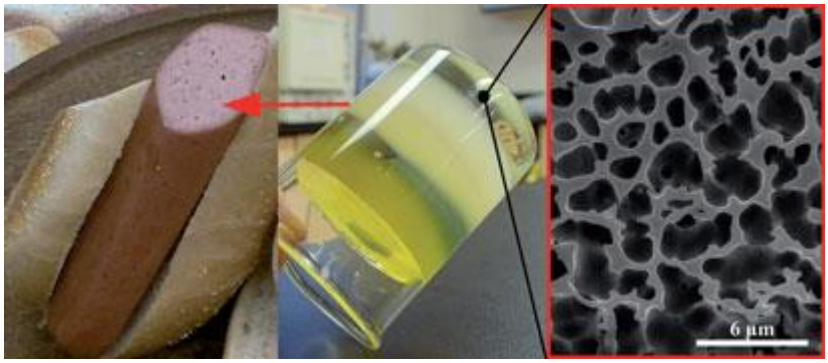

Figure 1. One of the oleogel applications in frankfurters-type sausages (Zetzl et al. 2012).

In recent years, the structuring of unsaturated fats (liquid oils) has been a promising approach to replace hard-stock fats and trans-fat in food products. Various systems have been developed to impart solid-like properties by entrapping liquid oil in the 3D matrix, such as crystalline particles (e.g. fatty acids, wax), self-assembled structures of low molecular weight compounds (e.g. sphingolipids, or phytosterols, such as β-oryzanol), polymer oleogelators (e.g. ethyl cellulose, EC) and structured emulsions (e.g. monoglycerides, MG) (Marangoni 2012; Marangoni and Garti 2015). For the application in foods (Figure 1), oleogelators are strictly regulated and the use of food polymers is preferred rather than other non-food oleogelators.

2.2. Oil structuring techniques using biopolymers

Recently, structuring liquid oils via the use of biopolymers could be done by two approaches, i.e. direct, using a hydrophobic polymer as oleogelator and indirect, using a structural template of hydrophilic or amphiphilic polymers (i.e. emulsion template) (Patel 2015). In the past few years, the exploration of hydrophobic polymer in oleogelation has been done extensively by using EC, the only known hydrophobic polymer allowed to use for edible purposes. EC is a semi-crystalline material obtained by chemical modification of cellulose by substituting the ethoxyl groups (Koch 1937).

The gelation of EC involves high temperature treatments above its glass transition temperature (Tg) of 130 °C (Davidovich-Pinhas, Barbut, & Marangoni, 2015), where EC can be fully dispersed in oils. When the temperature goes down, the inter-polymer

hydrogen bonds are gradually formed, resulting in a three-dimensional entangled polymer network in which the liquid oil is entrapped (Figure 1) (Laredo et al. 2011). Furthermore, the mechanical properties can be tuned in three steps: thermal annealing that involves reheating of EC oleogels below Tg (i.e. 80-100 °C), increase non-polarity of solvent resulting in improved binding affinity, and addition of surface active small molecules which improves the interfacial stability that can prevent oil expulsion from the gel matrix (Zetzl et al. 2012).

EC oleogels seem to be promising due to the fact that they can produce a high gel strength which may possess the textural properties of solid fats. EC oleogels have been applied in the reduction of saturated fats in processed meat products and the elimination of oil expulsion in model cream fillings (Stortz et al. 2012). However, the main drawback of these systems is that they require the use of high temperatures which can lead to oil quality deterioration as the unsaturated fats are very prone to oxidative damage induced by high temperature processing. In addition, consumers nowadays are more aware of using pure food ingredients than chemically synthetic ingredients or new food materials that have never been used before in foods. In this regard, indirect methods using food polymers can be a potential alternative to produce oleogels. These systems also have the ability to mimic both the functional and textural attributes of solid fats which would be an active research subject in the field of oleogelation.

2.3. Oil structuring using structured templates formed by hydrophilic biopolymers (indirect method)

Oil structuring using hydrophilic biopolymers, most of which have been recognized as daily food ingredients, is an emerging approach to produce food-grade saturated fat substitutes. Hydrophilic biopolymers can be used to form a structural framework to polar solvent-based gels, but they cannot be directly used to structure non-polar oils due to their limited dispersibility in non-polar solvents (Patel et al. 2014). The dispersibility of polymers in the solvent is essential to form stronger molecular interactions by extending and opening polymer conformation, therefore the structural framework can be assembled.

Thus, an indirect method is proposed via prehydration of the biopolymers in an aqueous phase, followed by their adsorption to the oil-water interface, after which an oil retaining structure in a dehydrated form is obtained (Patel et al. 2014).

The most forthright and conveniently applicable indirect method is using an emulsion as a template to obtain oleogels (Tavernier et al. 2017). Previously, emulsion templates for obtaining dried structures were commonly used to encapsulate flavors via spray-drying of oil-in-water emulsions to rapidly evaporate the aqueous phase. The stability of the systems against oil droplets coalescence during drying could be improved by the incorporation of polysaccharides (e.g. maltodextrin) as a filler to increase the viscosity of the water phase or by stiffening the oil-water interface via thermal, chemical or enzymatic crosslinking of adsorbed polymers (Klinkesorn et al. 2005). This system could not be defined as an oleogel because it contained for more than 30 wt% of non-oily component in the dried product, while to get a gel-like consistency, the percentage of liquid oil is usually well above 90 wt%. Thus, the percentage of structurant or oleogelator should be kept to the minimum (usually below 8 wt%) (Stortz et al. 2012).

Hydrophilic polymers such as proteins have been demonstrated to produce oil in gel structure via emulsion-templating. Previously, this approach used a chemical cross-linking process at the oil-water interface to stiffen the interfacial layer of protein, which is less accepted in food applications (Romoscanu and Mezzenga 2005). Likewise, oil gels could also be prepared by freeze-drying of emulsions stabilized by zein-stearate mixtures. This approach requires endless particle formation steps which seems less applicable for large scale production (Gao et al. 2014).

2.3.1. Preparation of emulsions stabilized by protein-polysaccharide as a template to obtain oleogels

Proteins and polysaccharides are the most common and abundant food ingredients. These two biopolymers are well documented as satisfactory stabilizing agents for food emulsions.

The emulsion stabilizing properties of proteins can even be enhanced by mixing them with suitable polysaccharides (Stone and Nickerson 2012). First, oleogels obtained by drying of emulsions stabilized by proteins and polysaccharides were introduced by Patel et al. (2014); in this approach the concentrated emulsions (40 wt%) were formed using the layer-by-layer (LbL) technique (one biopolymer is firstly introduced followed by subsequent adsorption of the second biopolymer) as shown in Figure 2. On the other hand, Tavernier et al. (2017) developed a method to produce oleogels using concentrated emulsions (oil concentration up to 60%) stabilized by preformed protein:polysaccharide mixtures as a template (Figure 3). Concentrated emulsions are generally used to produce high yield oleogels and reduce the drying time. However, the stabilization is foreseen less feasible using the LbL technique due to the difficulty of incorporating another viscous aqueous phase (polysaccharide solution) into a primary concentrated emulsion. This may lead to a phase separation during the mixing of the emulsion with the polysaccharide solution. Thus, the use of preformed protein:polysaccharide mixtures is preferred rather than the LbL techniques (Jourdain et al. 2008).

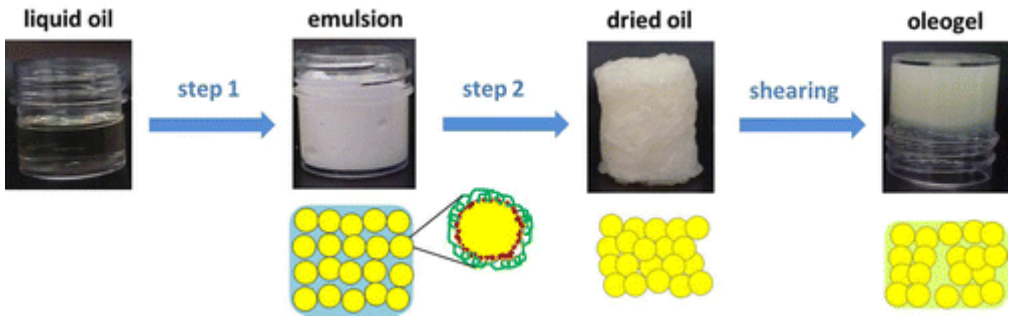

Figure 2. Schematic representation of the process where the oil-in-water emulsion is stabilized by an adsorbed layer of gelatin (red dots) and a layer of xanthan gum (curved green lines) (step 1) followed by the elimination of the aqueous phase through drying (step 2), where further shearing of the dried emulsion (oil droplets tightly packed) results in the formation of an oleogel (clusters of packed droplets) (Patel et al. 2014).

