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Comparison on the Effect of High-Methoxyl Pectin or Soybean-Soluble Polysaccharide on the Stability of Sodium Caseinate-Stabilized Oil/Water Emulsions

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The interactions of high-methoxyl pectin (HMP) and soybean-soluble polysaccharide (SSPS) with sodium caseinate-stabilized emulsions were investigated using a multitechnique approach, including dynamic light scattering (DLS), electrophoretic mobility measurements, transmission diffusing wave spectroscopy (DWS), and ultrasonic spectroscopy (US). At pH 6.8, both polysaccharides are negatively charged and did not adsorb onto caseinate-coated droplets due to electrostatic repulsion; however, SSPS showed a different behavior compared to HMP in the turbidity parameter $1/l^*$ and sound attenuation parameters measured by DWS and US, respectively. The present study brought the first evidence of the stabilization effect of SSPS in acidified sodium caseinate-emulsions. While destabilization occurred at low polysaccharide concentrations, probably via bridging flocculation, acid-induced aggregation of the oil droplet was completely prevented by 0.2% SSPS or HMP. However, the interaction behavior of SSPS during acidification was different from that of HMP. This was demonstrated by the different development of the parameter $1/l^*$, droplet sizes, sound attenuation, and velocity.

KEYWORDS: Sodium caseinate emulsions; high-methoxyl pectin; soybean soluble polysaccharide; ultrasound; diffusing wave spectroscopy.

INTRODUCTION

Many food products are oil-in-water emulsions, where proteins are generally used to stabilize the oil droplets. Polysaccharides are incorporated to control the bulk properties by increasing the viscosity of the aqueous phase or by the formation of a gel. They may also interact with protein adsorbed at the interface. Depending on the size and charge of the molecules, concentration of polysaccharide present, and the environmental conditions of the solution (pH and ionic strength), the protein−polysaccharide interactions can improve the stability of the emulsion, lead to destabilization by bridging flocculation, where a long chain polymer is present in small concentrations and adsorbs onto more than one colloidal particle, or depletion flocculation, where the intercolloidal region becomes depleted of polymer, creating a polymer concentration gradient, hence an osmotic pressure difference, which draws the particles closer to one another or, last, cause gelation (*1*, *2*). Understanding the assemblies occurring at the interface and the dynamics of the interactions is fundamental for engineering food emulsions.

Pectin and soybean-soluble polysaccharide (SSPS) are two acidic polysaccharides used in foods, having some similarities in their structures and functionalities. Pectin is negatively charged at pH > 3.5 and mainly consists of a backbone of galacturonic acid partly methylesterified and branches of arabinose, galactose, and xylose (*5*). High-methoxyl pectin (HMP) has a degree of methylesterification (DE) greater than 50%. SSPS is extracted from soy cotyledons and it contains 18% of galacturonic acid (GalA); this is a much lower amount than that found in pectin (often over 75% GalA) (*5*, *6*). SSPS has a comparable structure to that of pectin but a more branched configuration. The backbone of SSPS consists of homogalacturonan and rhamnogalacturonan; the rhamnogalacturonan portion is highly branched. It has been shown that SSPS has a radius of gyration of about 23.5 ± 2.8 nm and a globular shape (*7*). Our previous study found a gyration radius of 43.0 ± 2.5 and 45.2 ± 3.1 nm for the HMP and SSPS used in this work, respectively, measured by size exclusion chromatography and multiangle laser light scattering (SEC-MALLS) (*8*).

Sodium caseinate, a soluble mixture of four principal caseins (α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein), is widely employed as an emulsifier in the food industry. They adsorb rapidly at the oil/water interface and consequently stabilize emulsion droplets through a combination of steric and electrostatic interactions (*3*). When lowering the pH toward the isoelectric point of caseins, the interdroplet interactions change from repulsion to a net attraction, inducing droplet flocculation (*4*).

It is known that when added to sodium caseinate-stabilized emulsions at neutral pH, HMP does not adsorb onto the caseinate-coated droplet surface because of electrostatic repulsion. Above a critical concentration, nonadsorbing HMP can

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induce the destabilization of emulsion by depletion flocculation (*9*, *10*). Instability by depletion flocculation was also observed in whey protein isolate or soy protein isolate stabilized emulsions containing HMP at neutral pH (*11*, *12*).

It was shown that, at pH above or near the isoelectric point (pH 4.6) of sodium caseinate, HMP can adsorb and stabilize sodium caseinate-coated emulsion droplets against aggregation (*9*, *10*, *13*), which occurs in the absence of HMP due to the simultaneous loss of electrostatic and steric repulsion during acidification (*4*). In addition, at pHs 3 and 4, extensive droplet flocculation was observed in HMP-containing emulsions. The authors suggested that the adsorption of negatively charged HMP to the positively charged caseinate-coated droplets lead to charge neutralization, thereby reducing the electrostatic repulsion between droplets, and individual pectin molecules may adsorb onto more than one droplet causing bridging flocculation (*10*).

Compared to HMP, much less is known about the interactions of SSPS and milk proteins at oil/water interfaces. One study showed that SSPS does not seem to interact with sodium caseinate adsorbed at oil/water interfaces at pH 7 (*14*). However, the depletion flocculation observed in HMP-containing emulsions was not shown in the soy protein isolate-stabilized emulsion containing up to 0.3% SSPS (*12*). The addition of 2% SSPS to whey protein isolate-stabilized emulsion at pH 4 resulted in bridging flocculation (*14*). The behavior of SSPS with casein-stabilized emulsions during acidification has never been reported.

Previous studies have shown that, although both HMP and SSPS are capable of stabilizing casein particles in acid milk beverages, there are obvious differences on the mechanism of adsorption and stabilizing behavior (*8*, *15*, *16*). A comparison of the interactions of HMP and SSPS with caseins adsorbed at the oil/water interface will contribute to a more solid understanding of their behavior in dairy products, which is the objective of this study. In this work, the effect of HMP (DE 67.4%) and SSPS on the stability of sodium caseinate-stabilized emulsions at neutral pH and during acidification was studied. To better understand the dynamics of the interactions in bulk emulsions and identify critical concentrations of polysaccharide or pH of destabilization, a multitechnique approach, which includes diffusing wave spectroscopy (DWS), ultrasonic spectroscopy (US), dynamic light scattering (DLS), and electrophoretic mobility measurement, was employed. Noninvasive techniques DWS and US allow concentrated samples to be observed in situ and therefore obtain information that may be lost during sample manipulation.

