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Stabilization of Caseinate-Covered Oil Droplets during Acidification with High Methoxyl Pectin

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Polysaccharides are widely used in the food industry to modify the stability of protein-based drinks. However, an in depth knowledge of the interactions occurring in the system is still lacking. In this study, the interactions between sodium caseinate and high methoxyl pectin under acidification conditions were studied nondestructively and without dilution using transmission diffusing wave spectroscopy. Oil-in-water emulsions were prepared with 10% soybean oil and 0.5% sodium caseinate. Various concentrations of pectin (ranging from 0 to 0.2%) were added, and the emulsions were acidified with glucono-δ-lactone. With acidification, a "sol−gel" transition occurred and emulsions containing pectin were more stable at lower pH than those without pectin. Furthermore, the sol−gel transition of the mixtures was more sudden for control emulsions without pectin. While in control samples the final solidlike emulsion after gelation tended to be more inhomogeneous and more dissimilar to the starting emulsion, emulsions with pectin in solution gelled later under acidification. With a sufficient amount of pectin, the emulsions showed no aggregation and the destabilization pH varied depending on the amount of pectin present in the emulsions. At intermediate pH values (pH > 5.5), the emulsions displayed a decrease in particle size, more pronounced in samples containing pectin. The results collected using light scattering in concentrated systems, 10% (v/v) in our case, suggested that pectin stabilizes the emulsion oil droplets forming a network of oil droplets loosely connected by strands of pectin.

KEYWORDS: Emulsion; sodium caseinate; pectin; diffusing wave spectroscopy (DWS)

INTRODUCTION

Sodium caseinate is widely used as a food ingredient in emulsions, as its components readily adsorb onto the interface of oil droplets providing long-term stability against coalescence via a combination of electrostatic and steric stabilization. At neutral pH, constant volume fraction, and droplet size, an optimal concentration of sodium caseinate is needed to provide stability against flocculation and creaming of the oil droplets. At low concentrations of caseinate, bridging flocculation may occur, while at high concentrations oil droplets will lose their stability because of depletion (*1*).

Acidification converts the liquidlike dispersion of casein particles into a soft solidlike aggregated network. In emulsions stabilized with sodium caseinate, when the pH is lowered toward the isoelectric point of the proteins, the interdroplet interaction changes from a repulsive to a net attraction, inducing droplet flocculation, transforming the concentrated casein-stabilized emulsion into an aggregated emulsion gel (*2*). It has been reported that this aggregation behavior is dependent on temperature, pH, and calcium content (*3*).

Pectin is often incorporated in acidified protein-based drinks to improve the texture and stability of these systems because of its gelling, thickening, and stabilizing properties. High methoxyl pectin (HMP) prevents the formation of a clear serum layer in acid milk drinks by a combination of electrostatic and steric stabilization of the casein particles (*4*, *5*). At and below pH 5.0, HMP molecules adsorb on the casein particles and form multilayers, with a layer thickness that is dependent on pH (*6*). Our understanding of the mechanism of stabilization of HMP on casein dispersions derives from studies on model systems containing casein micelles diluted in HMP solutions or mixtures of caseins and HMP where the pH is reduced after mixing (*7*, *8*).

While the interactions between HMP and caseins during acidification of skim milk are at least partly understood, very little is known on the effect of HMP addition to emulsion droplets stabilized by sodium caseinate. It has been reported that very low concentrations of pectin can protect sodium caseinate-coated droplets against aggregation at pH < 5.0 (*8*). Pectin is incorporated with the protein on the oil−water layer at pH 5.5 (*9*) while not at pH 7.0. Even when pectin is not attached to the interface but present in the dispersed phase, it can have profound effects on the rheological properties and creaming behavior of the emulsions. It has been previously demonstrated that following the addition of HMP or low methoxyl pectin, at high pH and above a critical concentration,

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pectin causes phase separation by depletion, while at low pH the molecules may bridge between droplets causing flocculation (*9*, *10*).

The objective of this research was to study the interactions between HMP and casein emulsions during acidification using glucono-δ-lactone (GDL), under conditions as close as possible to realistic concentrations. Although it is possible to observe the changes occurring to the emulsions with the addition of pectin by rheology, very little is known in undiluted systems on how the presence of HMP affects the particle size and influences the interactions between oil droplets. In the present research, we employed diffusing wave spectroscopy (DWS) to study the effect of pH and concentration of HMP on the aggregation behavior of sodium caseinate emulsions. This light scattering technique has been recently employed to study droplet−droplet interactions in situ to distinguish differences between depletion and bridging flocculation in whey protein-stabilized emulsions as affected by the addition of polysaccharide (*10*).