Generally, there are three simple steps proposed to obtain final oleogel products via emulsion templates stabilized by protein:polysaccharide mixtures, i.e. the electrostatic mixture formation, stabilization of the emulsion by the electrostatic mixtures, and drying the emulsion followed by shearing the dried emulsion to obtain the oleogels. Recently, oleogels could be formed using emulsion templates ($\phi_{oil} \approx 0.6$) stabilized by preformed

SPI:κ-CG electrostatic mixtures. The elimination of the water phase by conventional drying resulted in dried emulsions which could be further sheared into oleogels (Tavernier et al. 2017).

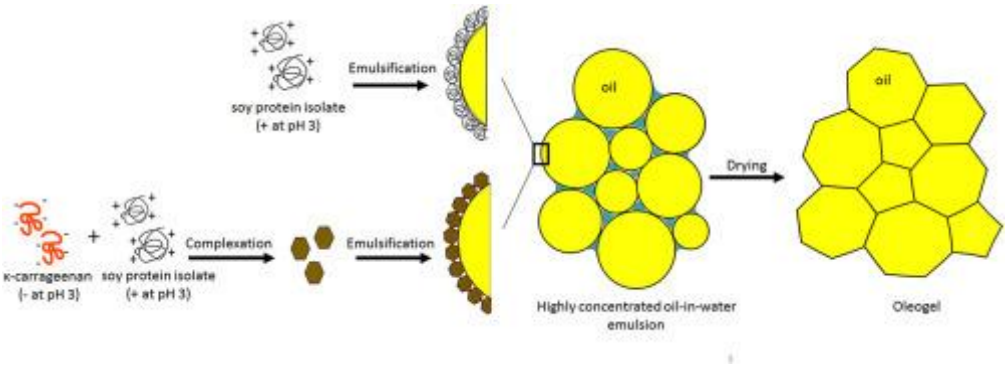

Figure 3. Schematic illustration of concentrated emulsion stabilization by SPI and SPI:κ-CG mixtures followed by the drying of the aqueous phase, resulting in the formation of an oleogel in which the oil droplets are hexagonally close-packed (Tavernier et al. 2017).

2.3.2. Factors affecting oleogel stabilization by protein:polysaccharide mixtures via emulsion templates

Since the interpolymeric interactions between proteins and polysaccharides are driven by some critical factors, these factors must be controlled to obtain a stable physical network for liquid oil entrapment. Some factors such as pH and biopolymer ratio and concentration play an essential role in the mixture formation to provide optimal emulsion and oleogel stabilization (Tavernier et al. 2017).

The formation of appropriate mixture for emulsion stabilization is mainly controlled by pH and protein:polysaccharide ratio. According to recent research that used SPI:κ-CG mixtures for oleogel stabilization via emulsion templates, the mixtures were optimally produced at a ratio of 15:1 and pH 3 to achieve stable emulsions and oleogels (Tavernier et al. 2017). The mixtures of SPI:κ-CG could be formed either at a pH lower than the IEP of SPI (pH < 4.5) or at a pH higher than the IEP (pH > 4.5). However, strong electrostatic interactions occur when κ-CG and SPI are oppositely charged (pH 2.5 (pKa of κ-CG) < pH of complexation < 4.5 (IEP of SPI)) (Ortiz et al. 2004). On the other hand, if SPI and κ-CG

carry a negative net charge which usually occurs in the higher pH ranges, segregative phase separation is expected due to strong electrostatic repulsive forces.

The success of stable oleogel formation against oiling-off was mainly determined by good physical stability of the mixtures and emulsions (Tavernier et al. 2017). Nanosized mixtures with high surface charge density were required to obtain stable emulsions. These nanomixtures were adsorbed and accumulated in the oil-water interface resulting in interfacial layer thickening, which prevents the occurrence of coalescence. Moreover, the rigidity of the emulsion interface and the stability of the emulsions are the determining factors in controlling textural properties (firmness) of oleogels using the emulsion-templated approach.

3. EXPERIMENTAL RESEARCH

3.1. Aim

The aim of this work is to produce oleogels via the HIPE-templating approach using two biopolymers, i.e. SC and ALG, as the stabilizers. The effect of SC:ALG ratios at different pH values on the formation of stable oleogels will be investigated. ζ-potentials and particle size distribution of the mixed dispersions containing two biopolymers will be characterized. The microstructure, volume-weighted average droplet size, droplet size distribution, and gravitational stability of the HIPEs will be characterized. The oil loss value of the oleogels will be investigated. Last but not least, investigation on the rheological properties and oil diffusion coefficency of both HIPEs and oleogels will also be carried out.

3.2. Materials

SC was obtained from Armor Proteines (35460 Saint-Brice-en-Cogles, France) and contained 91% protein, 4% minerals, 0.7% fat, and 0.2% carbohydrates. ALG (Algogel®) was received from Cargill R&D Vilvoorde, Belgium. Sodium azide, sodium chloride, and HCl were obtained from Sigma Aldrich (Saint-Louis, USA). NaOH was bought from VWR Chemicals (USA) and refined sunflower oil was obtained from a local supermarket. Milli-Q water was used throughout the study. All materials were used without further purification or modification, and all samples were formulated and reported on a weight-by-weight basis (g g^{-1}).

3.3. Preparation of biopolymer mixtures and conjugates

3.3.1. Preparation of stock solutions

SC and ALG powders were weighed into separate beakers at concentrations of 12% and 1%, respectively, in 100 mL Milli-Q water containing 0.02% sodium azide. These dispersions were then stirred continuously at room temperature (22–25 °C) till complete

dissolution was obtained. The solutions were then stored overnight at 5 °C to ensure complete hydration of the biopolymers.

3.3.2. Preparation of mixture dispersions

As a preliminary investigation, the stock solutions of SC and ALG were diluted and mixed together to obtain a mixed dispersion of SC:ALG with ratio 6:1. Afterwards, the effect of pH on the SC:ALG mixtures was assessed by adjusting the pH of the mixtures to 4.5, 5.5, 6.0, and 7.0 with 0.1 and 1 N HCl and 0.1 N NaOH. To investigate the effect of SC:ALG ratio (concentration of ALG was kept constant), colloidal dispersions of SC:ALG at ratios of 6:1, 8:1, 10:1, and 12:1 were prepared by diluting and mixing the stock solutions and then adjusted to pH 5.5, 6.0 and 7.0. During the adjustment of pH, the protein-polysaccharide mixtures were initially sonicated with a Sonifier 250 (Branson Ultrasonics,US) set at output 9 and duty cycle 90 (%) followed by magnetic stirring. The SC:ALG mixtures formed at pH 7.0 were also compared with SC:ALG conjugates formed by dry-heat treatment.

3.3.3. Conjugate formation

A dry-heat method was conducted for the formation of SC:ALG conjugates. The stock solution of SC was diluted and mixed with ALG solution to prepare a series of mixed dispersions of SC:ALG at ratios of 6:1, 8:1, 10:1, and 12:1 at pH 7.0. The dispersions were stored in the freezer for 8 hours. The frozen samples were then lyophilized in a freeze dryer ALPHA1-2 LDPlus (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) for 48 hours. The freeze-dried samples were transferred to a desiccator containing salt solution and placed in an oven at 60 °C for a 24-hour incubation under 74% relative humidity. After the dry-heat treatment, the SC:ALG conjugates were dissolved in Milli-Q water to obtain a series of dispersions with the same concentration series as described in section 3.2.2. The conjugate dispersions were treated with the same procedure mentioned in section 3.2.2. to facilitate the dissolution of the conjugates.

3.4. Mixture characterization

3.4.1. Z-average diameter

Measurements for particle size distribution were carried out using photon correlation spectroscopy (PCS) with a Spectrometer 100 SM (Malvern Instruments, Malvern, UK) equipped with a 15 mW He–He laser K7032 multi 8-bit correlator (Malvern Instruments, Malvern, UK). Analyses were carried out only for SC:ALG of 6:1 to investigate the effect of pH values on the mixture formation. The measurements were done at a scattering angle of 150° at 25 °C with 100 μm aperture. The Z-average mean diameter was obtained by cumulant analysis.

3.4.2. Zeta-potential characterization

ζ-potentials of the SC:ALG (6:1) mixtures formed at pH 4.5, 5.5, 6.0, and 7.0 were measured using electrophoretic light scattering with a Zetasizer IIC (Malvern Instruments, Malvern, UK) at 25 °C. The mean ζ-potential values were then directly calculated by the instrument software.

