

Self-Assembly and Physicochemical and Rheological Properties of a Polysaccharide–Surfactant System Formed from the Cationic Biopolymer Chitosan and Nonionic Sorbitan Esters

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The natural cationic polysaccharide chitosan was mixed with the nonionic surfactants sorbitan monolaurate, sorbitan monooleate, or sorbitan triooleate to produce a biopolymer–surfactant system with unique properties. The mixtures of chitosan and surfactant formed emulsion-like solutions and/or creams. The known properties of the components were considered (i.e., hydrophile–lipophile balance, molecular weight, structure, and density), and various physicochemical and rheological properties of the mixtures were measured. Specifically, the critical micelle concentration of the sorbitan esters in a chitosan solution was measured using both surface tension and fluorescence-based methods. The concentration-dependent morphologies of the aggregates within the chitosan–surfactant solutions were evaluated by optical microscopy and dynamic light scattering. A schematic depicting the possible molecular arrangement of chitosan and surfactant within the various formulations was produced from consideration of the experimental findings. The degree of interaction between chitosan and the individual surfactants was assessed by FTIR analysis. The rheological properties of the chitosan–surfactant emulsions were also investigated and found to be related to the observed morphologies. Overall, clear composition–property relationships were established for these chitosan–surfactant systems which have potential applications in the food and pharmaceutical industries.

Introduction

Biocompatible, biodegradable, and/or nontoxic emulsion-based formulations have great potential for applications in the food, cosmetic, and pharmaceutical industries.^{2,3} Emulsions are described as a suspension of small globules and are composed of at least two immiscible liquids. Polysaccharides and small-molecule surfactants are two of the predominant groups of amphiphilic materials that have been explored for the stabilization of emulsions.⁴ The polysaccharides that have been commonly employed are alginate, carrageenan, chitosan, pectin, rhaman, xanthan, dextran, carboxymethylcellulose, hydroxypropyl methylcellulose, and scleroglucan, while the small molecule surfactants most commonly used are sodium dodecyl sulfate (SDS), mono- and diglycerides, sorbitan esters, and phospholipids.^{2,5–12}

Few studies have examined the use of combinations of polymer and surfactant as stabilizers for preparation of emulsions. Furthermore, of the polymer–surfactant combinations examined, most have included a charged polymer (e.g., poly(acrylic acid), chitosan) and an ionic surfactant (e.g., alkyltrimethylammonium bromide, SDS).^{12,13} When polymers and surfactants are used in combination as the stabilizing component of a formulation they may interact to form complexes. The formation of polymer–surfactant complexes have been shown to lead to the destabilization of the emulsion system.¹⁴ The following four stages have been implicated in the destabilization of an emulsion system: flocculation, coalescence, creaming, and phase separation.² Flocculation refers to particle aggregation and the formation of a thin film between the particles. The film separating the particles can be diminished by several mechanisms including van der Waals forces producing large single droplets that characterize the coalescence stage.¹⁵ Depending on the density of the particles, a creaming or sedimentation process may occur.^{2,15}

This study examines sorbitan esters and chitosan as a small molecule surfactant–biopolymer combination for preparation of stable emulsions. Sorbitan esters, commonly referred to as Span or Tween, are nonionic surfactants that are formed from mixtures of partial esters of sorbitol and anhydrides in addition to fatty acids. The wide range of sorbitan esters that are commonly employed as surfactants in various industries differ in terms of their chemical (i.e., composition, structure), physical (i.e., hydrophile–lipophile balance (HLB), color, and state at room temperature), and functional properties (i.e., critical micelle

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concentration (CMC), density, viscosity).^{16–19} Sorbitan esters are currently approved for use by the FDA as an inactive excipient in inhalation, intramuscular, nasal, oral, ophthalmic, rectal, topical, transdermal, and vaginal formulations (FDA, 2005).