MATERIALS AND METHODS

Sample Preparation. Sodium caseinate (donated by New Zealand Milk Proteins, Mississagua, Ontario) solutions were prepared by dispersing 0.55% (w/w) sodium caseinate in MilliQ water with continuous stirring. The protein solution was filtered through a 0.8 μm filter (Millex-HV Millipore Co., Billerica, MA). A stock solution of 1% (w/w) pectin (72 DE, AMD 783, donated by DANISCO, Scarborough, ON) was prepared by adding the pectin to MilliQ water at 60 °C and continuously stirring at room temperature until complete solubilization. After overnight storage at 4 °C, the pH of the pectin solution was adjusted to 7.0 using diluted NaOH (Sigma, St. Louis, MO).

Emulsion Preparation. Ten percent oil-in-water emulsions were prepared by adding 10 mL of soybean oil to 90 mL of protein solution. The samples were premixed for 1 min using a high-speed blender (PowerGen 125, Fisher Scientific, Co., Nepean, ON). Emulsions were then prepared at room temperature using a laboratory scale high-pressure homogenizer (Emulsiflex C5, Avestin, Ottawa, ON) with two passes at 40 MPa. Experiments were carried out in triplicate. The particle size distribution of the emulsions was tested immediately after homogenization using integrated light scattering (Mastersizer X, Malvern Inst., Brookhaven, Ma) with a value of 1.06 for relative refractive index (droplet to solvent), 0.001 absorption, and an index of refraction of 1.33 for the solvent. The angular dependence of the scattered light was measured by diluting the emulsion in MilliQ water. The surface−volume mean diameter, $D_{3,2}$, obtained was 250 nm.

Viscosity Measurements. The viscosities of the pectin solutions were measured with an Ubbelohde viscometer (Industrial Research Glassware, NJ) type A859 size 1B. A range of concentrations, from 0 to 0.2%, were measured at 21 °C. This was repeated at least 10 times for each pectin concentration. The average flow time was recorded (in seconds) and was multiplied by the appropriate viscometer constant in order to obtain the results in Centistokes. The linear relationship obtained by plotting the values of viscosity obtained as a function of pectin concentration was used to calculate the appropriate solvent viscosity for a given concentration of pectin.

DWS. As laser light illuminates a colloidal suspension, each particle within it will scatter light. As the particles in the scattering volume move, the intensity of the scattered light will fluctuate with time. These fluctuations can be characterized by their temporal autocorrelation function given by

$$g_{(1)}(t) \equiv \left[\frac{1}{\beta}\left(\frac{\langle I(t)\, I(0)\rangle}{\langle I\rangle^2} - 1\right)\right]^{1/2} \tag{1}$$

where β is a constant determined primarily by the collection optics and $I(t)$ is the intensity at a given instant in time (*11*).

In samples such as ours, where the thickness of the sample $L \gg l^*$ (i.e., $L/l^* > 10$) (where l^* is the transport mean free path) and the time $t \ll \tau$ (the correlation time of the sample), the correlation function can be described by (*11*):

$$g_{(1)}(t) \approx \frac{\left(\frac{L}{l^*} + \frac{4}{3}\right)\sqrt{\frac{6t}{\tau}}}{\left(1 + \frac{8t}{3\tau}\right)\sinh\left[\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right] + \frac{4}{3}\sqrt{\frac{6t}{\tau}}\cosh\left[\frac{L}{l^*}\sqrt{\frac{6t}{\tau}}\right]} \tag{2}$$

This correlation function obtained is nearly exponential in time, with a characteristic decay time of

$$\tau = \tau_o\left(\frac{l^*}{L}\right)^2 \tag{3a}$$

where

$$\tau_o = (Dk_o^2)^{-1} \tag{3b}$$

where D is the particle diffusion coefficient and $k_o = 2\pi/\lambda$, the wave vector of the light.

For a turbid solution, where all photons are multiple scattered, l^* can be calculated from the transmission of light as described in detail elsewhere (*12*):

$$T_i = \frac{I}{I_0} = \frac{5l^*/3L}{1 + 4l^*/3L} \tag{4}$$

where I and I_0 are the initial and transmitted intensities of the laser light. The calibration to find the value of I_0 (since the intensity of the laser cannot be measured directly) was carried out using 343 nm diameter latex spheres (Portland Duke Scientific, Palo Alto, CA) at a concentration where interparticle interactions are negligible. Once the value of l^* is known, the diffusion coefficient can be calculated via eqs 3a and 3b. The radius of the scatterer, R, is then related to the diffusion coefficient via the Stokes−Einstein relation.

$$D = \frac{kT}{6\pi\eta R} \tag{5}$$

where k is Boltzmann's constant and η is the measured viscosity of the medium. This equation is based on a hard sphere assumption. In the case where the scattering particles are not perfectly spherical, such as in aggregating systems, the value of the radius obtained will be that of an average perfect sphere, which best fits the aggregate.