3.5. Preparation of HIPEs stabilized by SC-ALG mixtures and conjugates

HIPEs were prepared with sunflower oil (concentration of 80%, φ_{oil} = 0.82) and the previously prepared colloidal mixtures (concentration of 20%). As a preliminary investigation on the pH effect, HIPEs were obtained by homogenizing sunflower oil (φ_{oil}=0.82) in SC:ALG (6:1) aqueous dispersions formed at pH 4.5, 5.5, 6.0, and 7.0. After the selection of the pH values used, the series of SC:ALG concentrations were prepared, i.e. 6:1, 8:1, 10:1, and 12:1 at pH 5.5, 6.0 and 7.0 to investigate the effect of different ratios of SC:ALG. Finally, the same series of SC:ALG conjugates formed by dry heating was also prepared for the comparison with SC:ALG mixtures at different pH values.

All mixtures of sunflower oil and colloidal dispersions were homogenized first at 6500 rpm till the colloidal dispersions occupied the oil phase, followed by homogenization at 9500 rpm for 2 min. A dispersing tool, UltraTurrax IKA® T25 Basic (IKA-Werke GmbH

& Co. KG, Germany) equipped with a dispersing rotor S25KV-25G was used here. The resulting self-standing emulsion gels were then stored at 5 °C prior to analysis on the following day.

3.6. Characterization of HIPEs

3.6.1. Droplet size distribution

The volume-weighted average droplet size (D[4,3]) of emulsions was determined using a MasterSizer 3000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) equipped with a wet sample dispersion unit (Malvern Hydro MV, UK). Before the measurement all samples were diluted 20 times using a buffer of the same pH. In the sample port, the samples were dispersed in Milli-Q water at 1500 rpm until an obscuration of 2% to 6% was obtained. The background and sample integration times were 20 and 10 seconds, respectively. The optical properties were defined as refractive index 1.47 (sunflower oil), 1.330 (dispersant water) and absorption index 0.01, with the normal instrument calculation sensitivity and general purpose spherical particle shape selected. Results were calculated with the Mastersizer Version 5.54 software (Malvern, UK) to obtain the particle size distributions and the volume-weighted average droplet size (D[4,3]). All measurements were done in triplicate.

3.6.2. Microstructure studies

Optical microscopy techniques were utilized to study the microstructure of the samples. Optical microscopy was carried out on a Leica DM2500 microscope (Leica Microsystems, Belgium).

3.7. Preparation of oleogels from HIPE templates

HIPE samples (25 g) were dried in the oven at 37 °C for 24 h. Oleogels were prepared by shearing the dried samples for 20 s at 6500 rpm with UltraTurrax IKA® T25 Basic (IKA-Werke GmbH & Co. KG, Germany) equipped with a dispersing rotor S25KV-25G.

3.8. Characterization of oleogels

3.8.1. Oil loss value

The oil-loss value of oleogel samples was measured by centrifuging a 1.5 mL plastic centrifuge vial containing a known mass of sample (approximately 1.5g) for 12 min in an Eppendorf Centrifuge 5410, at the speed of 10000 rpm. Centrifuge vials were weighed before and after the addition of gels so that the actual sample mass could be obtained. After centrifugation, the released oil at the surface was decanted and weighed. These values were calculated as the percentage of decanted oil after centrifugation:

$$Oil\ Loss\ value\ (OL) = \frac{Amount\ of\ oil\ loss}{Initial\ amount\ of\ oil\ in\ sample}\ x\ 100\%$$

The OL value for each gel was calculated as the mean of three replicates, with standard errors for each calculated value. A high OL value suggests that the network contains a significant proportion of weakly bound oil, and that the short-term oil binding capacity of the network is low.

3.8.2. Small amplitude oscillatory shear rheology

Dynamic rheological measurements were carried out on an advanced rheometer AR 2000ex (TA Instruments, USA) equipped with a Peltier system for temperature control. A parallel plate cross-hatched geometry of 40 mm diameter was used and the geometry gap was set at 1000 μm. A range of experiments including an amplitude sweep (stress=

0.1-1000 Pa for emulsions and stress = 0.1–3000 Pa for oleogels, frequency = 1 Hz), and frequency sweeps (0.1–100 Hz, oscillatory stress = 5 Pa) were carried out at 5 °C.

3.9. Stability studies

3.9.1. Photo analytical centrifugation

The stability of the HIPE samples was evaluated by measuring the O/W phase separation under centrifugal force with an analytical photo-centrifuge (LUMiFuge 116, L. U. M. GmbH, Germany). In this study, 2 g of the sample in a 5 mL glass tube (≈20 mm measurement region) was centrifuged at 3000 rpm at 25 °C for 3 h. During the centrifugation, the near-infrared sensor scanned the sample cells over the total length. A charge coupled device (CCD) line sensor received light transmission through the sample, which showed a pattern of light flux as a function of the radial position, giving a transmission profile of the sample at a given time, from which emulsion instability, such as creaming, sedimentation, and coalescence could be detected. Quantitative data were obtained by integration of the transmission profiles between the 105–114.5 mm radial positions from the rotation center. The total integrated area was plotted against the centrifugation time. The slope of this graph was used to express the separation rate and thus the stability, with its lower value indicating a better creaming stability. The slope was directly obtained by using SEP View 4.0 Lumifuge software, through the calculation of linear regression during the initial period of steep rise of transmission before the plateau was reached. The time period chosen for each slope calculation may vary among the individual samples.

3.9.2. Nuclear Magnetic Resonance (NMR) analysis

The physical stability of the HIPE and oleogel samples was also analyzed by measuring the oil diffusion signal (PGSTE) using pulsed field gradient (pfg)-NMR. NMR measurements were performed at 20 °C on a benchtop Maran Ultra spectrometer (Oxford Instruments, Abingdon, UK) operating at 0.55 T (23.4 MHz for 1H). The measurement parameters used

in this analysis include relaxation delay (RD = 12 s), diffusion delay time (Δ = 200 ms), list of gradient strength (G from 0 to 3.17 T/m), and gradient duration (δ=1.5 ms). The profiles of oil diffusion were presented by using the signal intensity (I) as a function of q^2 (a function of the gyromagnetic ratio γ (2.675×10^8 s^{-1} T^{-1}), the gradient duration δ and the gradient strength G, $q^2 = (\delta \cdot \gamma \cdot G)^2$). The relationship between I and diffusion coefficient (D) in the sample is described as below:

$$I = I_0 * \exp\left(-D * q^2 * \left(\Delta - \frac{\delta}{3}\right)\right)$$

Whereby I_0 is the echo intensity in the absence of gradient pulses.

The value of the diffusion coefficient can be calculated using a modified equation below:

$$D = -\left(\left(\frac{Ln\left(\frac{I}{I_0}\right)}{q^2 * \left(\Delta - \frac{\delta}{3}\right)}\right)\right)$$

Hence, the mean oil diffusion coefficient (D) of each sample can be obtained by calculating the respective linear regressions of the slow decaying part of the profiles with natural logarithm values of (I/I_0) as dependent variable y and $q^2 * \left(\Delta - \frac{\delta}{3}\right)$ as independent variable x. The fast decay at the beginning of profile was mainly caused by water diffusion in the samples. Thus, only the data of the slower decay (where the water signal was completely decayed while the oil signal was still large) was used for the calculation of the oil diffusion coefficient.

3.10. Data analysis

All experiments were carried out at least in duplicate using freshly prepared samples. All data analyses were done using MS-Excel 2010 and Origin 8.0. The results are reported as means and standard deviations.

4. RESULTS AND DISCUSSION

4.1. HIPE properties

4.1.1. Macrostructure

The SC:ALG mixtures were adjusted to pH 4.5, 5.5, 6.0, and 7.0. Meanwhile, SC:ALG conjugates were formed at pH 7.0 by controlled dry heat treatment (60 ºC, 74% RH for 24 hours). For the emulsification process, the dispersions were mixed with sunflower oil (dispersion/oil mass ratio = 1:4), followed by homogenization. Self-standing emulsions were obtained due to the fact that oil droplets were tightly packed together (beyond the maximum close packing of monodispersed spherical particles, φ=0.74).

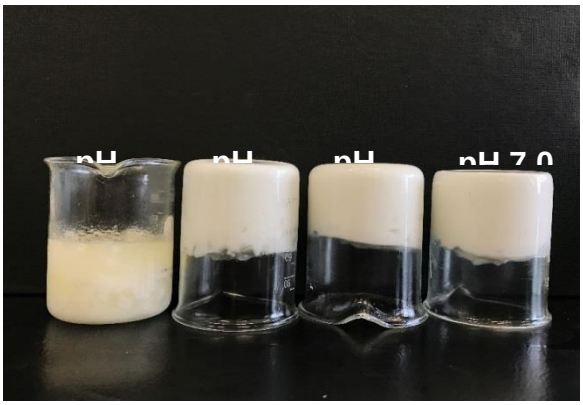

Figure 4. Visual appearance of the HIPEs stabilized by SC:ALG (6:1) mixtures at pH 4.5, 5.5, 6.0, and 7.0.