Chitosan is a natural polysaccharide that can also be obtained synthetically by the deacetylation of chitin to produce poly-(1,4- β -D-glucopyranose) molecules. The properties of chitosan such as molecular weight and degree of deacetylation may be varied to suit formulation design.²⁰ The solubility of chitosan is dependent on both the pH of the medium and the degree of deacetylation. A 1% acetic acid or lactic acid solution (pH < 6) is commonly used to dissolve chitosan.²¹ Chitosan is GRAS listed for use in food preparations (FDA, 2001) and has also been employed in numerous environmental and pharmaceutical applications.^{20,22–25} Clinical trial evaluation of the biocompatibility of chitosan-based materials has revealed no inflammatory or allergic responses following implantation, injection, topical application, and ingestion in the human body.^{26,27}

To date, sorbitan esters have only been employed as a surfactant for preparation of chitosan-based beads and microspheres.^{28–31} For the first time, chitosan is mixed with sorbitan esters to form a surfactant–biopolymer complex that produces stable emulsion and cream formulations. Combinations of chitosan and three sorbitan esters were evaluated in order to determine the influence of the chemical structure of the sorbitan ester on the physicochemical and rheological properties of the emulsion. The stability of the emulsions was investigated in terms of shear stress or steady shear rate. The CMC of the sorbitan esters in the presence of chitosan was measured by both surface tensiometry and a fluorescence-based method. The surface tension measurements also provided an indication of the influence of the different surfactant molecules on the surface activity of chitosan. The interactions between the chitosan and the sorbitan esters were evaluated using FTIR analysis. The morphology of the chitosan–surfactant droplets were visualized by optical microscopy and found to be related to the rheological properties. To date, most emulsions have only been examined at dilute concentrations; however, in this study, a wide range of concentrations were investigated in order to observe the creaming effect. The onset of creaming and the rheological properties of the chitosan–surfactant formulations were evaluated. The physicochemical properties of the mixtures provided some insight into the

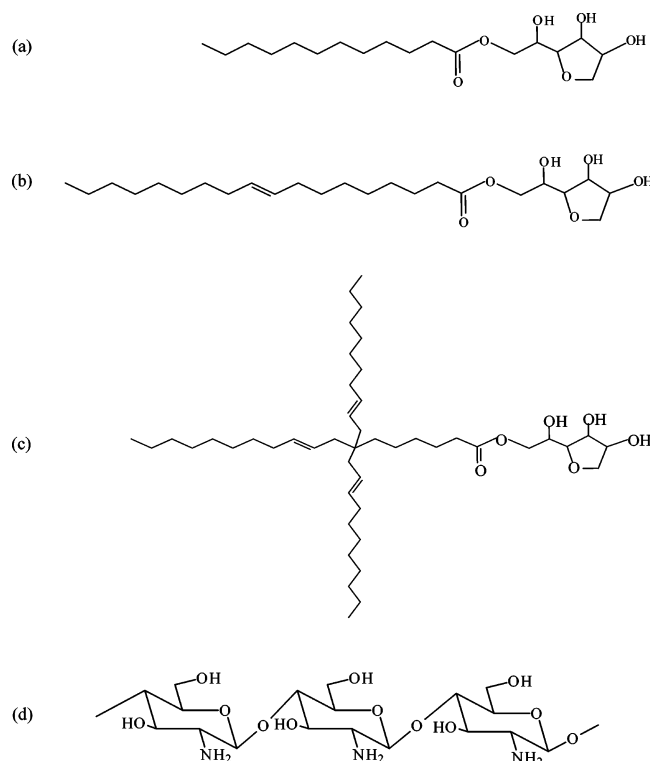


Figure 1. Chemical structures of (a) sorbitan monolaurate, (b) sorbitan monooleate, (c) sorbitan triooleate, and (d) three monomer units of chitosan. (Note: the structural arrangement of each molecule does not represent the thermodynamically favorable conformation).

orientation or organization of the chitosan and sorbitan esters at the molecular level. Overall, these studies established the composition–property relationships for dilute and concentrated solutions of chitosan and sorbitan ester. In addition, this novel biopolymer–surfactant complex may be used as an emulsion system for food and drug-delivery applications.