MATERIALS AND METHODS

Sample Preparation. Sodium caseinate (New Zealand Milk Proteins, Mississagua, Ontario) solutions were prepared by dissolving 0.55% (w/w) sodium caseinate and 0.02% sodium azide (as antimicrobial) in ultrapure water with continuous stirring and the pH of the solution was then adjusted to pH 6.8. After filtering through a 0.8 μm filter (Millex-HV Millipore Co., Billerica, MA), a stock emulsion was prepared containing 10% (w/w) soybean oil. The samples were premixed for 1 min using a high-speed blender (Power Gen 125, Fisher Scientific, Co., Nepean, ON) and then homogenized with a high-pressure homogenizer (Emulsiflex C5, Avestin Inc., Ottawa, ON) with three passes at 40 MPa. Emulsions were then stored at 4 °C overnight. The average droplet size of the emulsion prepared (D (3, 2), as measured with Mastersizer 2000, Malvern Instruments Ltd, UK) was 0.43 ± 0.01 μm.

A stock solution of 1% (w/w) pectin (67.4% DE unstandardized, CpKelco, San Diego, CA) or 1% SSPS (DA300S, Fuji Oil Co. Ltd. Japan) was prepared in ultrapure water at 70 °C and then cooled with continuous stirring at room temperature until complete solubilization was obtained. The stock solutions were stored at 4 °C for 24 h, and before mixing with the emulsion, the pH of the solutions was adjusted to pH 6.8 using NaOH.

Samples containing emulsion droplets and polysaccharide were prepared by dilution of the stock emulsion with SSPS or HMP solutions to a final oil concentration of 6% and various amounts of polysaccharides: 0.01%, 0.03%, 0.05%, 0.07%, 0.08%, 0.1%, 0.15%, 0.2%, and 0.3% (w/w) SSPS or HMP for experiments at pH 6.8; 0.05%, 0.1%, and 0.2% SSPS or HMP for acidification experiments. These emulsions were compared to control emulsions in which, instead of pectin solution, an equivalent amount of water was added.

The effect of HMP or SSPS on the emulsions at pH 6.8 was monitored in parallel by DLS, DWS, US, and electrophoretic mobility measurements. Samples of 1.5 mL at pH 6.8 were placed in a cuvette and then measured over 30 min using US or DWS (details described below), the same sample was then diluted in 10 mM imidazole buffer at pH 6.8, and the ζ potential of the droplets and the average apparent size were determined by electrophoretic mobility and DLS test, respectively (Zetasizer Nano ZS ZEN3600, Malvern Instruments Ltd, Malvern, UK). The acidification experiment was induced by slow decrease of the pH using 0.3% (w/v) glucono-δ-lactone (GDL). The dynamics of the interactions of HMP or SSPS with sodium caseinate-stabilized emulsion droplets were continuously followed by pH measurement in parallel with the DLS, DWS, and US experiments.

All experiments were repeated three times at 23 °C (with three separate emulsions and polysaccharide solutions). Statistical analysis of variance was carried out to determine the effect of pH, polysaccharide type, and concentration on the changes in the $1/l^*$ parameter, droplet diameter, velocity, and attenuation (measured at the various frequencies). Significant differences were determined with the least-square means procedure and the general linear model procedure using SAS (version 8.0, SAS Institute, Cary, NC). Significance was considered for $p < 0.05$.

Dynamic Light Scattering during Acidification. The average hydrodynamic diameter of the emulsion droplets with no polysaccharide, 0.05%, 0.1%, and 0.2% HMP or SSPS during acidification were measured using DLS, after extensive dilution, as follows. Immediately after the addition of GDL and throughout the experiment, the emulsions were continuously circulated through a laboratory scale ultrafiltration cartridge (Millex-HV CDU F001LG, Millipore, Billerica, MA, nominal MW cut off of 10 000 Da), and the pH of the emulsion was measured at the same time. Permeate and retentate were recirculated to avoid concentration. At particular pH values, 2 mL of permeate were collected, and the corresponding emulsion was then diluted in the permeate and measured using DLS (Zetasizer Nano ZS, ZEN3600, Malvern Instruments Ltd, UK).

Transmission Diffusing Wave Spectroscopy. A DWS test of 30 min at 5 min intervals was carried out on each sample containing up to 0.3% HMP or SSPS at pH 6.8. For the acidification experiments, immediately after the addition of 0.3% GDL, emulsions were poured in a cuvette and measured with DWS over 4 h at 5 min intervals as previously described (*17*). The light source used was a monochromatic solid-state laser with a wavelength of 532 nm and 100 mW of power (model 532-100MBS, Omnichrome, Chino, CA). The sample of 1.5 mL, contained in an optical glass cuvette with a path length of 5 mm, was immersed in a water tank maintained at a constant temperature of 23 °C. After the laser light passed through the sample, the scattered light was collected and fed into two separate photomultipliers (HC120-03, Hamamatsu, Loveland, OH). The signals from the photomultipliers were amplified and fed to a correlator (FLEX2K-12 × 2, Bridgewater, NJ), which performed a cross-correlation analysis. The time-correlation function of multiply scattered light is given by (*12*):

$$g_{(1)}(t) \approx \frac{\left(\frac{L}{l^*}+\frac{4}{3}\right)\sqrt{\frac{6t}{\tau}}}{\left[\left(1+\frac{8t}{3\tau}\right)\sinh\left(\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right)\right]+\left[\frac{4}{3}\sqrt{\frac{6t}{\tau}}\cosh\left(\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right)\right]} \quad (1)$$

where the thickness of sample $L \gg l^*$ (i.e., $L/l^* > 10$), and the time $t \ll \tau$ (the correlation time of sample), In this case, the correlation function obtained has a characteristic decay time of $\tau = \tau_0(l^*/L)$.[2] Where

$\tau_o = (Dk_o^2)^{-1}$, D is the particle diffusion coefficient, $k_o = 2\pi n/\lambda$ is the wave vector of the light, and n is the refractive index of the solvent. The correlations were measured at intervals of 5 min. Two parameters were derived from the DWS data, radius, and the factor l^* (explained below).

