Stabilization of Model Beverage Cloud Emulsions Using Protein–Polysaccharide Electrostatic Complexes Formed at the Oil–Water Interface

THEPKUNYA HARNSILAWAT,[†] RUNGNAPHAR PONGSAWATMANIT,[†] AND
DAVID J. MCCLEMENTS*,[‡]

Department of Product Development, Faculty of Agro-Industry, Kasetsart University,
Bangkok, Thailand 10900, and Department of Food Science, University of Massachusetts,
Amherst, Massachusetts 01003

The potential of utilizing interfacial complexes, formed through the electrostatic interactions of proteins and polysaccharides at oil−water interfaces, to stabilize model beverage cloud emulsions has been examined. These interfacial complexes were formed by mixing charged polysaccharides with oil-in-water emulsions containing oppositely charged protein-coated oil droplets. Model beverage emulsions were prepared that consisted of 0.1 wt % corn oil droplets coated by β-lactoglobulin (β-Lg), β-Lg/alginate, β-Lg/ι-carrageenan, or β-Lg/gum arabic interfacial layers (pH 3 or 4). Stable emulsions were formed when the polysaccharide concentration was sufficient to saturate the protein-coated droplets. The emulsions were subjected to variations in pH (from 3 to 7), ionic strength (from 0 to 250 mM NaCl), and thermal processing (from 30 or 90 °C), and the influence on their stability was determined. The emulsions containing alginate and carrageenan had the best stability to ionic strength and thermal processing. This study shows that the controlled formation of protein−polysaccharide complexes at droplet surfaces may be used to produce stable beverage emulsions, which may have important implications for industrial applications.

KEYWORDS: Emulsion; β-lactoglobulin; alginate; carrageenan; gum arabic; complexation; interfaces; stability

INTRODUCTION

The term "beverage emulsion" normally refers to non-dairy-based beverages that are drunk cold, such as fruit, soft, and cola drinks (*1*, *2*). This group of products has a number of common manufacturing, compositional, and physicochemical features. Beverage emulsions are normally prepared by homogenizing an oil and aqueous phase together to create a concentrated oil-in-water emulsion, which is later diluted with an aqueous solution to create the finished product. The oil phase in beverage emulsions normally contains a mixture of nonpolar carrier oils (e.g., terpenes), flavor oils, and a weighting agent, whereas the aqueous phase contains water, emulsifier, sugar, acids, and preservatives (*1*, *2*). The aqueous phase in finished beverage emulsions is normally quite acidic (pH 2.5−4.0). Finished beverage products have slightly turbid or "cloudy" appearances because they contain relatively low oil droplet concentrations (typically 0.01−0.1 wt %). They also have rheological characteristics that are dominated by the continuous phase, rather than the presence of the droplets. Beverage

emulsions are thermodynamically unstable systems that tend to break down during storage through a variety of physicochemical mechanisms, including creaming, flocculation, coalescence, and Ostwald ripening (*3*). The long-term stability of beverage emulsions is normally extended by adding a variety of stabilizers to retard these processes, for example, emulsifiers, thickening agents, and weighting agents.

Gum arabic is one of the most common emulsifiers currently used to stabilize beverage emulsions (*1*, *2*). Gum arabic is derived from the natural exudate of *Acacia senegal* and consists of a number of different biopolymer fractions (*4*−*8*). The surface-active fraction is believed to consist of branched arabino-galactan blocks attached to a polypeptide backbone. The hydrophobic polypeptide chain is believed to anchor the molecules to the droplet surface, whereas the hydrophilic arabino-galactan blocks extend into the solution, providing stability against droplet aggregation through steric and electrostatic repulsion (*5*, *6*, *9*, *10*). One disadvantage of gum arabic as an emulsifier is that is has to be used at relatively high concentrations compared to other biopolymer emulsifiers. In addition, there are often problems associated with fluctuations in the quality and cost of gum arabic ingredients over time. Consequently, a number of researchers have examined alterna-

* Author to whom correspondence should be addressed [telephone (413) 545-1019; fax (413) 545-1262; e-mail mcclements@foodsci.umass.edu].
† Kasetsart University.
‡ University of Massachusetts.

Stabilization of Model Beverage Cloud Emulsions

J. Agric. Food Chem., Vol. 54, No. 15, 2006 **5541**

tive emulsifiers to replace gum arabic, such as modified starch and proteins (*1, 11−15*).

Globular proteins, such as those from milk, eggs, or soy, can be used at much lower levels that gum arabic to stabilize oil-in-water emulsions. For example, as much as 20% gum arabic may be required to produce a stable 12.5 wt % oil-in-water emulsion, whereas <1% whey protein can be used (*15, 16*). On the other hand, globular protein stabilized emulsions are much more susceptible to pH, ionic strength, and thermal processing than gum arabic stabilized emulsions (*15*). It would therefore be beneficial to have a natural emulsifier than could be used at a low level to create a beverage emulsion but that also provided good stability against environmental stresses.

Recently, several studies have shown that a relatively simple interfacial engineering technique can be used to improve the stability of protein-stabilized emulsions to environmental stresses (*17−22*). This technique involves the electrostatic deposition of charged polysaccharides onto the surfaces of oppositely charged protein-coated oil droplets. Relatively thick and highly charged interfaces can be produced using these protein−polysaccharide complexes, which means that the steric and electrostatic repulsion between droplets can be increased, thereby improving droplet stability to aggregation. In addition, the polysaccharide concentration required to form these interfacial complexes is much lower than that required when polysaccharide emulsifiers are used in isolation. We hypothesize that this interfacial engineering technology could be used by the food industry to create beverage emulsions with good physical stability at relatively low total emulsifier levels.

The objective of the present study is to determine whether model beverage cloud emulsions can be created using a globular protein (β-lactoglobulin) and various charged polysaccharides (alginate, carrageenan, and gum arabic) that are stable to solution and environmental conditions normally found in practice (i.e., acid pH, minerals, and thermal processing).