Materials and Methods

Materials. Chitosan (medium molecular weight (MW \approx 400 000) was purchased from Fluka BioChemika (Buchs, Switzerland). Sorbitan monolaurate (Span 20) and sorbitan monooleate (Span 80) were purchased from FisherBiotech (Fair Lawn, NJ). Sorbitan triooleate (Span 85) was purchased from Wiler Fine Chemicals (London, Canada). The chemical structures of chitosan and the sorbitan esters are shown in Figure 1. 1,6-Diphenyl-1,3,5-hexatriene (DPH) and acetic acid were obtained from Sigma (Oakville, Canada).

Emulsion Preparation. A 2% chitosan solution (w/v) was prepared by dissolving chitosan (85% deacetylated) in a 1% (w/v) acetic acid solution overnight. The chitosan solution was mixed with varying molar ratios (1:0.2 to 1:5 chitosan/surfactant) of sorbitan monolaurate, sorbitan monooleate, or sorbitan triooleate and homogenized for 30 min at 3000 rpm (Polytron PT-MR 3100, Kinematica AG, Switzerland).

Calculation of HLB. HLB values were calculated on the basis of the total mole percentage of the components present within each mixture.

Measurement of Critical Micelle Concentration. The CMC of the chitosan–surfactant mixtures was determined by an established fluorescence-based method.³² Briefly, DPH was dissolved in chloroform (1 mg/L) and added into glass vials which were then dried thoroughly under nitrogen and placed in a vacuum oven. The chitosan–surfactant solutions (prepared as described above) were diluted and added to the DPH-containing vials in concentrations that ranged from to 3.7×10^{-5} to 0.3 mol/L. All hydrated samples were

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stirred overnight in the dark at room temperature. The fluorescence emission of the samples was measured at 430 nm ($\lambda_{\text{ex}} = 350$ nm) with a dual-scanning microplate spectrofluorometer (Spectra GeminiXS, Molecular Devices, Sunnyvale, CA).

Measurement of Surface Tension. Surface tension (γ) of each chitosan–surfactant solution was measured with a surface tensiometer (Processor tensiometer K12, KRÜSS GmbH, Germany) at room temperature. To measure γ , each chitosan-based solution was diluted with deionized water, placed in a beaker, and then left for 10 min to stabilize the solution. The measurements were repeated in triplicate for each solution. The slope from the plot of γ versus concentration can be used to determine the value of surface excess (Γ) by means of the Gibb's absorption isotherm:

$$\Gamma = \frac{1}{RT} \left(\frac{d\gamma}{d \ln C} \right) \quad (1)$$

where R and T are the gas constant and absolute temperature, respectively. The calculated value for Γ can be used to obtain the mean molecular area (A) per molecule:

$$A = \frac{1}{\Gamma N_A} \quad (2)$$

where N_A is Avogadro's number.

Optical Microscopy and Measurement of Droplet Size. Images of the chitosan–surfactant emulsions (1:0.3 mol/mol) were captured using a Zeiss Axiovert 135 TV light microscope. The mean diameter of the particles was determined from the micrographs using a population size of 200 and Sigma Scan Pro software (Jandel Scientific, San Rafael, CA). Dynamic light scattering (DLS) was also used to determine the size of the chitosan–surfactant droplets (90Plus Particle Size Analyzer, Brookhaven Instruments Corp., New York, NY). The effective mean diameter of the droplets was determined using BIC Particle Sizing software.

FTIR Analysis. The FTIR spectra of chitosan, sorbitan esters and the mixtures were obtained using a universal ATR Spectrum-one spectrophotometer (Perkin-Elmer, Wellesley, MA). All spectra were an average of 16 scans at a resolution of 2 cm^{-1} and were repeated in triplicate. A background spectrum containing no sample was subtracted from all spectra. The positions of the spectral peaks, peak areas, and heights were obtained using a software package (Perkin-Elmer).