For completely noninteracting scatterers (completely uncorrelated spatially), the l^* value will depend on particle size, particle concentration, and index of refraction. However, in highly concentrated suspensions, there will be a correlation in position and velocities of the scattering particles. These correlations in position affect the angular distribution of the scattered light and hence the turbidity and l^*. For concentrated suspensions such as milk or food emulsions, where there is a substantial volume fraction ($\phi > 0.1$) of dispersed phase, then the dispersed particles are relatively close to one another, and spatial correlations may become important, especially if the particles are charged or otherwise affect one another. In such a case, assuming constant volume fraction and refractive index contrast between the particles and the continuous phase, a change in the value of l^* will indicate some degree of increase in the interactions between particles. This will occur when the emulsion droplets start to interact with one another and aggregate.

The detailed design and operation of the DWS spectrometer are explained in detail elsewhere (*13*). Briefly, DWS measurements were made in transmission mode where the laser impinges on the sample from one side and scattered light is collected from the other. Laser light of 488 nm in wavelength was shone through a rectangular, flat-faced glass cell of 2 mm path length. This cell was immersed in a water bath containing about 1 L of water, and the containment unit was equipped with two glass windows to ensure a clear passage of the laser into and out of it. The scattered light was collected by use of a single mode fiber optic cable bifurcated 50/50 at its outlet end, and

the split light signal was fed into two matched photomultipliers and a cross-correlator, which fed the cross-correlation function into a personal computer.

After the addition of the appropriate amounts of pectin, all final emulsions were diluted to 9% (w/w) oil. To determine their stability, the emulsions containing different amounts of pectin were placed in the DWS spectrometer and analyzed for 25 min at 23 °C. During the acidification experiments, different amounts of GDL were added and emulsions were gently stirred immediately before placing the sample in the sample holder. The effect of pectin concentration on the droplets aggregation was also studied, by adding HMP to the emulsions at concentrations ranging from 0 to 0.2% and following acidification using DWS. All size calculations were corrected for background viscosity. The values of viscosity were those measured with the viscometer (see above) assuming that most HMP was present in the dispersed phase. If some pectin was indeed interacting with the oil droplets, the average radius of the emulsion would be underestimated.

To determine the time dependence of the pH, another set of emulsions was placed in a beaker in a water bath maintained at 23 °C and the pH was measured every 30 s up to a reaction time of 10 min, then at intervals of 1 min up to 30 min, then every 5 min until the close of the experiments for 2 h. Plots of the measured pH against time were averaged and analyzed by curve fitting, so that it was possible to calculate the pH at any time during the reactions. The fitted parameters were used to provide interpolated values for pH for all of the experimentally measured points in the DWS equipment.

All experiments were carried out in duplicate. DWS readings were taken during 5 min at 1 s intervals. The data were collected and analyzed using Sigma Plot software, and eqs 2 and 5 were used via in-house written mathematical routines to determine the $1/l^*$ values and radii. The error bars shown in $1/l^*$ represent the standard deviation of the intensity during the 5 min interval of the measurement (for each experimental run). Because l^* is dependent on the volume fraction of the emulsion and we are not interested in the absolute value of the interactions between the particles but rather on the general dynamic trend, no absolute error was calculated between runs. By showing the variation of the value of $1/l^*$ within a run, it is possible to determine the ergodicity of the sample within short time intervals and ensure the applicability of our equations. When measuring the radius, the standard error between different runs was around 6%. The error was calculated only for the initial stages of the experiments, as when gelling starts to take place. Equation 2 can no longer be applied; therefore, eq 5 ceases to have physical meaning. The radii values are only approximate simply to give the reader a general feeling for the differences between the two systems at these pH values.

RESULTS

To evaluate the stability of the emulsions, DWS measurements were carried out over time at the initial pH (pH 7.0). Different amounts of pectin, from 0 to 0.15% (w/w), were added to the emulsion and observed over a period of 30 min (no GDL was added at any time) (**Figure 1**). While droplet−droplet interactions did not change in the emulsions with no pectin added and for a pectin concentration of 0.01%, when pectin was added at concentrations between 0.02 and 0.08%, changes in the $1/l^*$ parameter seemed to occur over time, reaching equilibrium only after 25 min after the addition. The reason for such behavior is yet to be understood, and it could be caused by a time-dependent diffusion effect of the pectin at intermediate concentrations. At higher concentrations, both apparent radius and $1/l^*$ values were constant over time. The apparent radii of the droplets (corrected for the viscosity of the pectin at the various concentrations) remained fairly constant at all of the pectin concentrations tested (**Figure 1B**), and the average size of the droplets measured with DWS was in agreement with the results obtained by integrated light scattering, which gave an approximate radius of the emulsion droplets of 125 nm.