The mixtures showed an increased turbidity as the pH decreased. At pH 4.5, the mixture had the most turbid appearance compared to the others because it formed strong complexation in proximity to the isoelectric point of casein (pH 4.6). The visual turbidity observation is also supported by ζ-potential measurement. The mixture at pH 4.5 showed the lowest ζ-potential value (-28 mV), while the mixtures at pH 5.5, 6.0, and 7.0 showed more repulsive values of ζ-potential (below -30 mV). Consequently, the mixture at pH 4.5 could not be used to stabilize the HIPE (Figure 4). Verwey and Overbeek (1946) summarized that large negative (below - 30 mV) or positive ζ-potentials (above 30 mV) contribute superiorly to emulsion stabilization. Hence, the poor emulsifying performance of the SC:ALG mixture at pH 4.5 might be attributed to the lower ζ-potential value. As the SC:ALG (6:1) mixture at pH 4.5

gave unsatisfying results, the optimization of HIPEs by increasing the concentration of SC was continued at pH 5.5, 6.0, and 7.0. In addition, the Z-average diameter of SC:ALG (6:1) mixtures at different pH values ranges from 120 nm to 365 nm.

4.1.2 Microstructure

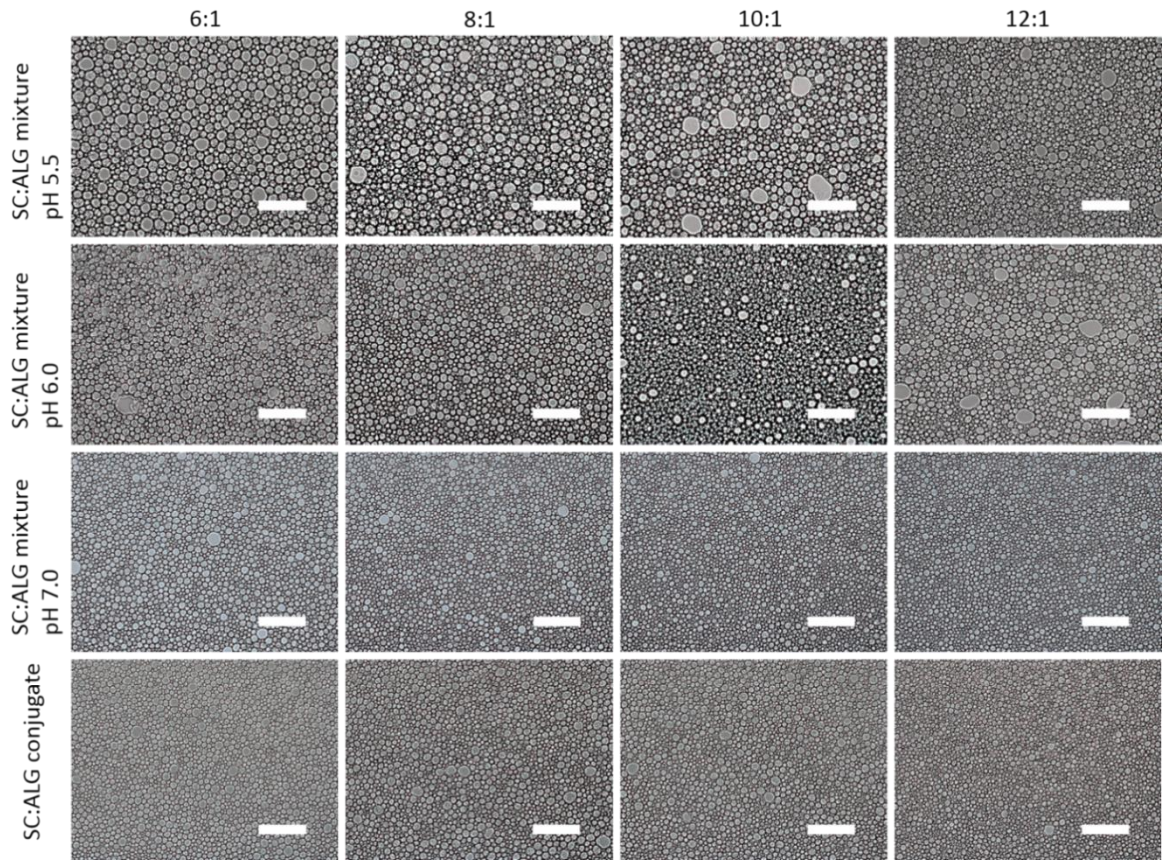

Figure 5. Microscopy images of HIPEs stabilized by SC: ALG mixtures and conjugates at pH 5.5, 6.0, 7.0, and SC:ALG conjugates. Scale bars = 50μm.

The microscopic appearance of HIPEs stabilized by SC:ALG mixtures at different ratios and pH values and SC:ALG conjugates at different ratios is shown in Figure 5. The analyses were carried out on the following day after the emulsion preparation. A significant decrease in the droplet size and close-packed oil droplets could be observed as the pH value and concentration of SC increased. HIPEs prepared using SC:ALG (12:1) mixture at pH 7.0 and SC:ALG (12:1) conjugate showed the finest oil droplet appearance.

4.1.3. Oil droplet size

Table 1.Volume-weighted average droplet D[4,3] of HIPEs stabilized by SC:ALG mixtures and conjugates.

SC:ALG	D[4,3] (μm)			
	Mixtures			Conjugates
	pH 5.5	pH 6.0	pH 7.0	
6:1	13.76 ± 0.19	9.89 ± 0.01	8.43 ± 0.01	8.01 ± 0.02
8:1	10.50 ± 0.00	9.88 ± 0.06	7.11 ± 0.01	8.12 ± 0.02
10:1	10.80 ± 0.08	10.15 ± 0.05	6.65 ± 0.03	7.77 ± 0.01
12:1	10.63 ± 0.05	9.03 ± 0.03	6.47 ± 0.00	6.35 ± 0.01

Table 1 lists the volume-weighted average diameter (D[4,3]) of the emulsions at different SC:ALG ratios and pH values. In general, the D[4,3] of the emulsions was around or smaller than 10 μm. For the emulsions stabilized with SC:ALG mixtures, there is a noticeable trend in the droplet size: the droplet size decreased as the pH value increased. For both emulsions stabilized by SC:ALG mixtures and conjugates, the increase of SC:ALG concentration resulted in a decrease of oil droplet size. Furthermore, compared to the emulsions stabilized at lower pH values (5.5 and 6.0), the ones stabilized by SC:ALG mixtures at pH 7.0 and SC:ALG conjugates showed smaller D[4,3] values (similar between the two series) at all ratios.

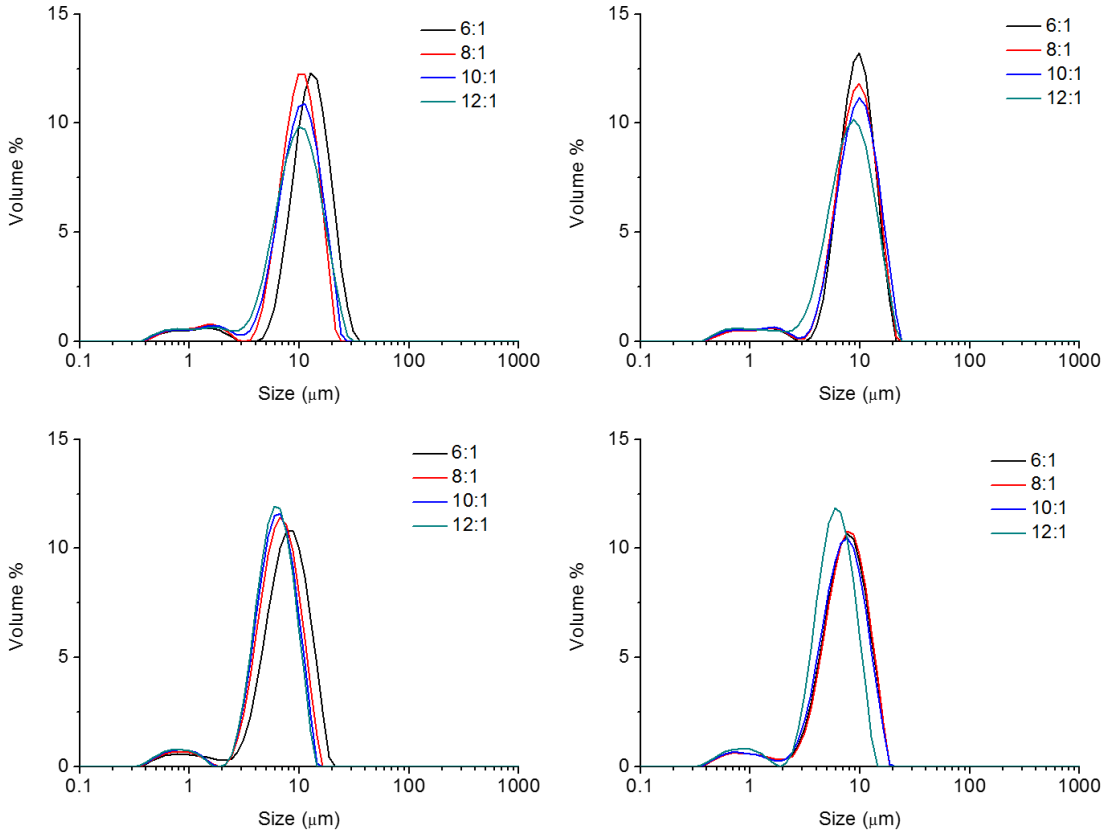

Figure 6. Droplet size distribution (μm) of HIPEs stabilized by SC:ALG mixtures at pH 5.5 (A), 6.0 (B), 7.0 (C), and SC:ALG conjugates (D).