Rheology Measurements. The rheological properties of a 2% chitosan solution, sorbitan esters, and chitosan–surfactant mixtures were measured using an AR2000 rheometer (T. A. Instruments, New Castle, DE) at 25°C . A 4-cm-diameter cone and plate geometry with a 2° angle was used for the liquid formulations. For the more viscous solutions, a 2-cm-diameter cone and plate geometry with a 4° angle was employed. The instrument was calibrated and rotational mapping was performed to ensure the accuracy of the measurements. A continuous ramping flow mode was used to measure the viscosity while increasing the shear stress from 0.1 to 500 Pa for the liquid solutions and from 1 to 500 Pa for the viscous emulsions. Finally, small-amplitude oscillatory shear tests were used to characterize the chitosan–surfactant solutions.

Results

1. Emulsion Preparation. Emulsion formulations were prepared from a chitosan solution, which served as the continuous phase, and sorbitan monolaurate, sorbitan monooleate, or sorbitan triooleate as the dispersed phase. The formulations are comprised of droplets or micelle-like particles that are formed from the sorbitan esters and surrounded by a chitosan coat. The chemical structures and physical properties of chitosan and the sorbitan-based molecules are shown in Figure 1 and Table 1. Upon chitosan and the surfactants being mixed (as described above), the formulations immediately had an off-white creamy consistency typical of o/w emulsions. Also, according to the high HLB values calculated for the chitosan–surfactant emulsions (i.e., HLB values

Table 1. Physical Properties of Chitosan and Sorbitan Esters

material	MW (g/mol)	HLB	density (g/cm ³)
chitosan (C)	400 000	36.7 ^a	1.0
sorbitan monolaurate (SML)	346.5	8.6	1.058
sorbitan monooleate (SMO)	428.6	4.3	0.994
sorbitan triooleate (STO)	957.5	1.8	0.952

^a HLB for chitosan was reported by Schulz et al.¹

ranged from 29.2 to 9.7 for the 1:0.3 to 1:5 (mol/mol) chitosan/sorbitan monooleate mixtures) the systems are classified as o/w (data not shown).²

2. Cream Preparation. Preparation of a viscous chitosan–surfactant cream was found to be dependent on numerous factors including the type of surfactant employed, the concentration of chitosan and sorbitan ester, the volume of the formulation, and the use of mechanical action for mixing. For example, chitosan flakes were dissolved in a 1% acetic acid solution and homogenized with the surfactant at varying molar concentrations with a total volume of 6 mL. For the chitosan/sorbitan monooleate mixture, a molar ratio of 1:1 was required for the onset of the creaming effect. As the concentration of sorbitan monooleate increased, the formulation became smoother and thicker in appearance. When a 10-mL final volume of the chitosan–sorbitan monooleate mixture was prepared, the onset of the creaming effect was only observed at a higher amount of surfactant (1:2 chitosan/sorbitan monooleate (mol/mol)). Furthermore, as the concentration of surfactant in the formulation was increased, the use of the homogenizer was not necessary to produce a viscous cream; the creaming effect was observed for the 1:5 (mol/mol) chitosan/sorbitan monooleate formulation upon mixing with a spatula. Similarly, the chitosan–sorbitan triooleate mixture was able to produce a smooth and viscous cream. The onset of the creaming effect only occurred at a higher concentration of surfactant (1:2 chitosan/sorbitan triooleate (mol/mol)) in comparison to the chitosan–sorbitan monooleate mixture when the total volume was 6 mL. However, for the chitosan–sorbitan monolaurate formulation, there was no apparent increase in viscosity under the same conditions (i.e., molar ratios, volumes, and mechanical actions) employed for preparation of creams from chitosan and the other sorbitan esters.