Changes in the background refractive index, changes in the size distribution of the particles, and droplet flocculation at high

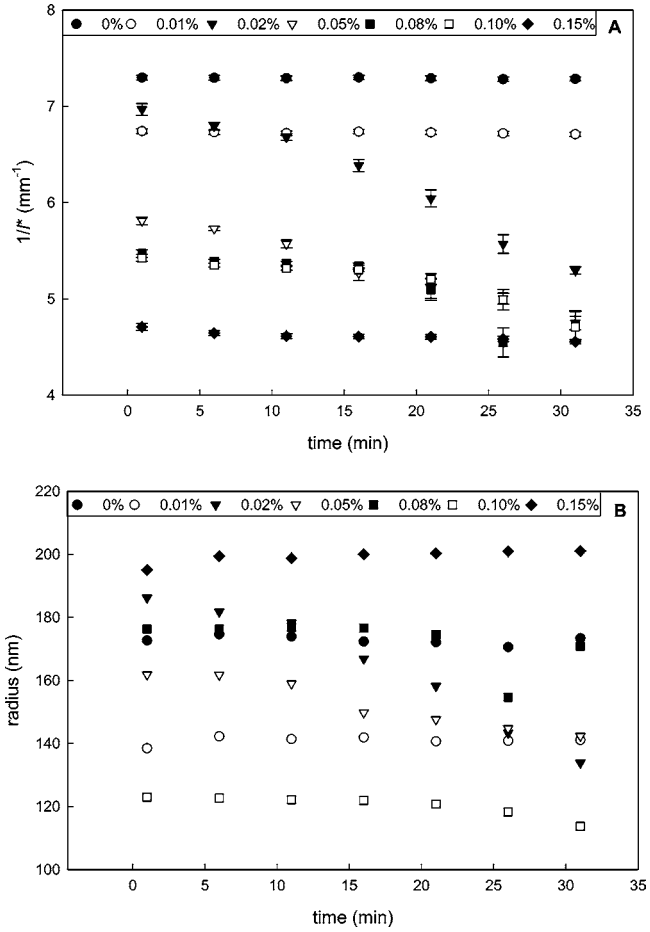

Figure 1. Changes in $1/l^*$ (**A**) and radius (**B**) over time as a function of pectin concentration for 10% oil-in-water emulsions stabilized with 0.5% sodium caseinate at pH 7.0.

pectin concentrations will affect the $1/l^*$ parameter, as explained in the theory section. The decrease in $1/l^*$ with increasing pectin concentration may be caused by the interactions occurring between the HMP and the sodium caseinate-covered emulsion droplets; however, this would be the first evidence of interactions between sodium caseinate emulsions and HMP at neutral pH and needs to be further assessed. The interactions between HMP and pectin at neutral pH and the relation between apparent radius and $1/l^*$ were beyond the scope of this work, and it is currently under investigation. When correcting the DWS data for background viscosity, it was assumed that most of the pectin molecules were present in the dispersed phase; as mentioned earlier, any adsorption of pectin onto the oil−water interface will result in the underestimation of their calculated radius.

To determine the effect of pH on the acidification behavior of the emulsion, different amounts of GDL were added. **Figure 2** shows the development of $1/l^*$ and apparent radius as a function of pH for three different concentrations of GDL (from 0.2 to 0.4%). In control samples (no pectin addition), the initial value of $1/l^*$ was fairly constant at about 6.7 mm^{-1} and remained constant until pH 5.1 (**Figure 2A**). After pH 5.1 a sudden drop in the $1/l^*$ was observed. The pH of aggregation did not change with different rates of acidification (different amounts of GDL up to 0.4%), indicating that the state of the emulsion is solely dependent on the pH of the system and not the rate of acidification. Furthermore, the addition of GDL did not change the optical parameters of the system, as there was no correlation between the amount of GDL added and the initial $1/l^*$ value. The particle size of control emulsions showed a