MATERIALS AND METHODS

Materials. Powdered β-lactoglobulin (β-Lg) was kindly supplied by Davisco Foods International (lot JE 001-3-922, Le Sueur, MN). The protein content was reported to be 98.3% (dry basis) by the supplier, with β-Lg making up 95.5% of the total protein. The moisture content of the protein powder was reported to be 4.9%. The fat, ash, and lactose contents of this product are reported to be 0.3 ± 0.1, 2.5 ± 0.2, and <0.5 wt %, respectively. Sodium alginate (lot 6724, TIC Pretested Colloid 488T) and gum arabic (lot no. 8475) (food grade) were donated by TIC gums. Food grade ι-carrageenan was donated by FMC BioPolymer (Philadelphia, PA) (lot 10325050). The manufacturers reported that this sample was in almost pure sodium form with a low amount of contamination from other minerals (<5%). Analytical grade hydrochloric acid, sodium hydroxide, sodium azide, and sodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO). Corn oil was purchased from a local supermarket and used without further purification. Distilled and deionized water from a water purification system (Nanopure Infinity, Barnstead International, Dubuque, IA) was used for the preparation of all solutions.

Solution Preparation. An emulsifier solution was prepared by dispersing 0.1 wt % β-Lg in 5 mM phosphate buffer (pH 7.0) and stirring for at least 2 h. Sodium alginate, gum arabic, and ι-carrageenan solutions were prepared by dispersing the appropriate amount of powdered polysaccharide into 5 mM phosphate buffer (pH 7.0) and stirring for at least 2 h. In the case of ι-carrageenan, the solution was then heated in a water bath at 70 °C for 20 min to facilitate dispersion and dissolution (*19*). Sodium azide (0.02 wt %) was added to each of the solutions to prevent microbial growth. After preparation, protein and polysaccharide solutions were stored overnight at 5 °C to allow complete hydration of the biopolymers.

Emulsion Preparation. In this study, the term "primary emulsion" is used to refer to the emulsion created using only the protein as the emulsifier, whereas the term "secondary emulsion" is used to refer to the primary emulsion to which a polysaccharide has also been added. It should be noted that the polysaccharide may or may not be adsorbed to the droplet surfaces in the secondary emulsions depending on solution conditions (e.g., pH and ionic strength).

A corn oil-in-water emulsion was prepared by blending 1 wt % corn oil and 99 wt % aqueous emulsifier solution (0.091 wt % β-Lg in 5 mM phosphate buffer, pH 7) for 2 min at room temperature using a high-speed blender (M133/1281-0, Biospec Products, Inc., Switzerland). This coarse emulsion was then passed through a two-stage high-pressure homogenizer (LAB 1000, APV-Gaulin, Wilmington, MA) three times to reduce the mean particle diameter: 4500 psi at the first stage and 500 psi at the second stage. The resulting emulsion was then diluted with phosphate buffer and sodium azide solution to obtain a dilute emulsion (0.2 wt % oil, 0.018 wt % β-Lg, pH 7.0). Finally, this dilute emulsion was diluted with different ratios of polysaccharide stock solutions (sodium alginate, ι-carrageenan, or gum arabic) and phosphate buffer solution to yield primary and secondary emulsions with the following compositions: 0.1 wt % corn oil, 0.009 wt % β-Lg, 0−0.012 wt % sodium alginate, or 0−0.012 wt % ι-carrageenan, or 0−0.05 wt % gum arabic (pH 7.0, 5 mM phosphate buffer). The primary and secondary emulsions were then stirred at room temperature for 30 min and adjusted to either pH 3 or 4 by adding 0.1 or 1 M HCl. Emulsions were then stored at room temperature before being analyzed (see below).

Emulsion Characterization. *Particle Charge Measurements.* The electrical charge of polysaccharide molecules in aqueous solutions was determined using a commercial instrument capable of electrophoresis measurements (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, U.K.). The electrical charge of the droplets in oil-in-water emulsions was determined using another commercial electrophoresis instrument (ZEM, Zetamaster, Malvern Instruments). These instruments measure the direction and velocity of molecular or particle movement in an applied electric field and then convert the calculated electrophoretic mobility into a ζ-potential value. The aqueous solutions and emulsions were prepared and stored at room temperature for 24 h prior to analysis.

Particle Size Measurements. The mean particle size of the emulsions was determined using a commercial dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments). This instrument infers the size of the particles from measurements of their diffusion coefficients. The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

Spectroturbidity Measurements. An indication of droplet aggregation in the emulsions was obtained from measurements of the turbidity versus wavelength because the turbidity spectrum of a colloidal dispersion depends on the size of the particles it contains (*23*). Approximately 1.5 g samples of emulsion were transferred into 5 mm path length plastic spectrophotometer cuvettes. The emulsions were inverted a number of times prior to measurements to ensure that they were homogeneous so as to avoid any changes in turbidity due to droplet creaming. The change in absorbance of the emulsions was recorded when the wavelength changed from 800 to 400 nm using a UV−visible spectrophotometer (UV-2101PC, Shimadzu Corp., Tokyo, Japan), using distilled water as a reference. We found that there was an appreciable increase in emulsion turbidity at 800 nm in those emulsions where droplet aggregation occurred. We therefore used turbidity measurements at this wavelength to provide an indication of the degree of droplet aggregation in the emulsions. The emulsions were prepared and stored at room temperature for 24 h prior to analysis.