3. Critical Micelle Concentration. An established fluorescence method was used to determine the CMC of mixtures of chitosan and sorbitan esters (Figure 2a). From the results, the mixture of chitosan and sorbitan monooleate had the lowest CMC of the combinations of chitosan and surfactants examined (Table 2). Similarly, Opawale and Burgess reported that the CMC of sorbitan monooleate at a mineral o/w interface at 25°C was lower than that of sorbitan triooleate.¹⁷ The authors speculated that the high CMC of sorbitan triooleate may be attributed to the bulkiness of this molecule. A relatively high CMC (5.6×10^{-4} M) was also found for the chitosan–sorbitan monolaurate mixture which may be attributed to its hydrophilic nature as reflected by its high HLB value (Table 1). Opawale and Burgess were unable to measure the CMC value for sorbitan monolaurate due to its high solubility in the aqueous phase.¹⁷

4. Surface Tension. The surface tension (γ) of the chitosan–surfactant mixtures was measured as a function of concentration at room temperature. As shown in Figure 2b, an increase in the concentration of the solution resulted in a decrease in γ due to the saturation of hydrophobic macromolecules at the air–water surface or interface (Figure 2b). When the molecules are close to saturation, γ slightly decreases or remains constant. From our observations, chitosan alone had the highest value for γ at saturation due to the hydrophilic effect of its protonated amine

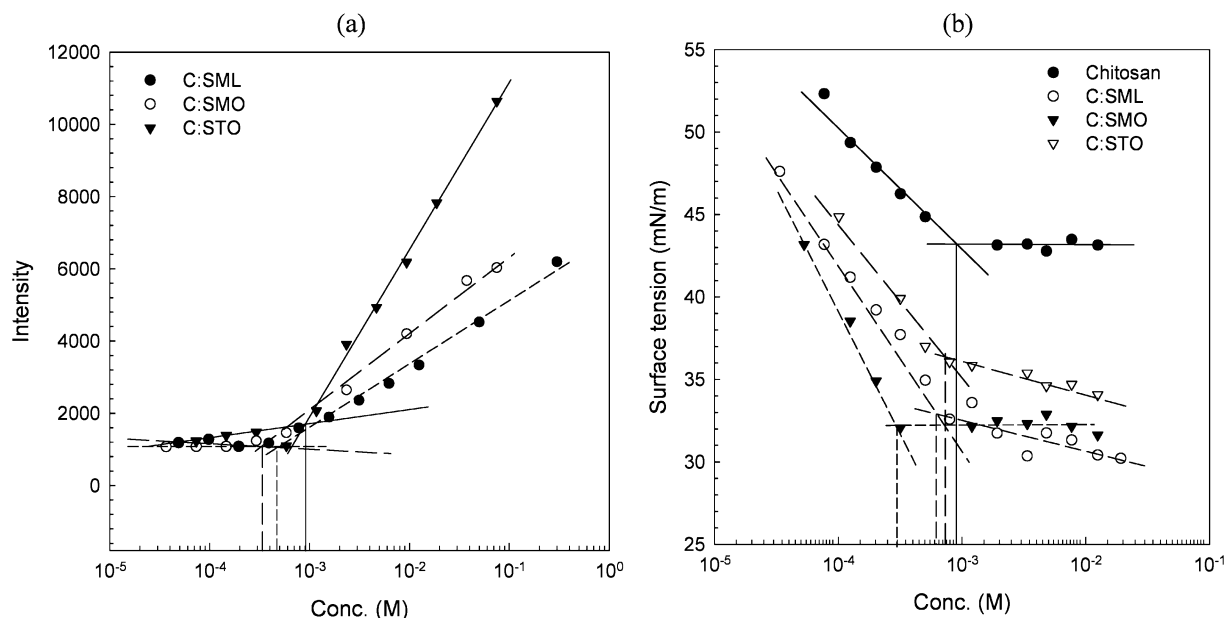


Figure 2. Semilogarithmic plot of the fluorescence emission intensity versus log of the chitosan–sorbitan ester (1:0.3 mol/mol) concentration (a) and surface tension values as a function of the log concentration of chitosan–sorbitan esters (b) (1:0.3 mol/mol) at room temperature. Chitosan–sorbitan monolaurate (C:SML), chitosan–sorbitan monooleate (C:SMO), and chitosan–sorbitan trioleate (C:STO).