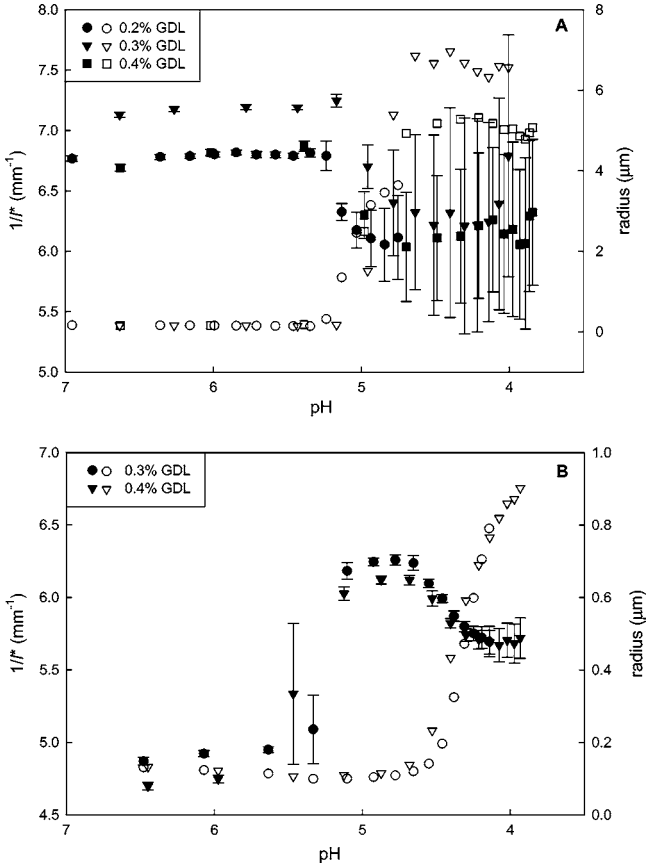

Figure 2. Effect of pH on 1/*l** (filled symbols, left-hand axis) and apparent radius (empty symbols, right-hand axis) for 10% oil-in-water emulsions stabilized with 0.5% sodium caseinate, after the addition of different amounts of glucono-*δ*-lactone. Control emulsions, with no pectin added (**A**) and emulsions containing 0.1% pectin (**B**). Error bars indicate the standard deviation of the 1/*l** value. Note the difference in scale of the *y*-axis.

similar trend, as the initial radius of the particles was similar and constant for the three emulsions with differing amounts of GDL. At pH 5.1, the radii for all emulsions showed a rapid increase of several orders of magnitude (**Figure 2A**). It is important to point out that the drastic increase in radius coincided with the sudden drop in 1/*l**. The addition of GDL clearly caused destabilization of the oil droplets covered with sodium caseinate, and the pH of destabilization, as shown by 1/*l** and a sudden increase in radius, was about 5.1. These results are in agreement with previous research published on the acid-induced gelation of sodium caseinate-stabilized emulsions (*3*).

When the same amount of GDL was added to sodium caseinate emulsions containing 0.1% pectin (**Figure 2B**), GDL did not affect the initial values of 1/*l**, confirming what was reported for control emulsions. However, it should be noticed that these initial values, although similar among themselves, were different from the initial 1/*l** values shown in **Figure 2A** for the control samples. The addition of pectin caused a decrease in 1/*l**, in agreement with the results shown in **Figure 1A**. Assuming that at the initial pH there are no interactions between the pectin molecules and the sodium caseinate, this decrease in 1/*l** could be attributed a change in the interactions between the droplets because of microphase separation at neutral pH.

When 0.1% pectin was present in the emulsion, the 1/*l** parameter remained constant until pH 5.5, after which 1/*l** started to increase (**Figure 2B**). This pH was significantly higher than in the control samples with no pectin. At pH 5.1, 1/*l** reached a quasi-plateau, which decreased slightly at a lower pH (about 4.6), reaching equilibrium at a 1/*l** value of approximately 5.7 mm^{-1}. While in the control samples the average size of the oil droplets and the 1/*l** changed at the same pH for all the concentrations of GDL tested, in emulsions containing 0.1% pectin, the aggregation behavior was different. The emulsions containing different amounts of GDL had the same initial droplet radius. Aggregation of the emulsion droplets did not seem to occur until the pH reached 4.6, while 1/*l** changed at a higher pH. Only at pH 4.6, the emulsion droplets showed a rapid increase in the average radius, although not to the extent shown for the control samples (**Figure 2**; notice the difference in the *y*-axis). It was concluded that the presence of pectin in solution changed the details of the emulsion behavior, with aggregation occurring at a lower pH and 1/*l** changing at higher pH than in control emulsions. It has been observed previously (ref) that at the isoelectric point of the proteins covering the emulsion droplets, pectin adsorbs onto the interface rendering further stability to the emulsion particles. This is probably the reason for the delayed aggregation point of the solution containing pectin (**Figure 2B**).

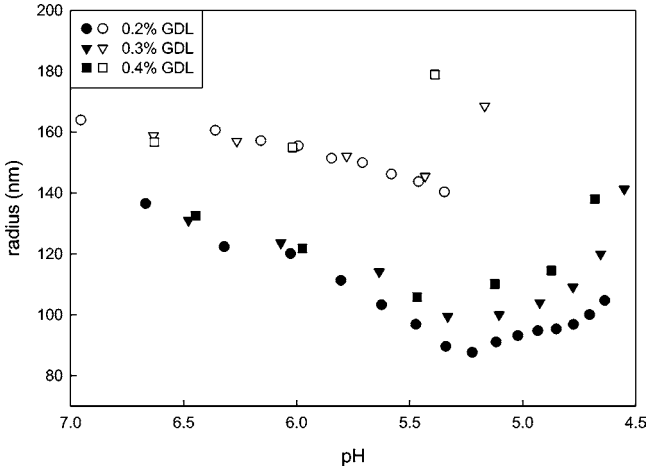

Figure 3. Detailed view (from **Figure 2**) of the initial stages of aggregation as a function of pH for control emulsions (open symbols) and emulsions containing 0.1% pectin (filled symbols).