Creaming Stability Measurements. Approximately 3.5 g samples of emulsion were transferred into 10 mm path length plastic spectrophotometer cuvettes and then stored at 30 °C for 7 days. The change in turbidity (τ) at 600 nm of undisturbed emulsions was measured during storage using a UV−visible spectrophotometer (UV-2101PC, Shimadzu Corp.) with distilled water being used as a reference. The light beam passed through the emulsions at a height that was ≈15 mm from the bottom of the cuvette, that is, ≈42% of the emulsion's height. The oil droplets in the emulsions tended to move upward with time due to gravity, which led to the formation of a relatively clear droplet-depleted
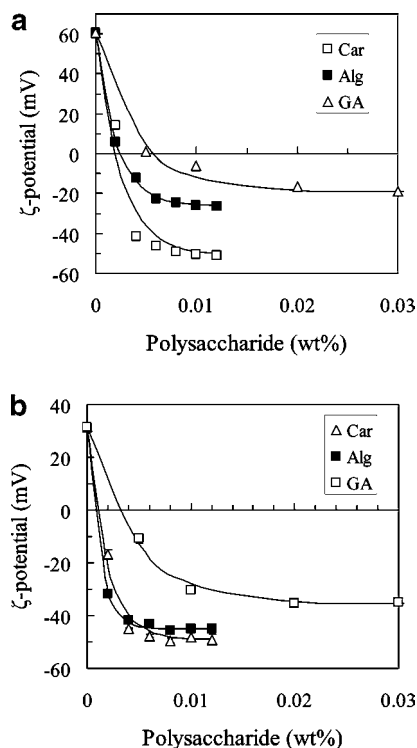
Figure 1. Dependence of droplet charge (ζ-potential) on polysaccharide concentration in 0.1 wt % corn oil-in-water emulsions containing different kinds of polysaccharides: (**a**) pH 3; (**b**) pH 4. The curves are based on predictions made using eq 1 and the parameters in **Table 1**.

serum layer at the bottom of the cuvette. The rate at which this serum layer moved upward provided an indication of the creaming stability of the emulsions: the faster rate, the more unstable the emulsions (*24*). An appreciable decrease in emulsion turbidity was therefore an indication of the fact that the serum layer had risen to at least 42% of the emulsion's height. The creaming stability was quantified in terms of the following expression: creaming stability (%) = 100 × τ(7 days)/τ(0 days), where τ(7 days) and τ(0 days) are the turbidity measurements made at days 0 and 7, respectively. A value of 100% therefore indicates no evidence of droplet creaming during 7 days of storage, whereas a value of 0% indicates that there was rapid creaming (i.e., all of the droplets have moved above the measurement point). It should also be noted that the turbidity of an emulsion depends on particle size as well as droplet concentration, so an observed change in creaming stability may also reflect changes in droplet aggregation as well as creaming.

Statistical Analysis. Each of the measurements described above was carried out using at least two freshly prepared samples, and the results are reported as the mean and standard deviation.

RESULTS AND DISCUSSION

Formation of Interfacial Complexes at Oil Droplet Surfaces. The purpose of this set of experiments was to determine whether various polysaccharides would adsorb to the surface of protein-coated oil droplets and to obtain some information about the electrical characteristics of the interfaces formed. Initially, we prepared β-Lg-stabilized emulsions at pH 7 in the absence (primary emulsions) and presence (secondary emulsions) of different types and concentration of polysaccharide. At pH 7, the protein and polysaccharides have similar electrical charges, and therefore we would not have expected the polysaccharides to have adsorbed to the surfaces of the protein-coated droplets. We then decreased the pH of the emulsions from pH 7 to either pH 3 or 4 and measured the particle ζ-potential of the resulting emulsions after 1 day of storage (**Figure 1**). At these pH values, the signs of the electrical charge

on the protein (positive) and polysaccharides (negative) are opposite, so that one would expect the anionic polysaccharides in the aqueous phase to be electrically attracted toward the cationic protein-coated droplets.

The electrical charge (ζ-potential) on the emulsion droplets was strongly dependent on final pH, polysaccharide type, and polysaccharide concentration (**Figure 1**). In the absence of polysaccharide, the electrical charge on the protein-coated emulsion droplets was positive, because the adsorbed β-Lg was below its isoelectric point (p$I \sim$ 5.0) (*25−27*). As the polysaccharide concentration in the aqueous phase of the emulsions was increased, the electrical charge on the droplets initially became less positive and then it became more negative, until it finally reached a plateau value (ζ_{Sat}). Similar results have been observed in previous studies, where the change in ζ-potential was attributed to progressive adsorption of anionic polysaccharides onto the surfaces of cationic protein-coated droplets, until the droplet surfaces had become saturated (*17−22*). The steepness of the initial change in ζ-potential with increasing polysaccharide concentration and the saturation ζ-potential depended on polysaccharide type and pH.

We modeled the ζ-potential versus polysaccharide concentration curves in terms of the empirical equation

$$\frac{\zeta(c) - \zeta_{Sat}}{\zeta_0 - \zeta_{Sat}} = \exp\left(-\frac{c}{c^*}\right) \qquad (1)$$

where $\zeta(c)$ is the ζ-potential of the emulsion droplets at polysaccharide concentration c, ζ_0 is the ζ-potential in the absence of polysaccharide, ζ_{Sat} is the ζ-potential when the droplets are saturated with polysaccharide, and c^* is a critical polysaccharide concentration. Mathematically, c^* is the polysaccharide concentration where the change in ζ-potential is 1/e of the total change in ζ-potential for saturation: $\Delta\zeta = \Delta\zeta_{Sat}/e$. The value of c^* is therefore a measure of the binding affinity of the polysaccharide for the droplet surface: the higher c^*, the lower the binding affinity. The binding of a polysaccharide to the droplet surface can therefore be characterized by ζ_{Sat} and c^*. Values for ζ_0, ζ_{Sat}, and c^* are given in **Table 1** for the three different polysaccharides at pH 3 and 4. The values of ζ_0 and ζ_{Sat} were determined from the ζ-potential measurements in the absence of polysaccharide and at the highest polysaccharide concentration used (where saturation was assumed). The c^* values were then obtained by finding the quantities that gave the best fit between eq 1 and the experimental data (using the Solver routine in Excel, Microsoft Corp.). There was good agreement between the experimental measurements and the ζ-potential values predicted for the secondary emulsions using eq 1 and the parameters listed in **Table 1** (**Figure 1**).