The turbidity parameter l^* is defined as the length scale over which the direction of the scattered light is completely randomized. For a turbid solution, where all photons are multiple scattered, l^* is directly related to the total scattered intensity calculated from the transmission of light (*18*):

$$T_i = \frac{I}{I_0} = \frac{5l^*/3L}{1 + 4l^*/3L} \quad (2)$$

where I_0 and I are the initial and transmitted intensities of the laser light and L is the path length. A calibration run was performed using 269 nm diameter latex spheres (Portland Duke Scientific, Palo Alto, CA). From eq 1 (using the fact that the radius is well-known), the l^* of the latex can be determined. From here, I_0 can be calculated, and by measurement of the scattered intensity of the sample, the derivation of l^* is trivial. Once the value of l^* has been determined, it is possible to calculate the diffusion coefficient, D, from the autocorrelation function obtained for the sample and shown in eq 1. Subsequently the radius, R, of the scatterer, via the Stokes−Einstein relation for a system of noninteracting spheres, can be calculated as follows.

$$D = \frac{kT}{6\pi\eta R} \quad (3)$$

where k is the Boltzmann constant, T is temperature, and the viscosity, η, of the polysaccharide solutions were measured with an Ubbelohde viscometer (type A859 size 1B, Industrial Research Glassware, NJ).

For particles that are completely noninteracting, i.e., uncorrelated spatially, the l^* parameter will depend on particle size, particle concentration, the refractive index contrast between the particles, and the dispersed phase. However, in concentrated and optically dense suspensions, the l^* value will also depend on the spatial correlation between the particles. Under constant volume fraction and no changes in refractive index contrast or size of the particles, a change in the l^* value will indicate a change in the interactions between particles.

Ultrasonic Spectroscopy. The changes in the velocity and attenuation of sound were measured at five different frequencies using a high-resolution ultrasonic spectrometer (HR-US102, Ultrasonic Scientific, Dublin, Ireland). Before measurement, the samples were equilibrated in a water bath at 23 °C. Emulsions of 1 mL were loaded in the two cells and kept at 23 °C by a programmable Haake F8 water bath (Thermo-Haake, Georgetown, ON). Samples at pH 6.8 were directly loaded in the sample cell. For acidification experiments, 0.3% GDL was added to the emulsions, which were then immediately loaded in the sample cell. In the second cell, the control emulsion (with no polysaccharide) without GDL was added as a reference. The sample and the reference were measured simultaneously. The instrument was calibrated with water at 23 °C and tuned to measure five frequencies (3, 5, 8.7, 14.5, and 15.5 MHz), and two independent ultrasound parameters, velocity, and attenuation were measured as a function of time (and pH during acidification).

Velocity of propagation of the sound wave is related to the compressibility of the medium as well as its density. Velocity of sound strongly depends on the molecular organization and intermolecular interactions in the medium. Changes in velocity are related to changes in volume and adiabatic compressibility of the dispersed particle and on the changes in the hydration state of the molecules. Velocity is very dependent on temperature; for this reason, its value is usually reported as a velocity difference from that of a reference (in this case reference emulsions without polysaccharide) to remove the contribution of temperature changes on propagation of the wave in the medium (*19*, *20*, *21*).

Attenuation of sound in our sampling regime is mainly caused by the sum of intrinsic losses, thermal and viscous losses (*22*). Intrinsic losses are caused by the interaction of the sound wave with the materials of the particles and the medium. Viscous and thermal losses are caused by the difference in densities of the particles and the medium as well as the temperature gradient generated near the particle surface. Because emulsions have a low density contrast between the oil droplets and surrounding medium, most attenuation derived from thermal losses that depend on the spatial arrangement of the droplets (*23*). The theory of acoustic for emulsions has been described previously (*22*, *23*).

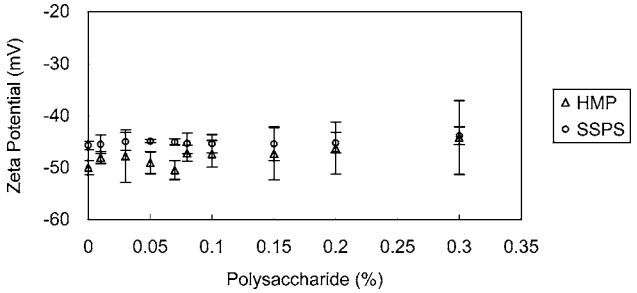

Figure 1. Zeta potential of emulsion droplets in the presence of HMP (△) or SSPS (○) at pH 6.8, as a function of polysaccharide concentration. The emulsions were diluted in 10 mM imidazole buffer at pH 6.8. Bars indicate standard deviation (three independent experiments).

RESULTS AND DISCUSSION

Interactions of HMP or SSPS with Caseins at Oil/Water Interfaces at pH 6.8. At pH 6.8, the effect of HMP or SSPS on the sodium caseinate-coated emulsions was studied using DLS, DWS, US, and electrophoretic mobility measurements. Compared to control emulsions (no polysaccharide), addition of up to 0.3% (w/w) HMP or SSPS to the emulsion had no significant effect on the ζ potential (**Figure 1**). The value of ζ potential was stable at −48 mV, with HMP or SSPS. At pH 6.8, both HMP and SSPS as well as the caseins adsorbed to oil droplets at the water/oil interface are negatively charged, and therefore, the strong electrostatic repulsion inhibits the adsorption of HMP or SSPS onto the emulsion droplets.

The stability of the emulsions containing various concentrations of HMP and SSPS was also evaluated by determining changes in average diameter of the emulsions as measured by DLS (under diluted conditions) and DWS (with no dilution) (**Figure 2**). While the apparent diameter by DLS suggested that the addition of HMP or SSPS to the emulsion droplets that contain adsorbed casein did not affect the size of the oil droplets (**Figure 2A**), measurements carried out with DWS showed that, at concentrations of HMP >0.08%, the diffusion coefficient of the scatterers decreased and the particle size of the oil droplets increased (**Figure 2B**). On the other hand, the addition of SSPS did not affect the size of the oil droplets as measured by both techniques. While the data collected with DLS was calculated using water viscosity at 23 °C and did not need to be corrected by viscosity of the medium, because the measurements were performed under extremely diluted conditions, DWS results were corrected for medium viscosity (viscosity of HMP or SSPS solutions). The average diameters reported in **Figure 2B** suggested that the addition of SSPS did not affect the size of the oil droplets in the emulsions, while HMP at high enough concentrations caused some aggregation, which could be measured by DWS. As HMP and casein-stabilized oil droplets are both negatively charged, it was concluded that the increase in size measured by DWS corresponded to depletion flocculation. However, the depletion interaction was reversible and was disrupted by the dilution step in DLS measurements. These results were in agreement with those reported in the literature (*9*, *10*, *14*), and showed for the first time that, by combining DWS and DLS measurements, it is possible to clearly identify depletion flocculation of an emulsion.