Figure 6 depicts the oil droplet size distribution curves of the HIPEs prepared by SC:ALG mixtures and conjugates. The small peaks on the left that appeared on all the curves might reveal the presence of unadsorbed particles in the bulk phase. A similar trend for all graphs shows a slight shift of the major peak to the left which means the HIPEs had a smaller droplet size when the concentration of SC:ALG increased. Moreover, Wijaya et al. (2017) stated that increasing the concentration of whey protein isolate:low-methoxyl pectin led to a droplet size reduction of HIPEs due to a better surface coverage of the mixtures.

4.1.4. Gravitational stability

To evaluate the stability of the HIPEs, analytical photo-centrifugation was carried out to measure the O/W phase separation. The analytical photo-centrifugation technique measures light transmission through the samples during the centrifugation time to detect the phase separation of the emulsion in real-time. The integral transmission profile of each sample was presented by plotting the percentage of light transmission against centrifugation time. The slopes of the transmission profiles were also calculated using a specific time period when the integral transmission sharply rises before reaching the plateau. These slopes could be interpreted as the creaming rates.

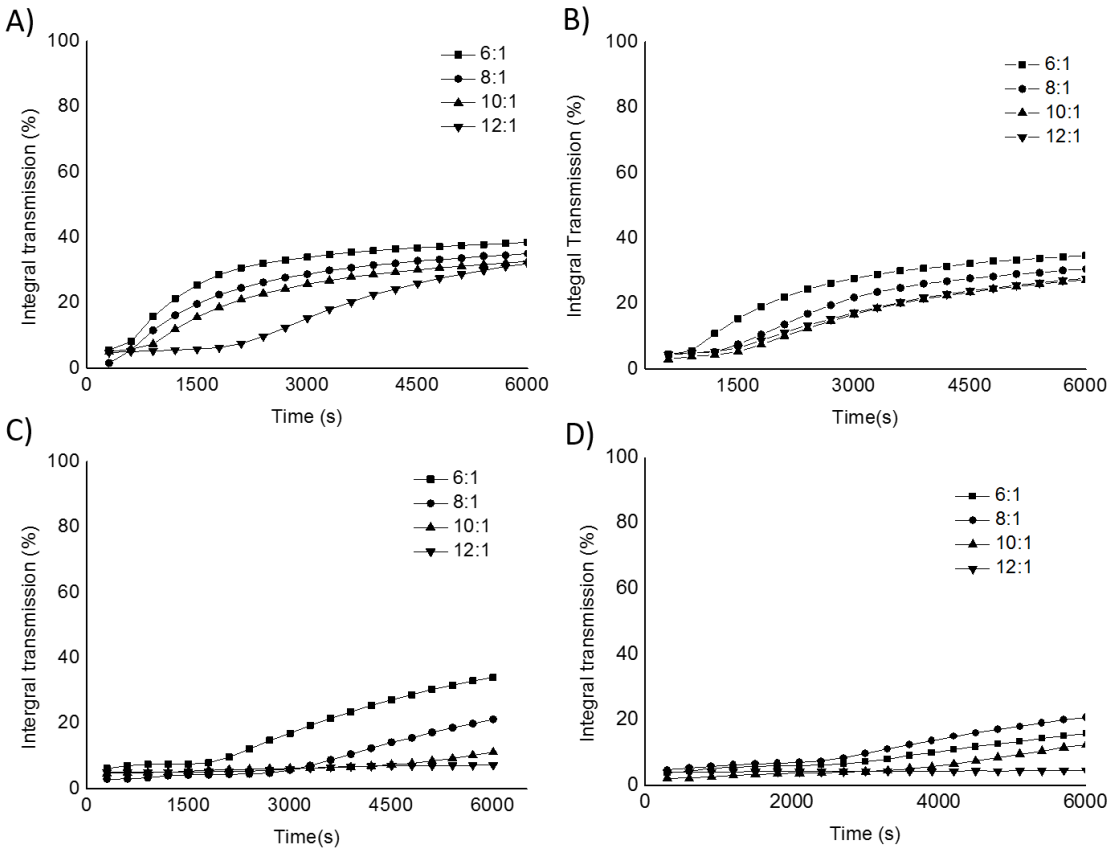

Figure 7. Integral transmission profiles by analytical photo-centrifugation of HIPEs stabilized by SC:ALG (6:1, 8:1, 10:1, and 12:1) mixtures at pH 5.5 (A), 6.0 (B), 7.0 (C), and SC:ALG (6:1, 8:1, 10:1, and 12:1) conjugates (D).

Figure 7 shows the integral transmission profiles of HIPE samples stabilized by SC:ALG mixtures (at different ratios and pH values), and by SC:ALG conjugates (at different ratios).

In general, emulsions stabilized by the polymers at higher ratios (10:1 and 12:1) exhibited a lower degree of light transmission, indicating an improved creaming stability. When comparing figure 7 A, B, and C, we found that as the pH of the mixtures increased, the overall transmittance of the samples decreased. The transmission profiles of the emulsions stabilized with conjugates had a relatively lower steepness compared with the other samples, suggesting lower creaming rates of these samples.

Table 2. Slopes of integral transmission profile of HIPEs stabilized by SC:ALG mixtures and conjugates. Except the marked results, the other slopes were calculated using the time period: from 300 to 3000 s.

	Slope [%/1000s]			
	Complexes			Conjugates
SC:ALG	pH 5.5	pH 6.0	pH 7.0	
6:1	12.14 ± 1.23	9.74 ± 0.70	6.39 ± 0.20*	2.89 ± 0.05**
8:1	11.02 ± 0.94	7.95 ± 0.48	5.30 ± 0.10**	3.69 ± 0.09**
10:1	9.00 ± 0.51	6.18 ± 0.38	1.61 ± 0.18**	2.88 ± 0.12**
12:1	6.45 ± 0.27*	5.82 ± 0.36	0.35 ± 0.05**	0.08 ± 0.01**

*time period selected for slope calculation was 1800 ~ 6000 s;

**time period selected for slope calculation was 3000 ~ 6000 s.

As seen from table 2, a significant decrease of the slopes can be observed in all sample series when increasing the pH, suggesting an improved creaming stability. As the concentration of SC increased, the slopes also shifted towards lower values, especially for HIPEs stabilized by SC:ALG mixtures. HIPEs stabilized by SC:ALG conjugates showed a relatively high creaming stability at all ratios. The conjugation of protein and polysaccharide by dry-heat treatment might yield strong and permanent complexation which might also improve the interfacial properties, hence a better creaming stability. HIPEs stabilized by SC:ALG (12:1) mixtures at pH 7.0 and SC:ALG conjugates displayed no remarkable rise of light transmission (figure 7 C and D) and the lowest slopes, and could be indicated as the most stable samples.

4.2. Oleogel properties

4.2.1. Preparation and visual observation of oleogels

According to the previous section (analytical centrifugation), SC:ALG at specific pH and sufficient concentrations could produce HIPEs with high stability. The HIPEs were subjected to drying to obtain a structural framework entrapping the oil after the water removal. The HIPEs were dried in an oven at 35 °C to selectively evaporate the water phase, until a constant weight of the samples was reached.