Figure 3 illustrates in detail the oil droplets size changes before the steep increase in radius at the pH of aggregation. While there was a decrease in radius of about 24 nm in the control emulsions between pH 6.8 and pH 5.5, when 0.1% pectin was added to the sodium caseinate-stabilized emulsions, the oil droplets showed a much larger decrease in radius, up to 50 nm. In both emulsion systems, the minimum was reached at pH 5.3, although the steep increase in radius was observed at a different pH for pectin-containing emulsions and control emulsions. The overall trend was similar for both sets of emulsions; however, the emulsions containing pectin showed a larger decrease in size than the control emulsions, and the steep increase occurred at a lower pH (**Figure 2**).

Figure 4 illustrates the effect of pectin concentration on the behavior of 1/*l** and the radius of the emulsions as a function of pH after the addition of 0.3% GDL. For each pectin concentration in the range from 0.01 to 0.2%, the interaction parameter 1/*l** remained constant until a threshold pH of 5.3. This confirmed what was previously observed in **Figures 1** and **2**, that the initial value of 1/*l** decreased with pectin concentration possibly due to microphase separation. On the other hand, the final 1/*l** at pH < 5.2 for all pectin concentrations reached a value of about 6 mm^{-1}.

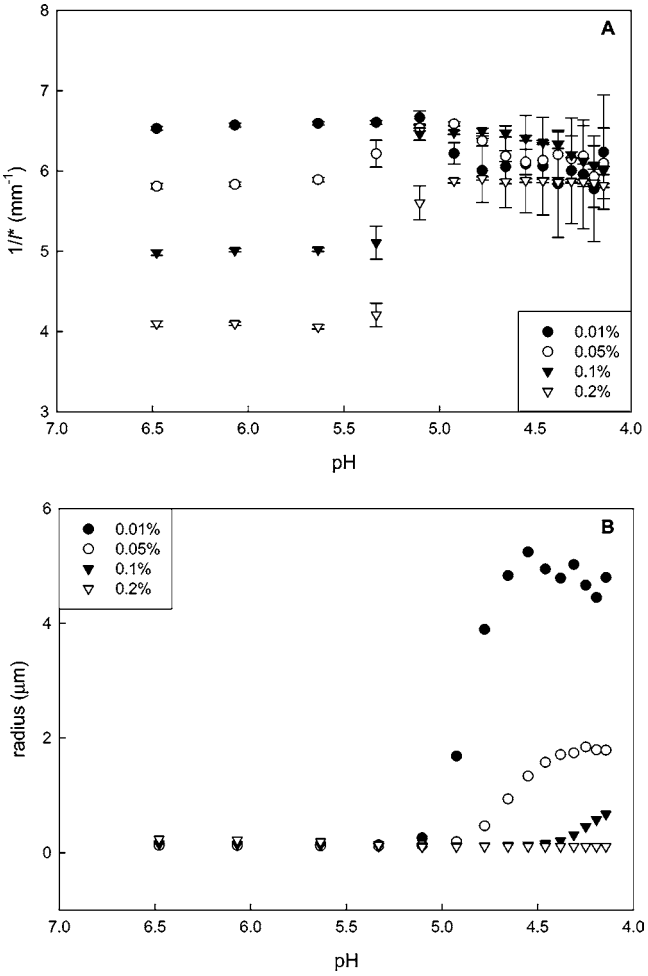

Figure 4. Effect of pH on 1/*l** (**A**) and apparent radius (**B**) for emulsions stabilized with sodium caseinate, after the addition of different amounts of pectin (from 0.01 to 0.2%). All samples contained 0.3% GDL.

While the amount of pectin strongly affected the initial values of 1/*l** but not the final state of the emulsions, the emulsions stability and aggregation rate depended on the amount of pectin present in solution (**Figure 4B**). The emulsions containing 0.1 and 0.2% pectin were more stable at low pH values than those with less pectin. In addition, the initial pH of aggregation seemed to depend on the amount of pectin added. Sodium caseinate emulsions containing 0.01% pectin showed a dramatic increase in size at pH 5.1. The pH of aggregation decreased with increasing concentration of pectin, with a value of 4.8 and 4.5 for emulsions containing 0.05 and 0.1% pectin, respectively. Emulsions containing 0.2% pectin were stable with pH.

As previously discussed, the pH of aggregation decreased with pectin concentration; however, a more detailed look at the apparent radius as a function of pH showed other differences in behavior (**Figure 5**). For the emulsions containing a small amount of pectin, up to 0.05%, the average radius was constant until the emulsion reached the destabilizing pH. When a larger amount of pectin was added, the radius showed a decrease with pH before the aggregation pH. When 0.1% pectin was added, the initial average radius was 150 nm and decreased by 40 nm before the onset of aggregation at pH 4.6. When enough pectin was added to the sodium caseinate emulsions (0.2% HMP), the initial radius was larger than for the other emulsions. In addition, during acidification, a decrease in radius of around 100 nm was observed, after which the value reached a plateau. This behavior confirmed the results shown in **Figure 3** for varying concentrations of GDL, where a decrease in droplet radius was observed before the onset of aggregation at low pH.