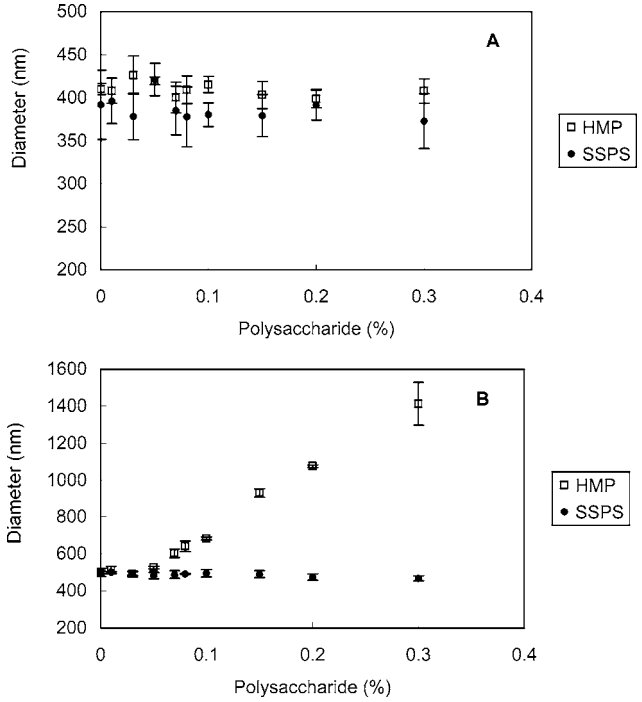

Figure 2. Average hydrodynamic diameter measured by dynamic light scattering (A) and the average diameter measured by diffusing wave spectroscopy (B) for emulsion droplets at pH 6.8 as a function of HMP (□) and SSPS (●) concentration. Values are average of three independent experiments, bars represent standard deviation.

Figure 3 illustrates the changes in the 1/*l** parameter measured by DWS and the ultrasonic velocity and attenuation measured by ultrasonic spectrometry (US) in the emulsions at pH 6.8 as a function of the amount of HMP or SSPS added. When HMP was added to the emulsion, the 1/*l** parameter showed a decrease with HMP concentration up to 0.07% before leveling off when further increasing the HMP concentration to 0.3% (**Figure 3A**). The changes in 1/*l** can reflect changes in refractive index of medium, particle size, and/or spatial correlation between particles, as explained in the Theory section. Using theoretical calculations involving Mie scattering theory and Percus−Yevic closure relation (a detailed explanation of these calculations is beyond the scope of this paper but can be found in Romer et al.), it was determined that the change in refractive index contrast in the medium to obtain such a decrease in 1/*l** would need to be much higher than that possible under these conditions; therefore, it was concluded that the steep decrease in 1/*l** at the low HMP concentrations (<0.07%) was caused by the rearrangement of emulsion droplets in space due to the strong electrostatic repulsion between pectin and oil droplets. This rearrangement of the oil droplets was shown quite clearly by DWS, and eventually, at concentrations >0.07% HMP, the depleted domains were clearly defined and depletion flocculation occurred. At these HMP concentrations, an increase in diameter was also noted (**Figure 2B**). Further increasing HMP concentration from 0.07% to 0.3% simply made these domains more crowded, but the overall spatial distribution and subsequently the 1/*l** parameter showed no significant change (**Figure 3A**). Moreover, we did observe a visible creaming on the samples containing more than 0.05% HMP at 24 h, confirming that the changes in 1/*l** are the indication of early stage of depletion flocculation of HMP-containing emulsions. Similar observations of changes in 1/*l** for emulsions undergoing depletion flocculation has been reported previously (*24*).

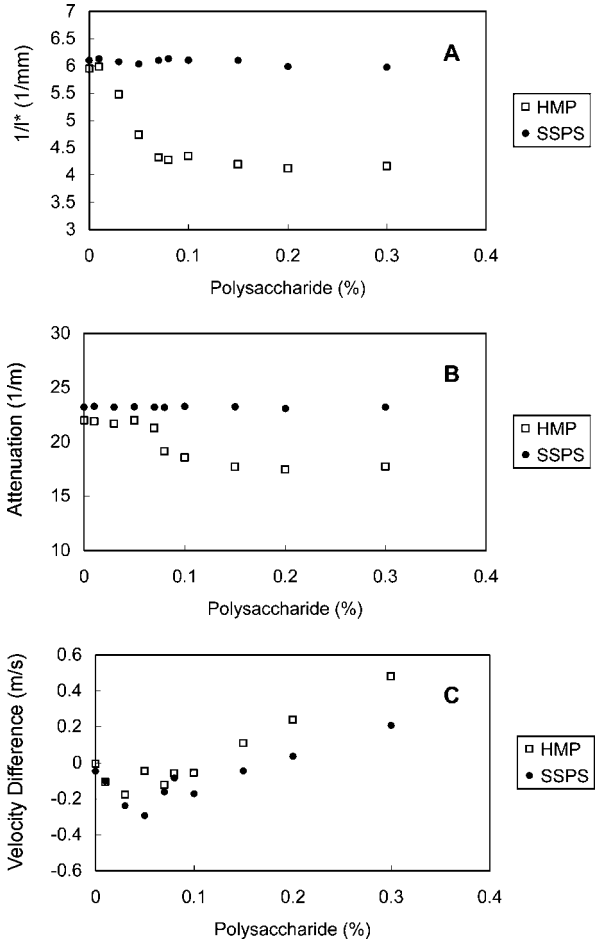

Figure 3. Values of 1/*l** measured by DWS (A), ultrasonic attenuation (B), and ultrasonic velocity difference (C) for emulsions at pH 6.8 as a function of HMP (□) and SSPS (●) concentration. Ultrasonic parameters shown for measurements at 5 MHz. Data are least-square means of three independent experiments.