The binding affinity was dependent on polysaccharide type and solution pH (**Table 1**). At both pH 3 and 4, the c^* values were appreciably lower for alginate and carrageenan than for gum arabic, which suggested that they had stronger binding affinities for the droplet surfaces. For carrageenan and gum arabic the binding affinities were fairly similar at pH 3 and 4, but for alginate the binding affinity was considerably higher (lower c^*) at pH 4 than at pH 3. The saturation value of the ζ-potential was also dependent on polysaccharide type and solution pH (**Table 1**). The protein/carrageenan-coated droplets had the highest negative charge and had similar ζ_{Sat} values at pH 3 and 4 ($\zeta_{Sat} \approx$ −50 mV). The protein/alginate-coated droplets had a high negative charge at pH 4 ($\zeta_{Sat} \approx$ −45 mV), but were appreciably less charged at pH 3 ($\zeta_{Sat} \approx$ −26 mV). The protein/gum arabic-coated droplets had the smallest negative

Stabilization of Model Beverage Cloud Emulsions

J. Agric. Food Chem., Vol. 54, No. 15, 2006 **5543**

Table 1. Parameters Characterizing the Binding of Polysaccharides to Protein-Coated Droplet Surfaces Determined from ζ-Potential versus Polysaccharide Concentration Measurements at pH 3 and 4 Using Equation 1

parameter	ι-carrageenan		sodium alginate		gum arabic	
	pH 3	pH 4	pH 3	pH 4	pH 3	pH 4
ζ_0 (mV)	60.6 ± 0.7	31.4 ± 0.9	60.6 ± 0.7	31.4 ± 0.9	60.6 ± 0.7	31.4 ± 0.9
ζ_{Sat} (mV)	-51.1 ± 1.9	-49.2 ± 2.0	-26.2 ± 2.0	-45.1 ± 2.6	-19.2 ± 0.4	-35.4 ± 0.4
$\Delta\zeta_{Sat}$ (mV)	112 ± 2	80.6 ± 2.2	86.8 ± 2.1	76.5 ± 2.8	79.8 ± 0.8	66.8 ± 1.0
c^* (wt %)	0.0025	0.0019	0.0021	0.0012	0.0042	0.0046
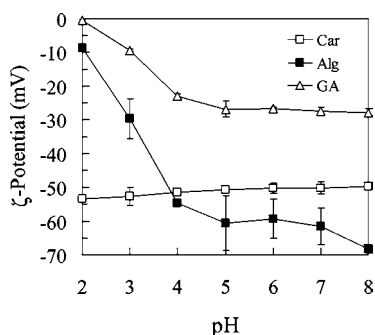
Figure 2. Dependence of the effective ζ-potential of polysaccharide molecules in aqueous solutions on pH.

charge at both pH values, but the negative charge was appreciably higher at pH 4 ($\zeta_{Sat} \approx -35$ mV) than at pH 3 ($\zeta_{Sat} \approx -19$ mV).

We postulated that the difference in the electrical characteristics of the protein/polysaccharide-coated droplets was due to differences in the electrical charge densities of the polysaccharide molecules. Consequently, we measured the electrical characteristics (ζ-potential versus pH) of 0.1 wt % aqueous polysaccharide solutions (**Figure 2**). These measurements show that the ζ-potential of the polysaccharide molecules (ζ_{PS}) follows the same trend as the ζ_{Sat} values of the emulsion droplets coated by protein/polysaccharide complexes: $\zeta_{PS} = -53$, -30, and -9 mV at pH 3 and $\zeta_{PS} = -51$, -55, and -23 mV at pH 4 for carrageenan, alginate, and gum arabic, respectively (**Figure 2**). The electrical charge on the carrageenan molecules and protein/carrageenan-coated droplets is highly negative at both pH 3 and 4. The electrical charge on the alginate molecules and protein/alginate-coated droplets is highly negative at pH 4 but less so at pH 3. The electrical charge on the gum arabic molecules and protein/gum arabic-coated droplets is considerably less negative than for the other two polysaccharides and is appreciably lower at pH 3 than at pH 4. Thus, it appears that the electrical characteristics of the protein/polysaccharide-coated droplets are largely determined by the electrical characteristics of the polysaccharide molecules.

It is also insightful to examine the overall change in the ζ-potential when the protein-coated droplets are saturated with polysaccharide: $\Delta\zeta_{Sat} = \zeta_0 - \zeta_{Sat}$ (**Table 1**). For carrageenan, the overall change in ζ-potential is considerably higher at pH 3 ($\Delta\zeta_{Sat} \approx 112$ mV) than at pH 4 ($\Delta\zeta \approx 81$ mV), even though the final ζ_{Sat} values are fairly similar at both pH values ($\zeta_{Sat} \approx -50$ mV). The electrical charges on the carrageenan molecules were fairly similar at pH 3 and 4 (**Figure 2**); hence, we can postulate that more carrageenan molecules adsorbed to the droplet surfaces at pH 3 than at pH 4. A possible explanation for this observation can be given in terms of the electrical interactions between a charged polysaccharide and a charged surface that it is approaching. Studies of the adsorption of synthetic polyelectrolytes onto oppositely charged surfaces have reported that the final ζ-potential is largely independent of the
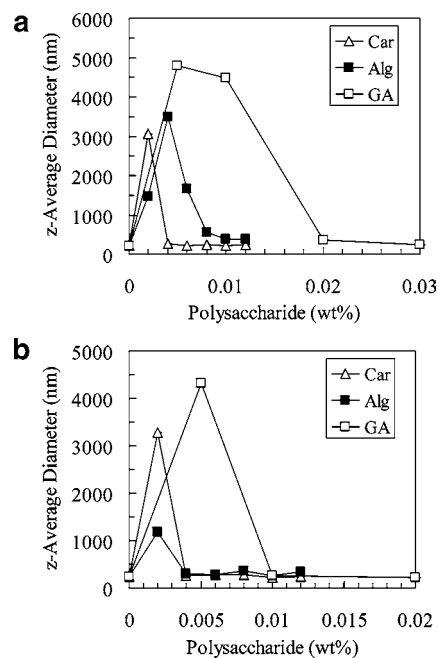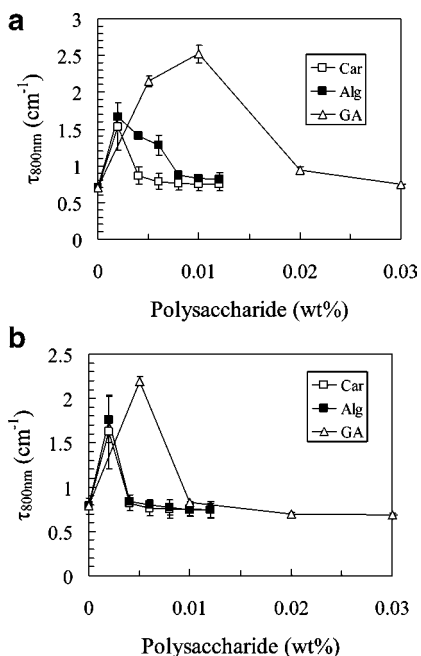