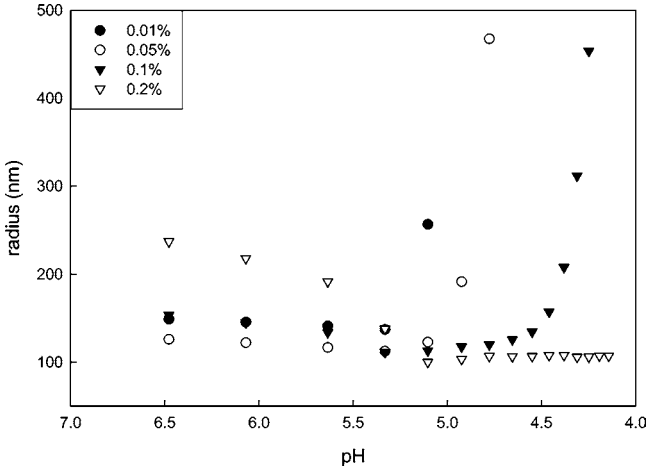

Figure 5. Detailed view (from **Figure 4**) of the initial stages of aggregation as a function of pH for emulsions containing different amounts of pectin.

DISCUSSION

DWS is an excellent technique for the study of highly turbid media, and it was employed to observe in situ the interactions of emulsion droplets occurring during acidification, without emulsion dilution. The 1/*l** parameter obtained from the turbidity experiment is an indication of both the physical aspects of a scatterer and the "state of correlation" of the system. A change in size of the oil droplets or a change in refractive index contrast will affect 1/*l**. If no changes of the physical parameters (such as size or index of refraction) occur in the particles themselves, the change in 1/*l** will signify a change in the correlation state of the system or the "awareness" of the particles of the presence of immediate neighbors.

At neutral pH, sodium caseinate is an effective stabilizer for the oil droplets. At this pH, the proteins are negatively charged and the pectin molecules in the dispersed phase, also negatively charged, will not interact with the oil droplets. DWS measurements of emulsions containing different concentrations of pectin (**Figure 1**) indicated a decrease in the 1/*l** value with pectin concentration. This decrease may have been caused by an increase in the correlation of the scattering droplets due to the presence of pectin in solution (because of a depletion mechanism at the high pectin concentrations). More work needs to be done in this area to properly understand what mechanisms are at play between the emulsion droplets and the pectin in solution.

As the pH approached the isoelectric point of the proteins, the screening charges at the oil/water interface decreased significantly and the emulsion droplets began to aggregate. This behavior was clearly observed by the DWS measurements of control emulsions. At a pH around 5.1, the 1/*l** parameter decreased sharply and there was a severe increase in the effective radius of the particles. However, when pectin was added to the emulsions, there was a marked difference in behavior: the 1/*l** parameter increased at pH 5.5 while the radii remained constant (in stark contrast with the control case where both changed at the same time) to then decrease slightly, at pH 4.6, until it reached a plateau. The decrease of 1/*l** was observed at the same pH as the increase in radius also for pectin-added emulsions. With the decrease in pH, the protein coat decreased its charge and the positively charged patches on the casein molecules interacted with the pectin molecules. As the pectin

molecules adsorbed onto the surface of the emulsion droplets, they changed the overall index of refraction of the particle. As stated previously, the $1/l^*$ parameter depends on size, refractive index contrast, and correlation of the particles. It is clear from **Figure 3** that in emulsions containing pectin between pH 5.1 and 4.6 the size of the particles changed very slightly, not enough to cause the change in $1/l^*$ shown in **Figure 2B**. Indeed, if we calculate the corresponding $1/l^*$ value for the droplet radii obtained between pH 5.1 and 4.6, assuming no interparticle interactions [or $S(q) = 1$], the value obtained is around 5.0 mm^{-1}, clearly not sufficient to explain the drastic increase in $1/l^*$ observed in the experiment. Therefore, the $1/l^*$ changes at pH > 5.1 must signify the coating of the emulsion particles with pectin molecules (with a subsequent small increase in size). When pectin was added to the emulsions, aggregation was delayed and, if a sufficient amount of pectin was added, aggregation was altogether prevented. These results confirmed earlier reports on the stabilizing effect of HMP when binding with caseins at pH 5.5 (*7*−*9*).

There are further differences between the $1/l^*$ behavior between the control emulsion and that containing pectin. As already mentioned, in control emulsions, there was a large change in $1/l^*$ (around 11% of the initial value) corresponding to the aggregation point (**Figure 2A**), and it may indicate that the average optical characteristics of the emulsion−pectin gel were quite different to those of the initial control emulsion stabilized by sodium caseinate, since turbidity measurements are related to the optical characteristics of the system (*11*). The emulsion containing pectin, however, showed an increase in $1/l^*$ at higher pH (>5.1), while after the aggregation point (pH 4.6) there was a smaller change in $1/l^*$ value (**Figure 2B**), indicating that the optical properties of the final aggregated emulsion were similar to those of the preaggregation stages. In addition, these final values of $1/l^*$ were independent from the amount of pectin added (**Figure 4A**), and the decrease in $1/l^*$ at pH 4.6 was not shown in samples containing high pectin concentration (with no apparent change in radius). If we assume, as it has been previously suggested in the literature (*9*, *10*), that aggregation is caused by bridging of the pectin molecules from one droplet to a neighboring one, we can conclude that the gel state of this system might be a very loose network of pectin-connected emulsion droplets, still existing as fairly intact individuals in the overall gel. This is further corroborated by the relatively small change in particle radius seen in the emulsions containing pectin; **Figure 2B**).