The least-square means of the ultrasonic attenuation and velocity difference obtained by US at 5 MHz are also shown in **Figure 3**. The same trend was observed for all five frequencies used in our study, although the absolute value of attenuation increased with increasing frequency. When the sound propagated through the emulsions, the attenuation as a function of HMP concentration showed a similar behavior to 1/*l**: the attenuation decreased up to 0.1% HMP and then leveled off at high HMP concentrations (**Figure 3B**). The presence of the nonadsorbed HMP resulted in spatial rearrangement of the oil droplets. Depletion flocculation in droplet-rich domains will occur at high enough HMP concentrations, leading to an overlap of their thermal waves. This caused reduced thermal losses and subsequently the attenuation of sound. However, the attenuation leveled off at higher HMP concentration. This could suggest that adding more HMP did not change the overall structural properties of the system but simply put more droplets in the separated phases. A similar observation in attenuation was reported for depletion flocculation of emulsion droplets with added xanthan (0−0.2%) (*25*). The ultrasonic velocity difference (the difference between the sample velocity and that of a reference emulsion with no polysaccharide) remained relatively constant (**Figure 3C**) (not statistically significant differences at $p < 0.05$) until 0.15% HMP and then increased with HMP concentrations, indicating that the compressibility of the system decreased. This may be caused by the higher amount of solids

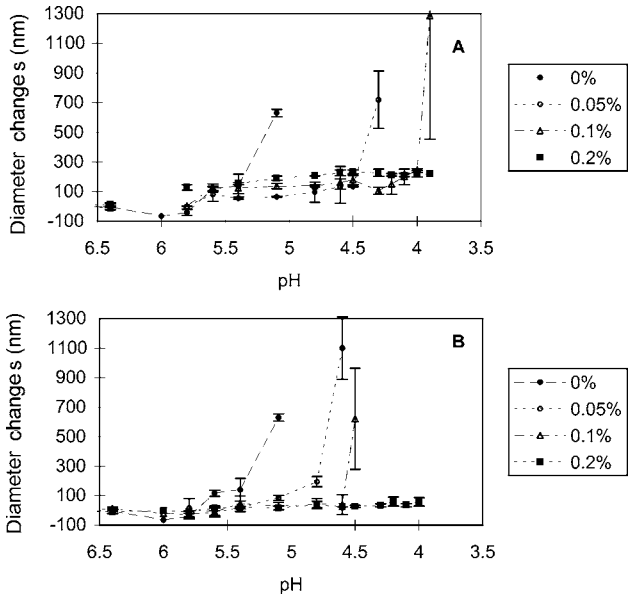

Figure 4. Changes in diameter measured by DLS during acidification. The emulsions were diluted in permeate from UF filter (see methods for detail). Average hydrodynamic diameter for emulsions with HMP (A) or SSPS (B) with different amounts of polysaccharide added. Results are the average of three independent experiments, bars represent the standard deviation

present compared to the original emulsion, as well as the high hydration of the pectin molecules present in solution.

Addition of SSPS to the emulsions showed a dramatic difference in the behaviors of the parameters measured by DWS or US compared to the emulsions with HMP (**Figures 2,3**). With increasing SSPS concentration up to 0.3%, the droplet diameter, the parameter $1/l^*$, and ultrasonic attenuation remained constant. These results indicated that the structural arrangement and optical properties of the system were not significantly different from the original emulsion without SSPS and confirmed the conclusions from DLS and ζ potential measurement that SSPS does not adsorb onto the surface of droplets. The ultrasonic velocity difference also showed an increase in emulsions containing SSPS (at the high concentrations) (**Figure 3C**), indicating an increase in compressibility due to the changes in the composition of the sample. While the presence of nonadsorbed HMP caused creaming in the emulsions at 24 h, there was no visible creaming observed in emulsions with SSPS over the same period of time. These differences between SSPS and HMP observed at pH 6.8 were caused by the difference in their structural properties. SSPS contains less galacturonic acid, the ζ potential of SSPS has been shown to be lower than HMP and the charge distribution on the backbone is different for the two polysaccharides (*15*). The electrostatic repulsion between SSPS and droplets therefore may be smaller than that between HMP and oil droplets. It has also been shown theoretically that, at fixed volume fraction, the magnitude of the depletion attraction between particles increased with the degree of nonsphericity of the macromolecular species (*26*). Hence, nonadsorbing HMP with extended and linear structure may be especially effective at inducing depletion effect rather than SSPS, which has a more branched structure and globular shape (*7*). These results were in agreement with those previously reported (*12*), where the addition of up to 0.3% SSPS did not cause destabilization of soy protein isolate-stabilized emulsion at neutral pH.

Interactions of HMP or SSPS with Caseins at Oil/Water Interfaces during Acidification. Figure 4 illustrates the apparent change in diameter, from those measured at pH 6.8, of the emulsions measured by DLS as a function of pH during acidification for emulsions containing HMP (**Figure 4A**) and SSPS (**Figure 4B**). In this case, the samples were extensively diluted in the corresponding permeate (collected from ultrafiltration), and the viscosity of permeate was used in the diameter calculations (see eq 3). The control emulsion showed a large extent of aggregation at pH 5.4 (large change in diameter), with a decrease in size at about pH 6.0 (negative change in D). The decrease in size before acid-induced aggregation of sodium caseinate-covered oil droplets has been previously reported (*24*). As seen in **Figure 4A**, in emulsions containing HMP, an increase in the droplet diameter between pH 6.0 and 5.5 was measured and the diameter rapidly reached a plateau. This increase in diameter indicated the adsorption of HMP at the interface. When not enough pectin was present, the pH of destabilization depended on the amount of HMP present: the emulsions containing 0.05% and 0.1% HMP showed destabilization at pH 4.3 and pH 3.9, respectively. At these pHs, the polysaccharide has opposite charge to that of the protein at the interface. For this reason, there will be a net attractive force between the two of them, which will result in the polysaccharide binding on to one or more emulsion droplets at the same time. If not enough pectin is present, two droplets might "share" the polysaccharide, giving rise to bridging flocculation. At high enough concentration of HMP (0.2%), the oil droplets were stable to acidification, as no aggregation occurred after the apparent diameter increase of about 100 nm.

Destabilization also occurred in emulsions containing 0.05% or 0.1% SSPS (**Figure 4B**). At these concentrations, a rapid increase in diameter was shown. The destabilization pH of these emulsions was higher than that observed in HMP-containing emulsions. Emulsions containing 0.2% SSPS did not show aggregation and a small increase in size was shown, however, not statistically significant. The difference in size at pH < 5.5 between the control emulsion (aggregated at this pH) and 0.2% SSPS-containing emulsions (with a size comparable to that of the emulsion at the starting pH) suggested that SSPS is present at the interface and helps in stabilizing the oil droplets against acid-induced aggregation.