Figure 3. Dependence of the mean particle diameter on polysaccharide concentration in 0.1 wt % corn oil-in-water emulsions containing different kinds of polysaccharides: (**a**) pH 3; (**b**) pH 4.

charge density of the adsorbing polyelectrolyte, provided that its charge density is not too low (*28*). This phenomenon was attributed to the fact that once the surface charge has reached a certain value, there will be a strong electrostatic repulsion between the surface and similarly charged polyelectrolytes in the aqueous phase, which limits further adsorption of the polyelectrolyte. Hence, we postulate that the carrageenan molecules adsorbed to the protein-coated droplet surfaces until a certain ζ-potential was reached (≈ -50 mV), and then the electrostatic repulsion was strong enough to prevent further polymer adsorption.

Stability of Emulsions Stabilized by Interfacial Complexes. The purpose of these experiments was to examine the influence of polysaccharide type, polysaccharide concentration, and pH on the stability of oil-in-water emulsions containing β-Lg-coated droplets. As explained above, we prepared β-Lg-stabilized emulsions at pH 7 in the absence (primary emulsions) and presence (secondary emulsions) of different types and concentrations of polysaccharide, and then the pH was reduced to either 3 or 4 by adding acid. The stability of the emulsions to droplet aggregation and creaming was then determined using light scattering, turbidity, and creaming stability measurements (**Figures 3–5**).

The stability of the emulsions to droplet aggregation and creaming was highly dependent on polysaccharide type, polysaccharide concentration, and solution pH (**Figures 3–5**). In the absence of polysaccharide, the primary emulsions appeared stable to droplet aggregation (low z-diameter, low τ_{800}) after

Figure 4. Dependence of the turbidity at 800 nm on polysaccharide concentration in 0.1 wt % corn oil-in-water emulsions containing different kinds of polysaccharides: (**a**) pH 3; (**b**) pH 4. An increase in turbidity is indicative of particle aggregation.
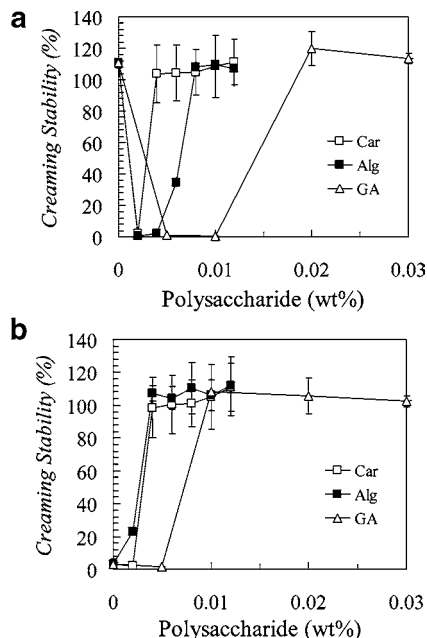
Figure 5. Dependence of the creaming stability on polysaccharide concentration in 0.1 wt % corn oil-in-water emulsions containing different kinds of polysaccharides: (**a**) pH 3; (**b**) pH 4. A decrease in creaming stability is indicative of particle aggregation.

24 h of storage at pH 3 and 4. Presumably, the positive charge on the protein-coated droplets was sufficiently high to prevent droplet aggregation by generating a strong interdroplet electrostatic repulsion (*3*). The primary emulsion at pH 3 was also stable to creaming after 7 days of storage at room temperature, which indicated that droplet aggregation did not occur. On the other hand, the primary emulsion at pH 4 was unstable to creaming after 7 days of storage, which indicated that some droplet aggregation had occurred over time. The reason that the primary emulsion was unstable to creaming at pH 4 may have been because this pH is fairly close to the isoelectric point
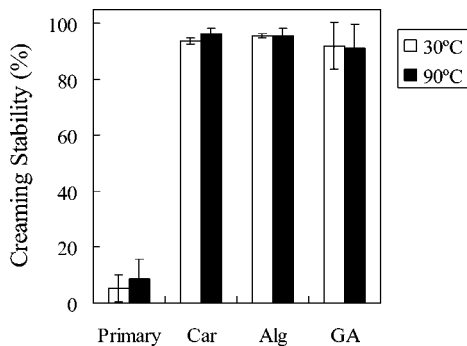
of the adsorbed β-lactoglobulin molecules, so that there may not have been a sufficiently strong electrostatic repulsion between the droplets to prevent aggregation during long-term storage.