Figure 8 reveals the macroscopic appearance of the oleogels stabilized with different ratios of SC:ALG mixtures at different pH values and SC:ALG conjugates formed by dry-heat treatment. The higher the concentration of stabilizing agents, the more solid-like appearances with less or no oil leakage the oleogels have. The pH also affected the macrostructure of the oleogel samples; at lower pH (pH 5.5 and 6.0), the oleogel samples showed an evidence of oiling off even at the highest ratio. Apparently, oleogels stabilized by SC:ALG mixtures at pH 7.0 and SC:ALG conjugates showed no oiling off at ratio of SC:ALG of at least 10:1. By increasing the ratio of SC:ALG and the pH value, we alleviated the oil leakage from the oleogels and obtained more solid-like structures.

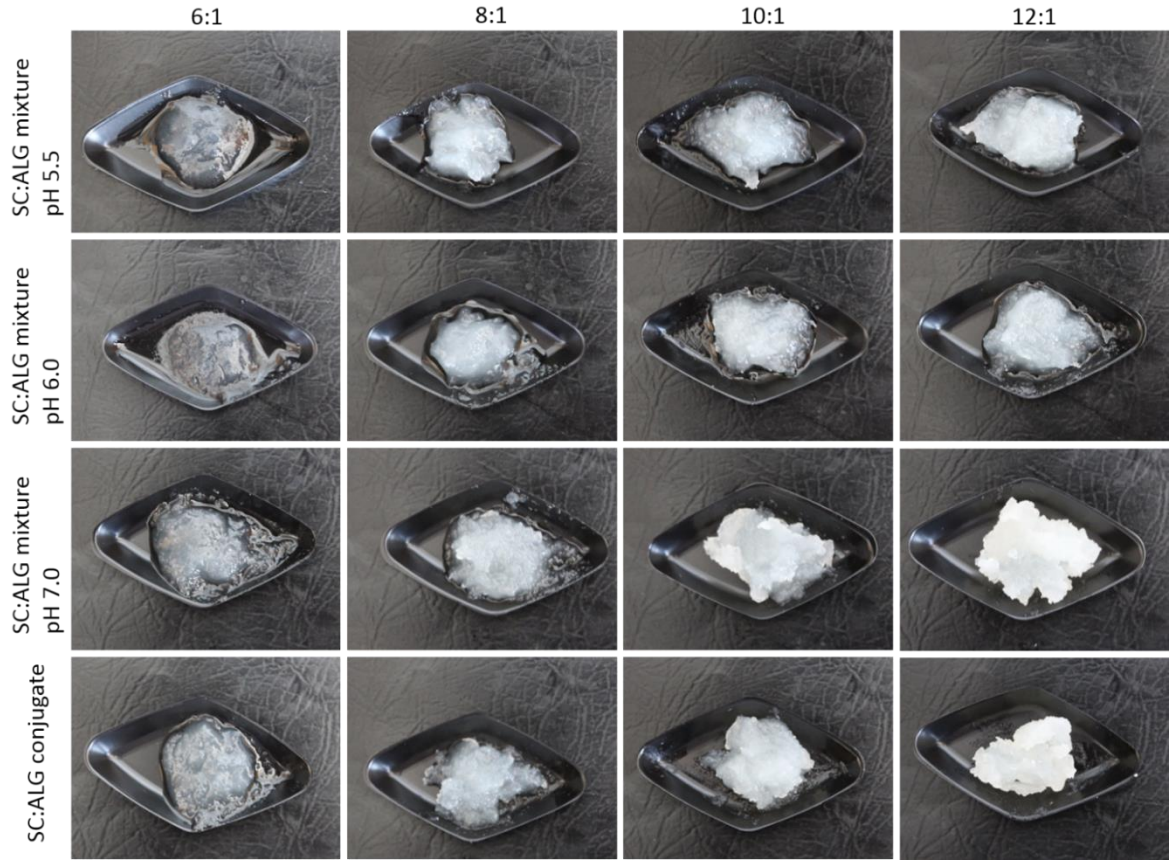

Figure 8.Photographs of oleogels obtained by oven drying the HIPEs stabilized by SC: ALG mixtures formed at pH 5.5, 6.0, 7.0, and SC:ALG conjugates.

4.2.2. Oil loss value

Table 3. Oil loss value of oleogels stabilized with SC: ALG mixtures formed at pH 5.5, 6.0, 7.0, and SC: ALG conjugates.

	Oil-loss value (OL)			
	Mixtures			Conjugates
SC:ALG	pH 5.5	pH 6.0	pH 7.0	
6:1	0.33 ± 0.00	0.35 ± 0.01	0.15 ± 0.01	0.11 ± 0.01
8:1	0.19 ± 0.02	0.18 ± 0.01	0.09 ± 0.00	0.05 ± 0.02
10:1	0.12 ± 0.01	0.13 ± 0.01	0.03 ± 0.01	0.04 ± 0.00
12:1	0.10 ± 0.01	0.11 ± 0.00	0.02 ± 0.01	0.04 ± 0.00

In the current physical stability assessment of oleogels, oil loss can be evaluated using various gravimetric techniques that destabilize the system to provoke syneresis. The oil loss is determined by measuring the amount of oil expelled from the system after the destabilization. In general, the destabilization can be induced by centrifugation. In this analysis, the oil is separated from the network because of density differences. Oil, which

has the lower-density, will rise above the high-density solid components of the system that are sedimented to the bottom of the centrifuge tube. The sample is centrifuged for a known amount of time, then the supernatant is decanted and weighted, giving an OL value which can also be indicated as the oil binding capacity (Razul et al. 2014)

The amount of separated oil collected after centrifugation (10000 rpm for 12 min) of oleogel samples, was expressed as the amount of OL (Table 3). Lower OL values reveal a better oil-binding capacity of the oleogels. Oleogel samples stabilized by SC:ALG at pH 5.5 and 6.0 showed higher OL values. The series of SC:ALG mixtures at pH 7.0 and SC:ALG conjugates displayed relatively lower OL values indicating an improved oil-binding capacity. The oleogel sample with the highest concentration of SC:ALG (12:1) at pH 7.0 had the lowest OL value which indicates a high oil binding capacity. Oil binding capacity may be defined as the ability of a system to retain its liquid oil phase (MacDougall et al. 2012), which in this case is the ability of the interpolymeric networks of protein and polysaccharide to hold the liquid oil.

4.3. Rheological study of HIPEs and oleogels

HIPE and oleogel samples were subjected to small amplitude oscillatory measurements (i.e. amplitude stress sweeps and frequency sweeps) to characterize their viscoelastic behavior according to the linear viscoelastic regions (LVR). The pH, SC:ALG ratios and complexation method (mixtures or conjugates) were expected to affect the LVR, in which the gel strength can be judged based on the behavior of G′ and G″, the value of G′ in the LVR, and the length of the linear region of the curve.