Table 2. Physical Properties of Chitosan–Surfactant Mixtures

	CMC ^a ($\times 10^{-4}$ M)	CMC ^b ($\times 10^{-4}$ M)	$d\gamma/d\ln C$	Γ ($\times 10^6$ mol/m ²)	A (\AA^2)
C (chitosan)	10	—	−3.85	1.55	107
C–sorbitan monolaurate	6.5	5.60	−4.60	1.85	90
C–sorbitan monooleate	3.0	1.25	−6.25	2.52	66
C–sorbitan trioleate	9.0	4.55	−4.44	1.79	93

^a Surface tension measurements. ^b Fluorescence measurements.

groups. However, for the chitosan–surfactant mixtures, chitosan–sorbitan trioleate had the highest saturated value for γ followed by sorbitan monooleate and then sorbitan monolaurate. The CMC of the chitosan–surfactant droplets can also be determined by the intercept of the two slopes. Similar to the fluorescent method, the lowest CMC value was found for chitosan–sorbitan monooleate. Furthermore, a similar CMC value was obtained for the chitosan–sorbitan monolaurate droplets (Table 2). Interestingly, a higher CMC value was obtained for the chitosan–sorbitan trioleate mixture using this method, in comparison to the value obtained using the fluorescence-based method.

The slope from the plot of γ versus concentration was used to calculate the Gibbs's absorption isotherm which in turn was used to determine the molecular area (A) of the sorbitan esters in the monomolecular film regions. As shown in Table 2, the value for A was smallest for chitosan–sorbitan monooleate and largest for chitosan–sorbitan trioleate. Therefore, the chitosan–sorbitan monooleate system is more favorable for formation of droplets within the chitosan solution when compared to the other surfactants. From the literature, at the o/w interface, A of sorbitan monooleate ($32.5 \text{ \AA}^2/\text{mol}$) is smaller than that of sorbitan trioleate ($45.3 \text{ \AA}^2/\text{mol}$).¹⁷ The higher A value for sorbitan trioleate may be attributed to the bulkiness and hydrophilicity of the three double bonds in the molecular chain (Figure 1).¹⁶

5. Droplet Size and Morphology. DLS was performed on dilute chitosan–surfactant solutions (0.02 M). As shown in Table 3, at this concentration a similar mean diameter of $2.2 \mu\text{m}$ was observed for the chitosan–sorbitan ester droplets. As the concentration of the chitosan–surfactant solutions was increased to 0.2 M, significant differences in the mean diameter and morphology were observed by optical microscopy (Figure 3).

Table 3. Droplet Sizes in Chitosan–Surfactant Solutions

sample	0.02 M	0.2 M
chitosan–sorbitan monolaurate	$2.4 \pm 0.7 \mu\text{m}$	$2.9 \pm 1 \mu\text{m}$
chitosan–sorbitan monooleate	$2.1 \pm 0.5 \mu\text{m}$	$22.2 \pm 5 \mu\text{m}$
chitosan–sorbitan trioleate	$2.1 \pm 0.3 \mu\text{m}$	$8 \pm 6 \mu\text{m}$

Specifically, the chitosan–sorbitan monooleate droplets had two populations: small, independent particles and large aggregates with mean diameters of 2.0 ± 1 and $22.2 \pm 5 \mu\text{m}$, respectively. By contrast, the chitosan–sorbitan trioleate droplets were mostly single, large, and irregularly shaped with a mean diameter of $8 \pm 6 \mu\text{m}$. For the chitosan–sorbitan monolaurate emulsion, less coalescence and no flocculation was observed, as only small single droplets with a mean diameter of $2.9 \pm 1 \mu\text{m}$ were present.