At intermediate polysaccharide concentrations, the secondary emulsions were highly unstable to droplet aggregation (high z-diameter, high τ_{800}) and creaming. This phenomenon can be attributed to charge neutralization and bridging flocculation affects (*29–31*). When there is insufficient polysaccharide present to completely cover the protein-coated droplets, there will be regions of positive charge and regions of negative charge exposed at the droplets surfaces, which will promote bridging flocculation. In addition, the overall net charge on the droplets was relatively small ($|\zeta| < 15$ mV), so that the electrostatic repulsion between the droplets would have been insufficient to overcome the attractive interactions (e.g., van der Waals and hydrophobic). At high polysaccharide concentrations, the secondary emulsions were stable to droplet aggregation (low z-diameter, low τ_{800}) and creaming at both pH 3 and 4. This restabilization can be attributed to the fact that the droplet surfaces were completely covered with polysaccharide and the droplet charge was relatively high (**Figure 1**). In addition, the interfacial thickness will have increased due to the adsorption of the polysaccharide to the droplet surfaces. Hence, there would be a strong electrostatic and steric repulsion between the protein/polysaccharide-coated droplets that should oppose their aggregation.

The range of intermediate polysaccharide concentrations at which the emulsions were unstable to droplet aggregation and creaming depended on polysaccharide type and pH (**Figures 3–5**). For example, emulsions containing protein-coated droplets to which carrageenan was added were unstable at only 0.002 wt % at pH 3 and 4; those to which alginate was added were unstable at 0.002 wt % at pH 4 but from 0.002 to 0.006 wt % at pH 3, and those to which gum arabic was added were unstable from 0.002 to 0.006 wt % at pH 4 but from 0.002 to 0.01 wt % at pH 3. These differences in droplet aggregation behavior can be attributed to the differences in droplet charge (**Figure 1**). In general, the emulsions were stable to droplet aggregation provided the magnitude of the ζ-potential was high and the droplets were sufficiently covered with polysaccharide.

Stability of Emulsions to Environmental Stresses. The purpose of this series of experiments was to determine whether the secondary emulsions containing protein/polysaccharide-coated droplets had better stability to environmental stresses than the primary emulsions containing protein-coated droplets. ζ-Potential measurements were used to assess the interaction of the polysaccharides with the protein-coated droplets, and creaming stability measurements were used to assess the overall stability of the emulsions. Primary and secondary emulsions (0.1 wt % corn oil-in-water emulsions, pH 4) with different salt concentrations (0, 50, or 100 mM NaCl), sugar concentrations (0 or 10 wt % sucrose), and heat treatments (30 or 90 °C) were analyzed. The polysaccharide concentration in the secondary emulsions was selected so that (i) it was sufficient to saturate the protein-coated droplet surfaces as determined from ζ-potential measurements (**Figure 1**) and (ii) it was just above the minimum amount needed to produce secondary emulsions that were stable to droplet aggregation and creaming (**Figures 3–5**). For this reason, the secondary emulsions were prepared using 0.004 wt % carrageenan, 0.004 wt % alginate, or 0.02 wt % gum arabic.

The influence of thermal processing (30 or 90 °C for 30 min) on the stability of the emulsions is shown in **Figure 6**. Previous

Stabilization of Model Beverage Cloud Emulsions

J. Agric. Food Chem., Vol. 54, No. 15, 2006 **5545**
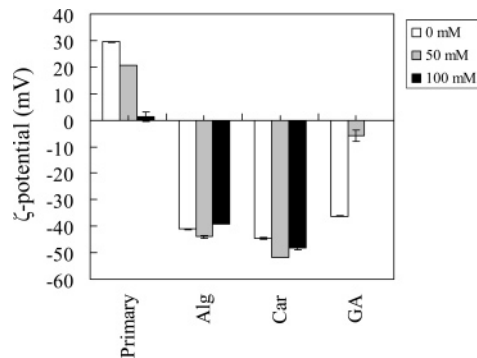

Figure 6. Influence of thermal processing on the stability of 0.1 wt % corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharides.
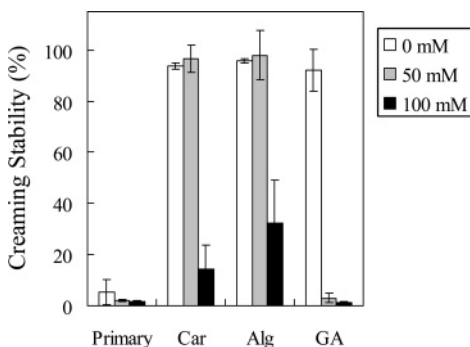
Figure 7. Influence of NaCl on the stability of 0.1 wt % corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharides.

studies have shown that heating β-Lg-stabilized emulsions to 90 °C can promote droplet flocculation due to thermal denaturation of the adsorbed proteins (*32*). The unheated and heated primary emulsions were both unstable to heating because the pH was fairly close to the isoelectric point of the adsorbed β-Lg so that there was not a sufficiently strong electrostatic repulsion between the droplets to prevent aggregation. On the other hand, all of the secondary emulsions were stable to heat treatment (**Figure 6**). We propose that the polysaccharides adsorbed to the surfaces of the protein-coated droplets and increased the steric and electrostatic repulsion between the droplets by increasing the thickness and charge of the interfaces. Our results suggest that heating did not cause the polysaccharides to be desorbed from the droplet surfaces; otherwise, the secondary emulsions would have become unstable to droplet aggregation like the primary emulsions. This hypothesis was confirmed by the ζ-potential measurements, which showed that the electrical charge on the droplets in the secondary emulsions changed by less than ± 2 mV upon thermal processing (data not shown). Hence, there was no evidence of desorption of the polysaccharides from the droplet surfaces induced by heating.