SSPS may interact with positively charged caseins patches through their negatively charged backbone via electrostatic interactions, as suggested for the adsorption of SSPS onto casein particles at low pH. It has been reported that SSPS covers the caseins particles in acid milk suspension with a thin monolayer (*27*). Usually, the adsorbed layer thickness is of the order of the polymer radius of gyration (*28*). The root-mean-square radius of the HMP and SSPS were 43.0 ± 2.5 and 45.2 ± 3.1 nm, respectively (*8*). Therefore the formation of a SSPS monolayer at the oil droplet surfaces should result in an increase in hydrodynamic radius less than 45 nm, which is in agreement with our result (**Figure 4B**). The adsorption of HMP caused an increase of 100 nm in hydrodynamic radius of the emulsion droplets (**Figure 4A**), suggesting the formation a HMP multilayer; however, more direct evidence is needed to prove this hypothesis.

Figure 5 illustrates the average size of the emulsions and the $1/l^*$ value measured by DWS as a function of pH during acidification. After addition of GDL, the pH slowly decreased toward the isoelectric point of the caseins, causing a sol−gel transition of sodium caseinate-emulsion (*4*). Acidification of the control emulsion resulted in a steep increase in droplet size and decrease of the $1/l^*$ value at pH 5.35, indicating extensive aggregation (**Figure 5A**).

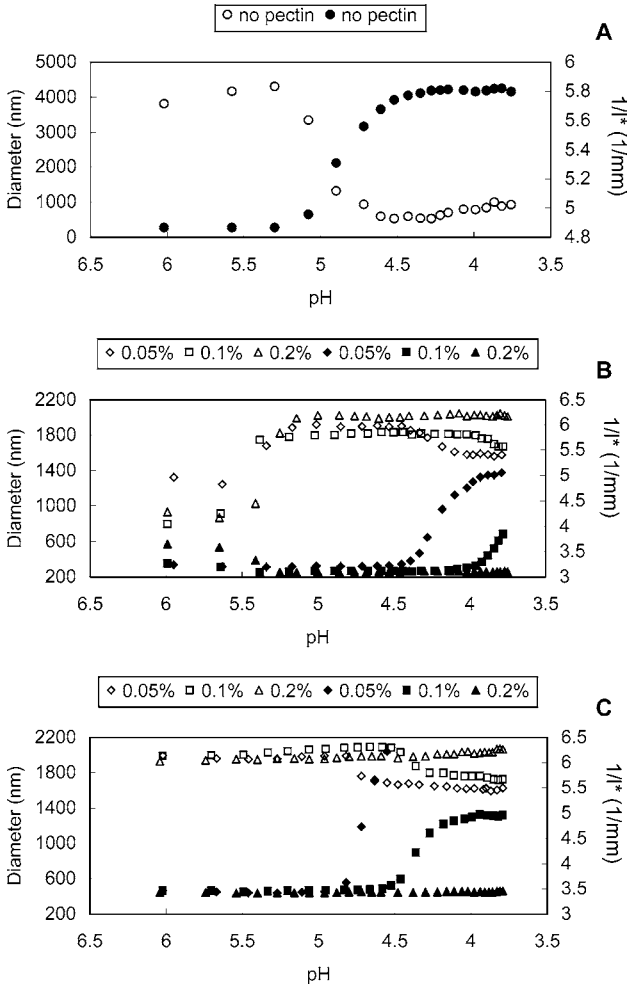

Figure 5. Changes in average diameter (filled symbols) and 1/*l** (empty symbols) of emulsions measured by DWS during acidification. No polysaccharide (A), HMP (B), or SSPS (C). Data are the least-square means of three experiments independent experiments.

When adding increasing amounts of HMP to the emulsion, 0.1 and 0.2% HMP caused lower initial 1/*l** value and higher droplet diameter than 0.05% HMP (**Figure 5B**), this was consistent with the observation at pH 6.8. During acidification, after remaining constant for a period of time, a rapid increase in 1/*l** was shown regardless of the HMP concentration tested. Statistical analysis showed no significant difference in the value of pH of onset between HMP concentrations and replications. This increase in 1/*l** at pH 5.6, occurring right before the aggregation of the control emulsion, could be caused by the adsorption of HMP onto the droplet surface. Upon lowering pH, the caseins-coated droplets gradually lost steric repulsion and had some positively charged patches appearing on the protein layer. Thus, negatively charged pectin adsorbed via electrostatic interaction. The adsorption of pectin from the serum phase to the interface changed the interactions between droplets, leading to the redistribution of oil droplets from microphase separation to redispersion, resulting in a spatial arrangement similar to that of control emulsion. This caused 1/*l** to increase to a value closer to that for control emulsion at neutral pH. The incorporation of HMP at pH 5.5 into the interface of caseins-stabilized emulsion has been observed previously (*9*, *24*). In the present work, it was assumed that the viscosity of the dispersed phase did not change during acidification, however, at pH <5.5, HMP adsorbed onto the droplet surface, resulting in less HMP molecules present in the dispersed phase. This may likely cause an underestimation of the diameter calculated from DWS measurements.

After 1/*l** reached a plateau at pH 5.1, 1/*l** and droplet diameter changed simultaneously for a given HMP concentration. In the emulsion containing 0.05% HMP, the increase in droplet size and decrease in 1/*l** occurred at the same pH (pH 4.55). The addition of 0.1% HMP shifted this transition to a lower pH (pH 4.0). This could result from the bridging of HMP between droplets at low pectin concentration (*10*, *24*). Low concentrations of HMP (0.05 and 0.1%) were not sufficient to fully stabilize the droplets and bridging occurred. Bridging flocculation changed the spatial correlation between droplets as well as their apparent size, as small "aggregates" of droplets now diffused together, resulting in a decreased 1/*l**. However, with the presence of 0.2% HMP, 1/*l** and droplet diameters kept constant at pH < 5.1, and the value of 1/*l** at pH 3.8 reached 6.1 mm^{-1}. This value was not significantly different from that of 5.9 mm^{-1} of control emulsion measured at pH 5.5−5.7. This suggested that the emulsion droplets were stabilized against acid-induced aggregation and had a structural arrangement similar to the original control emulsion. The stabilization effect of HMP has been attributed to the increased electrostatic and steric repulsion between droplets due to the adsorbed HMP (*10*).