6. FTIR Analysis. As mentioned above, to dissolve chitosan, an acidic solution (1% acetic acid, pH < 5) is necessary to protonate chitosan's NH_2 groups. It has been established that the FTIR spectra of a chitosan solution includes a NH_3^+ peak at 1533 cm^{-1} and a peak at 1635 cm^{-1} for the NH_2 (or CONH_2) groups (Figure 4a).³³ In the amine or amide region of chitosan, no interfering peaks were present for sorbitan monooleate. When sorbitan monooleate was mixed with the chitosan solution (1:2 mol/mol, chitosan/sorbitan monooleate), a significant shift in the NH_3^+ peak was observed from 1533 to 1558 cm^{-1} (Figure 4a, peak labeled 1). A similar peak shift from 1533 to 1557 cm^{-1} was also apparent for both the chitosan–sorbitan monolaurate and chitosan–sorbitan trioleate formulations at the same molar concentrations. A peak shift was also observed for the amide peak from 1635 cm^{-1} for chitosan alone to 1647 cm^{-1} for the

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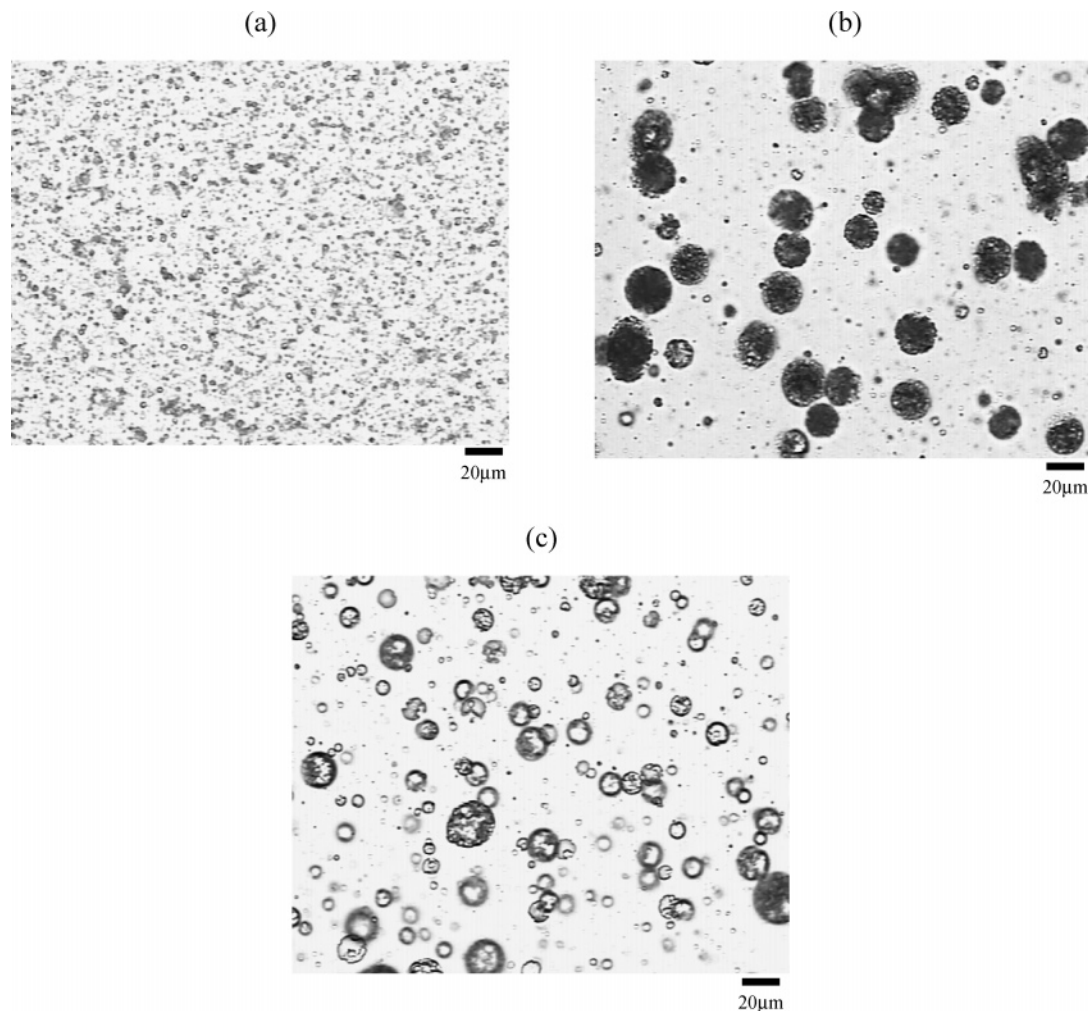