The influence of salt addition (0, 50, or 100 mM NaCl) on the stability of the emulsions is shown in **Figure 7**. The primary emulsion was unstable at all salt concentrations for the reasons mentioned above. The secondary emulsions containing alginate and carrageenan were stable to creaming at 0 and 50 mM NaCl, but were unstable at 100 mM NaCl. On the other hand, the secondary emulsions containing gum arabic were highly unstable to creaming at 50 and 100 mM NaCl. The addition of salt to the emulsions may have adversely affected their creaming stability in a number of ways. First, salt screens the electrostatic repulsion between charged droplets, which can promote droplet aggregation when the strength of the repulsive colloidal interac-

Figure 8. Influence of NaCl on the ζ-potential of 0.1 wt % corn oil-in-water emulsions (pH 4) in the absence and presence of different kinds of polysaccharides.

tions is no longer strong enough to overcome the attractive colloidal interactions (*3*). Second, the presence of salt in the emulsions may have weakened the electrostatic attraction between the polysaccharides and the protein-coated oil droplets, which may have led to partial or full desorption of the polysaccharide molecules. The fact that the ζ-potential of these emulsions did not change appreciably with increasing salt concentration (see below) suggests that the carrageenan molecules were not fully desorbed from the droplet surfaces. Nevertheless, weakening of the attraction between the polysaccharides and the protein-coated droplet surfaces may have led to bridging flocculation due to adsorption of a polysaccharide onto more than one droplet. At pH 4, the protein/gum arabic-coated droplets have an appreciably lower ζ-potential than the protein/carrageenan- or protein/alginate-coated droplets, which means that the electrostatic repulsion between the droplets is weaker. This would account for the fact that a lower amount of NaCl was needed to promote droplet aggregation in the gum arabic emulsions. In addition, the binding affinity of the gum arabic for the droplet surfaces was less than that of the carrageenan and alginate (**Table 1**), so it is also possible that the NaCl may have desorbed the gum arabic more easily. Measurements of the droplet ζ-potential were used to provide further insight into the physicochemical origin of the observed changes in emulsion stability with salt addition.

The influence of NaCl on the ζ-potential measurements was highly dependent on the polysaccharide type used to prepare the secondary emulsions (**Figure 8**). Normally, one would expect a progressive decrease in ζ-potential with increasing salt concentration due to electrostatic screening affects, because $\zeta \propto \kappa^{-1}$ (assuming constant surface charge density and no change in interfacial structure), where κ^{-1} is the Debye screening length (*3*). For aqueous solutions at room temperature, the Debye screening length is related to the ionic strength through $\kappa^{-1} \approx 0.304/\sqrt{I}$ nm, where I is the ionic strength of the solution expressed in moles per liter (*3*). Hence, one would expect that the droplet potential should decrease with increasing salt concentration in the following manner: $\zeta \propto 1/\sqrt{I}$.

For the protein-coated droplets there was a progressive decrease in ζ-potential with increasing salt concentration (**Figure 8**), which can be attributed to electrostatic screening effects. On the other hand, for the protein/carrageenan- and protein/ alginate-coated droplets the reduction in ζ-potential with increasing salt concentration was much less than expected. This type of behavior has also been observed for secondary emulsions containing β-lactoglobulin/pectin-coated droplets, where it was attributed to a change in the composition, thickness, or structure of the interfacial membrane with salt concentration (*21*).

5546 *J. Agric. Food Chem.,* Vol. 54, No. 15, 2006

Harnsilawat et al.

Changes in these interfacial properties as a result of salt addition may arise due to a reduction in the electrostatic interactions between adsorbed and non-adsorbed polysaccharides (repulsive), between two or more adsorbed polysaccharides (repulsive), or between adsorbed polysaccharides and proteins (attractive). Finally, the protein/gum arabic-coated droplets showed a much larger decrease in ζ-potential with increasing salt concentration than the protein/alginate- or protein/carrageenan-coated droplets, which suggested that some of the gum arabic may have desorbed from the droplet surfaces, thereby promoting instability at a lower NaCl concentration through charge neutralization and polymer-bridging effects. The different behaviors of the three polysaccharides may have been because of their different chemical compositions (functional groups) or their different molecular conformations. Carrageenan and alginate molecules would be expected to be more extended in structure than gum arabic molecules.

The influence of sugar addition (0 or 10 wt % sucrose) on the stability of the emulsions was also determined (data not shown). We found no change in droplet ζ-potential or creaming stability in the absence or presence of sucrose, which indicated that sucrose had no affect on interfacial composition or emulsion stability.

This study has shown that model beverage emulsions can be produced that contain oil droplets coated by protein/polysaccharide interfaces. These interfacial complexes were formed by electrostatic deposition of anionic polysaccharides onto cationic protein-coated droplets. The electrical characteristics of the interfaces formed appeared to be mainly determined by the electrical charge of the polysaccharides, which was governed by solution pH and polysaccharide type. The secondary emulsions formed were stable to thermal processing (90 °C for 30 min), sugar (10% sucrose), and salt ($\leq$50 mM NaCl). These results suggest that this interfacial engineering technology could be used by the beverage industry to replace traditional polysaccharide emulsifiers such as gum arabic and modified starch. The main advantages of the protein/polysaccharide complexes over traditional polysaccharide emulsifiers are that they can be used at much lower levels and that there may be less variation in price and quality in protein than in polysaccharide emulsifiers. On the other hand, there are also a number of potential disadvantages associated with this technology. First, there may be limitations in using some proteins in beverage emulsions due to labeling concerns associated with allergenicity or dietary restrictions (e.g., vegan, halal, and kosher). Second, proteins may react with any carbonyls in the flavoring system, which can result in undesirable flavor and color changes during processing and storage. Third, there may be additional costs associated with preparing beverage emulsions coated with protein−polysaccharide complexes, for example, extra ingredient or processing costs. Fourth, the sensitivity of some of the protein−polysaccharide systems to pH may limit their application in some beverage products. Further research is clearly needed to determine whether this promising interfacial technology can be turned into a commercial reality.

LITERATURE CITED

(1) Tan, C. T. Beverage flavor emulsion-a form of emulsion liquid membrane encapsulation. In *Food Flavors: Formation, Analysis and Packaging Influences*; Contis, E. T., Ed.; Elsevier: New York, 1998; p 29.

(2) Tan, C. T. Beverage emulsions. In *Food Emulsions*; Friberg, S. E., Larsson, K., Sjoblom, J., Eds.; Dekker: New York, 2004; pp 485−524.

(3) McClements, D. J. *Food Emulsions: Principles, Practices, and Techniques*, 2nd ed.; CRC Press: Boca Raton, FL, 2004; p 609.