SSPS showed a different behavior from HMP in the emulsion during acidification (**Figure 5C**). While initial 1/*l** and droplet size changed with HMP concentrations, adding increasing amounts of SSPS showed no significant difference in the initial value of 1/*l** and droplet diameters, which agrees with the results at pH 6.8. Both 1/*l** and droplet size changed simultaneously during acidification. At the beginning, these parameters remained constant and there was no obvious evidence of SSPS adsorption, as previously shown by an increase in 1/*l** in HMP-containing emulsion at pH 5.6. SSPS in solution has a much lower viscosity than HMP, therefore, adsorption of SSPS, if any, will not significantly affect the viscosity of the continuous phase. This could be the reason why the droplet size measured did not change at the beginning of acidification. This was confirmed by recalculating the diameter using the viscosity of water, and the resulting diameters were not significantly different from those calculated using the viscosity of SSPS solutions.

The emulsion containing 0.05% SSPS showed a decrease in 1/*l** and increase in droplet diameter at pH 4.9 (**Figure 5C**). This pH is much lower than the pH of aggregation of control emulsions (pH 5.4) (**Figure 5A**). At pH 4.9, negatively charged SSPS may interact with positively charged patches on the adsorbed casein layer, resulting in charge neutralization and bridging. The addition of 0.1% SSPS shifted the destabilization to a lower pH of 4.6. With 0.2% SSPS added, the values of 1/*l** and droplet sizes remained constant throughout acidification. This demonstrated that the acid-induced aggregation was inhibited by the presence of sufficient SSPS. The parameter 1/*l** at pH 3.8 had a value of 6.16 mm^{-1}, no different from that for 0.2% HMP-containing emulsion at pH 3.8 as well as of the original control emulsion (no statistical significant differences at $p < 0.05$). This indicated that the emulsions at pH 3.8 containing 0.2% SSPS have a similar structural property to those of control emulsions before acidification. This result is the first evidence of the stabilization effect of SSPS in acidified sodium caseinate emulsions. It has been reported that SSPS stabilizes casein particles in acidified milk dispersions mainly via steric repulsion (*8*, *15*). The mechanism of stabilization of SSPS is yet to be fully understood.

Both HMP and SSPS were able to delay the acid-induced destabilization of emulsion and shifted it to lower pH values

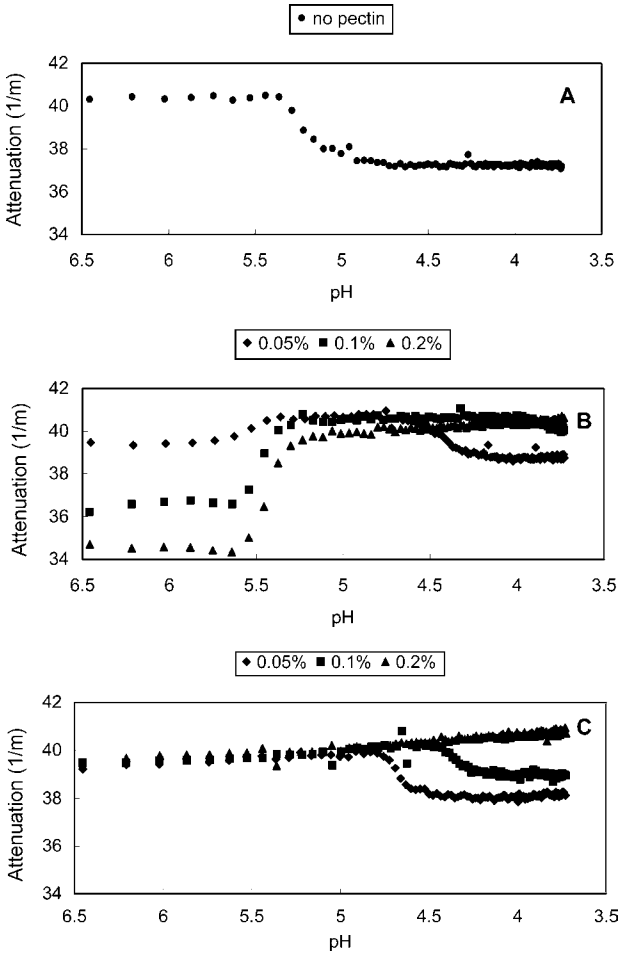

Figure 6. Changes in ultrasonic attenuation measured by ultrasonic spectroscopy at 8.7 MHz during acidification. No polysaccharide (A), HMP (B) or SSPS (C). Data are the least-square means of three independent experiments.

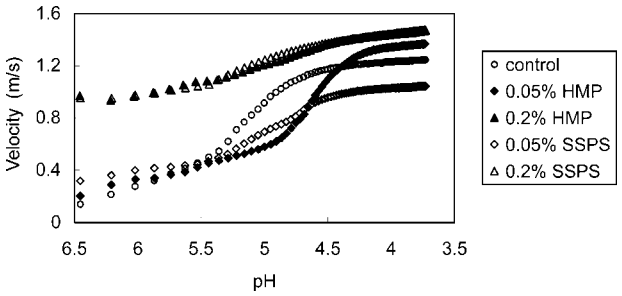

Figure 7. Changes in the ultrasonic velocity difference (difference between the velocity of the sample and that of emulsions with no polysaccharide and no GDL added) measured at 8.7 MHz during acidification. Data shown are representative of one experiment.

with increasing concentration, but the pH of destabilization caused by SSPS was higher than that caused by HMP bridging (**Figures 4,5**). This suggested that, at comparable concentrations, HMP was more effective than SSPS in stabilizing sodium caseinate-coated emulsions and shifting the pH of destabilization to lower values, in the pH range tested.