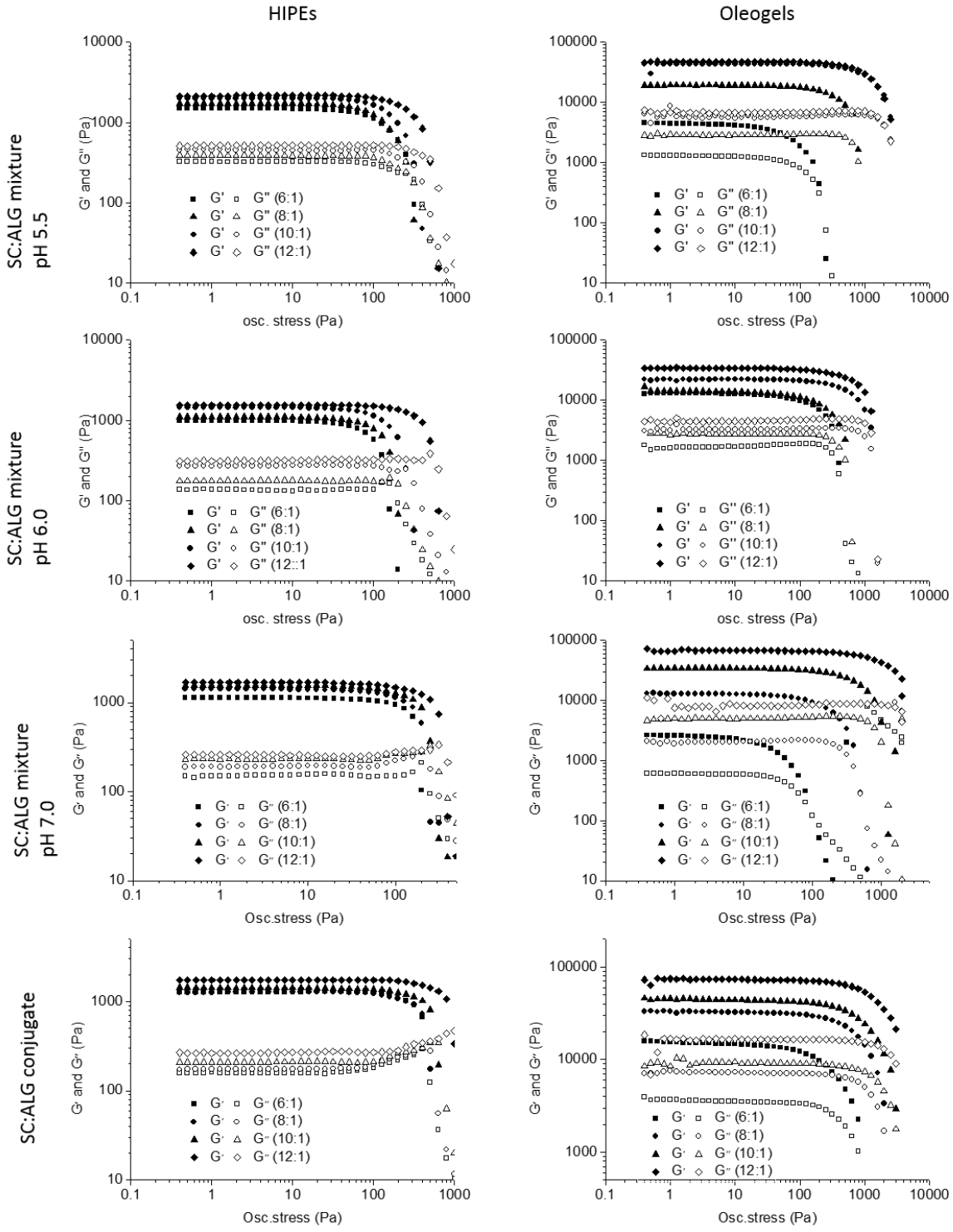

Figure 9. G′ and G″ as a function of oscillatory. stress for HIPEs and oleogels stabilized by SC:ALG mixtures and conjugates

Figure 9 shows the oscillatory sweeps of all HIPE and oleogel samples stabilized by SC:ALG mixtures (at different ratios of biopolymers and at different pH values) and conjugates (at different ratios). Generally, all samples showed a solid behavior (higher G′ than G″ within the LVR) with G′ over 1000 Pa and around 10000 Pa for HIPE and oleogel samples, respectively. By increasing the SC:ALG ratio, all samples demonstrated an increasing gel strength (increase of G′ and longer LVR). This may be associated with the formation of a stronger biopolymer network both at the interface and bulk phase at higher biopolymer concentrations. After the water removal, the oleogel samples showed a remarkable increase of G′ because the interpolymeric networks of protein:polysaccharide become more compact after the water removal and the oil droplets tightly pack together within the network. There is no significant difference in the effect of pH for all HIPE and oleogel samples. Moreover, at higher amplitudes, all the samples showed crossover points suggesting there was a structural breakdown at higher oscillatory stress. However, this did not change the solid properties of all the samples by visual observation after the measurement was done.

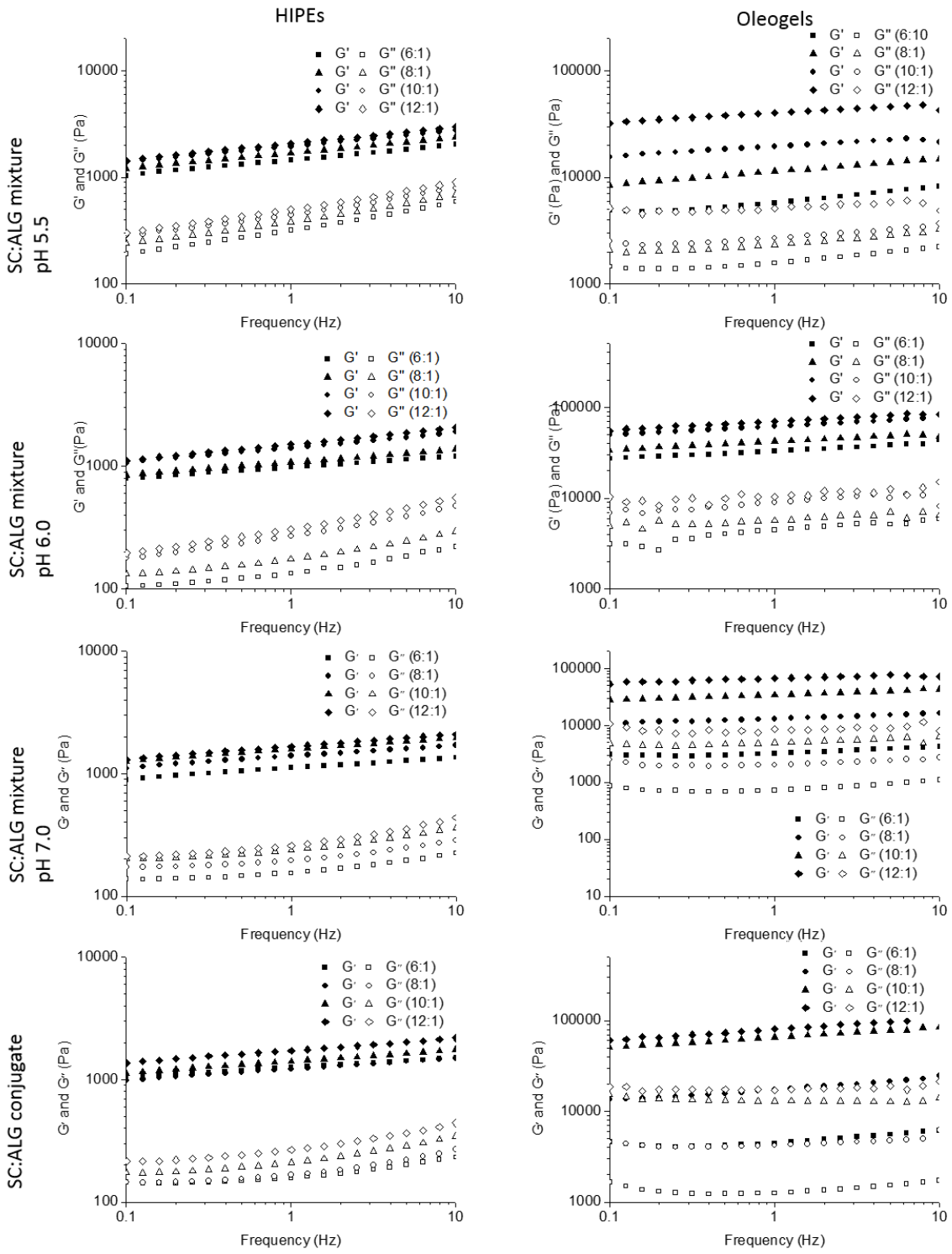

Figure 10. G′ and G″ as a function of frequency sweeps for HIPEs and oleogels stabilized by SC:ALG mixtures and conjugates.

Figure 10 shows the frequency sweeps of all HIPE and oleogel samples stabilized by SC:ALG mixtures (at different ratios of biopolymers and at different pH values) and conjugates. The dependence of the material to the rate of deformation was observed by subjecting HIPE and oleogel samples to a constant stress within the LVR (5 Pa), and the frequency was varied from 0.1 to 10 Hz. The frequency sweeps results were used to confirm the previous stress sweeps tests which can give more insight to the structure-viscoelastic behavior relationships of HIPE and oleogel samples. Generally, both HIPE and oleogel samples had a good resistance to the rate of deformation as can be observed from the slightly positive slope of G ′. The results from the frequency sweep confirmed the more solid-like properties of the HIPE and oleogel samples with a strong gel behavior ($G' > G''$), whereby much higher G' values were observed as the concentration of SC increased.