Another important difference that supports this hypothesis is the ergodic behavior of the aggregated emulsion droplets. In the control emulsions, the system became nonergodic (the time-averaged correlation function was different from the ensemble averaged correlation function) immediately after the aggregation point (pH 5.1), as shown by the large error in $1/l^*$ and a change in the shape of the correlation function (not shown). This could indicate that the aggregation of the casein-covered emulsion droplets formed a very inhomogeneous gel structure with domains of droplets in short-range, highly arrested motion, which could be intermeshed with domains of scatterers still able to diffuse. On the other hand, the emulsion containing pectin was ergodic until the end of the measurement. The aggregates formed by the sodium caseinate oil droplets and the pectin molecules were more homogeneous than those formed by control emulsion droplets: at least in the short spatial range, they were still able to move quasi-freely. These results further strengthened our hypothesis of emulsion droplets stabilized by loosely connected strands of pectin.

It could be hypothesized that the aggregates formed during acidification in the control emulsions were structurally different from those formed in emulsions containing pectin. Up to 0.2% pectin, for all the pectin concentrations tested, the final value of $1/l^*$ reached a constant value, which was not affected by the amount of pectin added (**Figure 4**). This may indicate that the physical characteristics of all the emulsions after acidification were similar. It could be speculated that a minimum amount of pectin, at a given pH, will induce the start of ordered packing between the droplets. Increasing the amount of pectin available in solution will increase the number of bridges between the droplets, not modifying its overall structure. This mechanism of stabilization is different from that hypothesized for acidified skim milk stabilized by pectin, where a pectin network between casein particles would be responsible for the stability of the system (*14*).

The change of $1/l^*$ in sodium caseinate emulsions during acidification is quite different from that reported for acid-induced aggregation of skim milk (*15*). In sodium caseinate emulsions (with or without pectin), there is a very narrow threshold pH after which the droplet particles aggregate and a sudden change in turbidity is observed. Previous studies on milk acidification have demonstrated that the changes in $1/l^*$ occur over a wide range of pH, as the casein micelles become aware of their surroundings and the system becomes more spatially organized [in light scattering terms, $S(q)$ starts to deviate from 1], while no changes occur in the size of the particles (*15*). While during acidification of milk, casein micelles begin to relate to one another well before any physical changes can be observed, and during acidification of sodium caseinate emulsions, the narrow pH window where l^* varies due to interparticle interactions correlates with a change in size of the oil droplets.

In all emulsions studied, a decrease in the radius of the emulsion droplet was observed at pH 5.3, well before the onset of the aggregation. This decrease in size could be related to the collapse of the protein layer on the surface of the oil droplet. In emulsions containing pectin, the decrease in droplet size was much larger than that of control emulsions. This could be caused by the thicker hydrodynamic layer around the droplet in the presence of pectin. In addition, there seemed to be a correlation between the amount of pectin and the initial size of the oil droplets. However, at high pH, there should be no interactions between the negatively charged protein-coated oil droplet and the negatively charged pectin. The increase in the value of $1/l^*$ at pH 5.3 and the decrease in radius at pH > 5.3, well before the aggregation pH, is in agreement with results recently reported on the particle size of casein micelles in the presence of pectin during acidification (*16*). While the present work was conducted in situ, the size of casein micelles during acidification was measured after dilution with dynamic light scattering (*16*). The changes observed on $1/l^*$ and radius in the emulsions containing pectin could also be caused by a change in the absorption state of the pectin: while at the initial pH HMP molecules are present unadsorbed in the dispersed phase, with lowering of pH, more molecules are interacting with the oil droplets, causing a change in the optical properties of the emulsion (changing the refractive index contrast) and affecting the viscosity of the dispersed phase.

In conclusion, the present work is the first light scattering study of the acid-induced aggregation of oil-in-water emulsions in a concentrated state. We have demonstrated the applicability and promising future that transmission DWS has on the study of undiluted oil-in-water emulsions. In particular, we were able to detect the coating of the pectin molecules on to the surface

of the emulsion droplets without the need of dilution or otherwise invasive methods. Furthermore, we could see, by means of the 1/*l** parameter and its relation to the changes in size, the structurally different gels created by acidification of oil-in-water emulsions in the absence and presence of pectin. The results brought evidence for a new hypothesis on the mechanism of stabilization of HMP in acidified casein-stabilized emulsions.

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