(4) Anderson, D. M. W.; Howlett, J. F.; McNab, C. G. A. The amino acid composition of the proteinaceous of *Acacia senegal* gum. *Carbohydr. Res.* **1985**, *2*, 104−114.

(5) Phillips, G. O.; Williams, P. A. Interaction of hydrocolloids in food systems. In *Ingredient Interactions: Effect on Food Quality*; Gaonkar, A. G., Ed.; Dekker: New York, 1995; pp 131−170.

(6) Jayme, M. L.; Dunstan, D. E.; Gee, M. L. Zeta potential of gum arabic stabilized oil-in-water emulsions. *Food Hydrocolloids* **1999**, *13*, 459−465.

(7) Goodrum, L. J.; Patel, A.; Leykam, J. F.; Kieliszewski, M. J. Gum arabic glycoprotein contains glycomodules of both extensin and arabinogalactan-glycoproteins. *Phytochemistry* **2000**, *54*, 99−106.

(8) Sanchez, C.; Renard, D.; Robert, P.; Schmitt, C.; Lefebvre, J. Structure and rheological properties of acacia gum dispersions. *Food Hydrocolloids* **2002**, *16*, 257−267.

(9) Islam, A. M.; Phillips, G. O.; Sljivo, A.; Snowden, M. J.; Williams, P. A. A review of recent developments on the regulatory, structural and functional aspects of gum arabic. *Food Hydrocolloids* **1997**, *11*, 493−505.

(10) Chanamai, R.; McClements, D. J. Impact of weighting agents and sucrose on gravitational separation of beverage emulsions. *J. Agric. Food Chem.* **2000**, *48*, 5561−5565.

(11) Trubiano, P. C. The role of specialty food starches in flavor emulsions. *Flavor Technol.* **1995**, *610*, 199−209.

(12) Kim, Y. D.; Morr, C. V.; Schenz, T. W. Microencapsulation properties of gum arabic and several food proteins: liquid orange oil emulsion particles. *J. Agric. Food Chem.* **1996**, *44*, 1308−1313.

(13) Tan, C. T. Beverage emulsions. In *Food Emulsions*; Friberg, S. E., Ed.; Dekker: New York, 1997; pp 491−524.

(14) Garti, N. Hydrocolloids as emulsifying agents for oil-in-water emulsions. *J. Dispersion Sci. Technol.* **1999**, *20*, 327−355.

(15) Chanamai, R.; McClements, D. J. Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: influence of pH, CaCl$_2$ and temperature. *J. Food Sci.* **2002**, *67*, 120−125.

(16) Tse, K. Y.; Reineccius, G. A. Methods to predict the physical stability of flavor−cloud emulsion. *Flavor Technol.* **1995**, *610*, 172−182.

(17) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of pH and ι-carrageenan concentration on physicochemical properties and stability of β-lactoglobulin-stabilized oil-in-water emulsions. *J. Agric. Food Chem.* **2004**, *52*, 3626−3632.

(18) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of ι-carrageenan on droplet flocculation of β-lactoglobulin-stabilized oil-in-water emulsions during thermal processing. *Langmuir* **2004**, *20*, 9565−9570.

(19) Gu, Y. S.; Decker, E. A.; McClements, D. J. Influence of pH and carrageenan type on properties of β-lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids* **2005**, *19*, 83−91.

(20) Gu, Y. S.; Regnier, L.; McClements, D. J. Influence of environmental stresses on stability of oil-in-water emulsions containing droplets stabilized by β-lactoglobulin−ι-carrageenan membranes. *J. Colloid Interface Sci.* **2005**, *286*, 551−558.

(21) Guzey, D.; Kim, H. J.; McClements, D. J. Factors influencing the production of o/w emulsions stabilized by β-lactoglobulin−pectin membranes. *Food Hydrocolloids* **2004**, *18*, 967−975.

Stabilization of Model Beverage Cloud Emulsions

J. Agric. Food Chem., Vol. 54, No. 15, 2006 **5547**

(22) Moreau, L.; Kim, H. J.; Decker, E. A.; McClements, D. J. Production and characterization of oil-in-water emulsions containing droplets stabilized by β-lactoglobulin−pectin membranes. *J. Agric. Food Chem.* **2003**, *51*, 6612−6617.

(23) McClements, D. J. Theoretical prediction of emulsion color. *Adv. Colloid Interface Sci.* **2002**, *97*, 63−89.

(24) Ogawa, S.; Decker, E. A.; McClements, D. J. Production and characterization of O/W emulsions containing cationic droplets stabilized by lecithin−chitosan membranes. *J. Agric. Food Chem.* **2003**, *51*, 2806−2812.

(25) Bromley, E. H. C.; Krebs, M. R. H.; Donald, A. M. Aggregation across the length-scales in β-lactoglobulin. *Faraday Discuss.* **2005**, *128*, 13−27.

(26) Das, K. P.; Kinsella, J. E. pH dependent emulsifying properties of β-lactoglobulin. *J. Dispersion Sci. Technol.* **1989**, *10*, 77−102.

(27) Sawyer, L.; Kontopidis, G. The core lipocalin, bovine β-lactoglobulin. *Biochim. Biophys. Acta* **2000**, *1482*, 136−148.

(28) Schonhoff, M. Layered polyelectrolyte complexes: physics of formation and molecular properties. *J. Phys.: Condens. Matter* **2003**, *15*, 1781−1808.

(29) Dickinson, E. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids* **2003**, *17*, 25−39.

(30) Friberg, S. E.; Larsson, K.; Sjoblom, J. *Food Emulsions*, 4th ed.; Dekker: New York, 2004; p 640.

(31) Von Goeler, F.; Muthukumar, M. Adsorption of polyelectrolytes onto curved surfaces. *J. Chem. Phys.* **1994**, *100*, 7796−7803.

(32) Kim, H. J.; Decker, E. A.; McClements, D. J. Influence of sucrose on droplet flocculation in hexadecane oil-in-water emulsions stabilized by β-lactoglobulin. *J. Agric. Food Chem.* **2003**, *51*, 766−772.