4.4. Oil diffusion by NMR

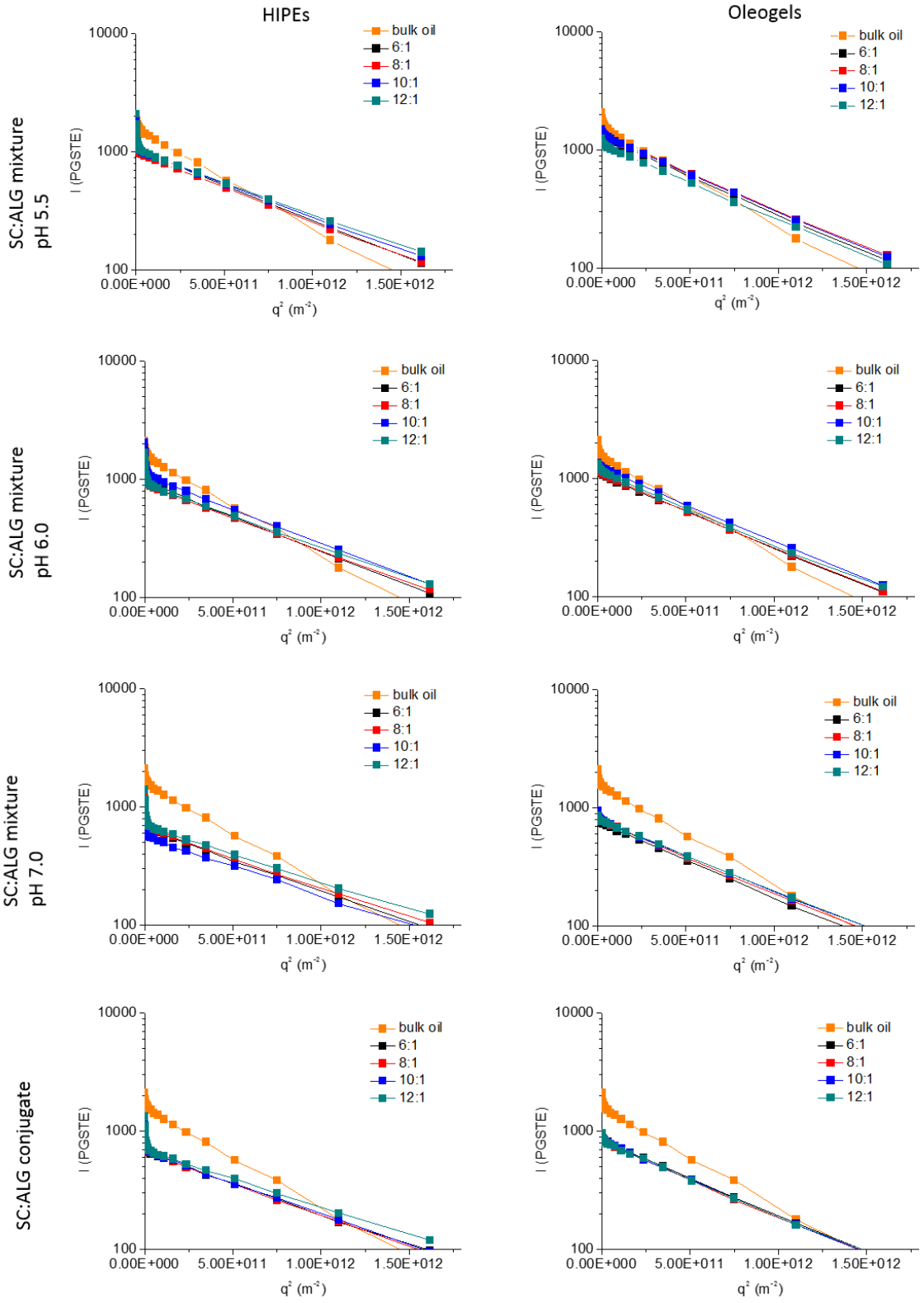

Figure 11. PGSTE diffusion signal of HIPEs and oleogels stabilized by SC:ALG mixtures and conjugates

Figure 11 shows the experimental signal intensity (I) data points as a function of q^2 for both the HIPEs and oleogels. The PGSTE signal of the bulk oil phase is also added to each series for comparison. All the HIPEs and oleogels showed an overall slower decay profile than the bulk oil phase. The slopes of the decay became even lower as the pH increased. The increase of the SC concentration showed a slight influence on the decay of the PGSTE signal among these samples which was observed in figure 11. The effect of different polymer ratios seemed to be even less noticeable in samples stabilized by conjugates when compared to samples prepared with SC:ALG mixtures. For further assessment, the mean oil diffusion coefficient (D) of each sample was calculated according to the method described in section 3.8.2.

Table 4. Oil diffusion coefficient (10^{-12} m^2/s) of HIPEs stabilized by SC:ALG mixtures and conjugates. Error refers to 95% confidence interval. The diffusion coefficient of bulk oil in our experiment was (9.50 ± 0.12) x 10-12 m2/s.

	HIPEs			
SC:ALG	Mixtures			Conjugates
	pH 5.5	pH 6.0	pH 7.0	
6:1	6.87 ± 0.02	6.85 ± 0.05	6.10 ± 0.03	6.05 ± 0.07
8:1	6.67 ± 0.04	6.39 ± 0.04	5.82 ± 0.06	6.21 ± 0.06
10:1	6.47 ± 0.04	6.64 ± 0.03	5.69 ± 0.09	6.10 ± 0.06
12:1	6.23 ± 0.05	6.11 ± 0.05	5.44 ± 0.06	5.50 ± 0.05

Table 4 lists the oil diffusion coefficient of emulsions stabilized by SC:ALG mixtures and conjugates. Generally, the increase of SC concentration and pH value led to lower D values in the emulsions. A thicker interfacial layer of protein:polysaccharide was formed at a higher concentration of SC which could arrest the oil droplets more tightly, and hence might hamper the mobility of oil. The lowest D values were obtained in HIPEs stabilized with SC:ALG mixture at pH 7.0 and SC:ALG conjugate at a ratio of 12:1. As previously discussed in section 4.1.3, HIPEs with higher SC concentration and pH values tend to have smaller droplet size. Therefore, our finding about oil diffusion is in line with the theory that if the oil droplets are small, the signal decay is slow. The small oil droplets signifies a dense biopolymer network formed, which entraps the oil droplets and induces better obstruction that lowers oil activity.

Table 5. Oil diffusion coefficient (10^{-12} m^2/s) of oleogels stabilized by SC:ALG mixtures and conjugates. Error refers to 95% confidence interval.

	Oleogels			
	Mixtures			Conjugates
SC:ALG	pH 5.5	pH 6.0	pH 7.0	
6:1	7.55 ± 0.05	7.19 ± 0.05	7.27 ± 0.07	7.26 ± 0.05
8:1	7.29 ± 0.05	7.23 ± 0.05	7.14 ± 0.05	7.25 ± 0.07
10:1	7.39 ± 0.05	7.26 ± 0.05	6.92 ± 0.05	7.32 ± 0.06
12:1	7.24 ± 0.03	7.15 ± 0.09	6.86 ± 0.04	7.18 ± 0.09

Table 5 lists the diffusion coefficient values of the oleogels, which were higher than that of HIPEs. It is noteworthy that the variations on pH values and polymer ratios had a negligible effect on the oil diffusion behavior in the oleogel samples, though a slight decline was seen when the SC level increased. Both HIPEs and oleogels exhibited a more hindered oil diffusion behavior than bulk oil, meaning that the use of the polymers SC and ALG did improve the oil retaining within the interpolymeric network of SC:ALG. However, the influence of pH and biopolymer ratio on oil diffusion in oleogels was less pronounced than in HIPEs.

5. CONCLUSIONS

We successfully fabricated oleogels from HIPE templates stabilized by SC and ALG. This approach includes the incorporation of a large amount of oil (80%) into the aqueous phase (20%) in the presence of SC:ALG mixtures (or conjugates) for the production of HIPEs, and transformation of HIPEs to oleogels by water removal and further shearing.

We found that the intrinsic properties of SC:ALG mixtures and conjugates played an important role during the fabrication of HIPEs and oleogels. The mixture at pH 4.5 appeared to be most turbid and was unable to form a stable emulsion. This could be attributed to the occurrence of aggregation caused by the complexation of the two polymers and/or by the interaction of the protein molecules at the proximity of the IEP of the protein. A stable mixture of the two biopolymers possessed a relatively low turbidity and high ζ-potential. Thus, they can form stable HIPEs and oleogels. The ratio of the biopolymers and the pH value had a great influence on the properties of HIPEs and the corresponding oleogels. HIPEs stabilized with SC:ALG mixtures and conjugates at higher concentrations exhibited smaller oil droplets, increased gel strength, better creaming stability, and slower oil diffusion decay. As the pH of the mixtures increased, a reduced droplet size and better stability of HIPEs were also observed. Similarly, by increasing the ratio of SC:ALG, we obtained firmer oleogels with less oil leakage, better stability towards oiling off, and stronger gel properties. Oleogels formed with biopolymers at higher pH also showed improved stability against oil loss. However, varying the pH of the mixtures resulted in no significant differences in the rheological properties for both HIPEs and oleogels. In the pfg-NMR measurement, both HIPEs and oleogels showed a more hindered oil diffusion decay than in bulk oil, revealing an enhanced oil retaining caused by the polymer network. In comparison to HIPEs, oleogels showed a higher diffusion coefficiency and their oil diffusion behavior appeared to be less affected by pH variation. In addition, HIPEs and oleogels stabilized by SC:ALG mixtures at pH 7 and SC:ALG conjugates at the highest ratio of SC:ALG (12:1) displayed similar properties with a relatively high stability compared to the other samples.

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