The dynamics of the interactions of HMP and SSPS with sodium caseinate-stabilized emulsions was also studied using US, a technique complementary to DWS. Only the results as a function of pH at 8.7 MHz are shown in **Figures 6** and **7**, as a similar behavior in ultrasonic attenuation and velocity was shown at all the five frequencies tested. When no polysaccharide was present, the attenuation of sound for control emulsions decreased at pH 5.4 (**Figure 6A**), which is similar to the behavior of $1/l^*$ in DWS (**Figure 5A**). A decrease in attenuation at these frequencies has been previously reported for flocculated emulsions (*11*, *25*). This decrease in attenuation was mostly caused by changes in viscous and thermal losses, related to the density and thermal gradient at the interface of the oil droplets. It has been suggested that in emulsions, low density contrast systems, the predominant form of attenuation at low frequencies is thermal losses that depend on the spatial arrangement of droplets (*22*, *29*, *30*). When droplets flocculated, the thermal waves of droplets in close vicinity overlapped. Therefore, the temperature gradient between a droplet and its surroundings decreased causing the thermal energy losses to be reduced, thereby leading to a lower attenuation of sound (*22*, *30*).

When HMP was added, the attenuation showed very similar behavior to the parameter $1/l^*$ in DWS (**Figures 5B** and **6B**). As shown in **Figure 6B**, at the starting pH, the addition of increasing amounts of HMP resulted in decreased initial attenuation. As discussed earlier (**Figure 3**), this was caused by the structuring of droplets due to electrostatic repulsion as well as the depletion flocculation of oil droplets at high pectin concentration. At pH 5.7, all the HMP-containing emulsions showed an increase in attenuation right before the aggregation of control samples, indicating the interactions of HMP with emulsion droplets, as shown previously by the increase in $1/l^*$ (**Figure 5B**). The adsorption of HMP changed the droplet surface as well as the interaction between droplets, resulting in the structural rearrangement of droplets and subsequently an increase in attenuation. In addition, at pH 4.6 and 4.0, a decrease in attenuation was observed in emulsions with 0.05 and 0.1% HMP, respectively. This decrease in attenuation corresponded to the increase in droplet sizes as measured by DWS. The bridging flocculation of emulsion droplets reduced thermal losses, thereby decreasing the attenuation. The decrease in attenuation caused by pectin bridging in whey protein-stabilized emulsion has been previously reported (*11*). After reaching a plateau at pH < 5.1, the attenuation of sound for emulsions containing 0.2% HMP did not change significantly and reached an end value of 39.90 m^{-1} at pH 3.8. This value was not statistically significantly different from that (39.87 m^{-1}) of the attenuation of control emulsions at neutral pH. This demonstrated that the emulsions containing 0.2% HMP at pH 3.8 have a structural organization similar to the emulsion control without HMP in which the individual droplets were separated from each other, indicating that HMP stabilized oil droplets against acid-induced aggregation as previously discussed (see **Figures 4,5**).

When SSPS was added (**Figure 6C**), the behavior of the ultrasonic attenuation was in agreement with the DWS results (**Figure 5C**) and different from that of HMP-containing emulsions (**Figure 6B**). The initial attenuation for emulsions containing various amounts of SSPS did not show significant differences from the emulsion control (no polysaccharide) at pH $>$ 5.4 due to the fact that SSPS does not induce depletion flocculation. In emulsions containing 0.05 or 0.1% SSPS, a decrease in attenuation was shown at pH 4.8 and 4.4, corresponding to the decrease in $1/l^*$ and increase in droplet diameter as measured by DWS (**Figure 5C**) and DLS (**Figure 4B**). This decrease in attenuation is most likely caused by decreased thermal and viscous energy losses when emulsion droplets flocculate due to the presence of SSPS. However, no decrease in attenuation was observed in emulsions with 0.2% SSPS

throughout acidification (**Figure 6C**), which corresponded to the constant $1/l^*$ and droplet diameter shown by DWS (**Figure 5C**). Compared to the deep decrease in the ultrasonic attenuation of the destabilized emulsion, the relatively constant attenuation here confirmed what was shown by the DWS results that the acid-induced aggregation was inhibited by SSPS. On the other hand, the emulsion reached an end value of attenuation of 41.05 m^{-1} at pH 3.8. This value was significantly higher than that for control emulsions (with no polysaccharide added) at neutral pH and emulsions containing 0.2% HMP at pH 3.8. This demonstrated that the energy losses in the SSPS-stabilized emulsion were higher, suggesting the SSPS-stabilized emulsion droplets may be different in their surface properties from HMP-sodium caseinate emulsions or control sodium caseinate emulsions. While HMP stabilized the emulsion droplets via steric and electrostatic repulsion, SSPS may provide stabilization mainly through its adsorption at the interface and steric repulsion. Because SSPS does not cause depletion flocculation and the adsorption of SSPS molecules onto the emulsion does not change the size or the index of refraction of the droplets significantly, the adsorption or stabilization behavior of SSPS would not be measurable using a static measurement such as the $1/l^*$ transmission parameter.

Figure 7 illustrates the changes in velocity of sound in emulsions during acidification measured at 8.7 MHz. Emulsions containing 0.2% HMP or SSPS at the starting pH showed a higher velocity difference than the control emulsion and emulsion with 0.05% polysaccharide due to the increased amount of molecules present in the samples. During acidification, the same overall pattern was shown at different frequencies and for HMP- or SSPS-containing emulsions: in all samples, the velocity difference increased with decreasing pH. However, the extent of change of velocity in control emulsions was higher than that in emulsions containing 0.05% polysaccharide, which in turn was higher than emulsions with 0.2% polysaccharide. The acid-induced aggregation in control emulsions or the bridging flocculation by the presence of 0.5% HMP or SSPS resulted in significant decrease in compressibility of the acidified system compared to the original emulsion. The increase in velocity caused by bridging flocculation in emulsions at low pH has been shown previously (*11*). The very small changes in velocity between the initial pH and acid pH in emulsions containing 0.2% HMP or SSPS indicated that the overall compressibility of the systems did not change. This was probably caused by competing changes occurring in the emulsions, such as rearrangement of droplets and adsorption of HMP, resulting in no significant changes in the ultrasonic velocity.

This study shows, for the first time, that high enough concentrations of SSPS can stabilize sodium caseinate emulsions against acid-induced aggregation. SSPS interacts with the protein-stabilized oil droplets via electrostatic interactions, although very different in structure from HMP. Because of the differences in charge distribution and in their molar mass versus size (SSPS is more branched than HMP, which has a more elongated structure), the stabilizing behavior of the two polysaccharides is different, and at high pH, SSPS does not cause depletion flocculation at concentrations where HMP is instead effective at causing destabilization. The comparison of data collected in this work clearly indicated that both DWS and US are excellent techniques for the study of destabilizing systems and they both can identify changes in the physical aspects of the particles as well as the state of correlation of the system.

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