Figure 3. Optical micrographs of the chitosan–surfactant mixtures. Photomicrographs of the 1:0.3 (mol/mol) (a) chitosan–sorbitan monolaurate, (b) chitosan–sorbitan monooleate, and (c) chitosan–sorbitan triooleate mixtures at 20× magnification.

chitosan–sorbitan monooleate cream. The shift in the amide peak for chitosan–sorbitan monolaurate and chitosan–sorbitan triooleate were from 1635 to 1638 and 1652 cm^{-1} , respectively. Therefore, the greatest shift in chitosan’s amide peak was observed for chitosan–sorbitan triooleate and only a slight shift was found for chitosan–sorbitan monolaurate. The peaks found in both acetic acid and the pure chitosan solution (1407, 1052, and 1012 cm^{-1}) were either shifted or absent from the chitosan–surfactant spectrum (Figure 4a). Therefore, the sorbitan esters may act to displace the acetic acid content from the chitosan solution.

A study by Hoffman’s group used FTIR to measure the peak area and peak height of chitosan’s amine group relative to the ether group at 1152 cm^{-1} .³⁴ This provided a measure of the acetate content in their films and demonstrated the interaction between the components in their film (i.e., chitosan and glycerol). In a similar manner as shown in Figure 4b, the addition of sorbitan ester to chitosan caused a decrease in the peak height ratio and peak area ratio of chitosan’s amine group relative to its ether group (1533 cm^{-1} /1152 cm^{-1}). The greatest decrease in peak height ratio and peak area ratio was obtained for chitosan–sorbitan triooleate followed by chitosan–sorbitan monooleate and then chitosan–sorbitan monolaurate. Interestingly, the same order was also found for the greatest shift in chitosan’s amide peak.

7. Rheological Measurements. The rheological properties of the chitosan–surfactant solutions were analyzed by steady shear measurements and small amplitude oscillatory shear tests.

7.1. Steady Shear Measurements. The steady shear viscosity was analyzed for freshly prepared solutions of chitosan, sorbitan esters, and chitosan–surfactant mixtures as a function of steady shear rate at 25 °C. As shown in Figure 5, a Newtonian flow behavior (i.e., the viscosity was independent of shear rate and time) was observed for the surfactants. Specifically, sorbitan monolaurate had the highest viscosity value of 4.63 Pa·s followed by sorbitan monooleate (0.91 Pa·s) and then sorbitan triooleate (0.23 Pa·s). According to the source of the sorbitan esters, the measured viscosity of each of the surfactants was in agreement. Chitosan and chitosan–sorbitan monooleate (1:0.5) showed different responses to shear rate; a Newtonian behavior was observed at low shear rates, and as the steady shear rate was increased above a critical value, the viscosity gradually decreased (shear-thinning behavior). The three-parameter Carreau model may be used to fit the relationship between viscosity and shear rate as follows³⁵

$$\eta = \frac{\eta_0}{[1 + (t_1 \dot{\gamma})^2]^{(1-n)/2}} \quad (3)$$

where t_1 is the longest relaxation time and n represents the material’s shear-thinning behavior (n is dimensionless). As the

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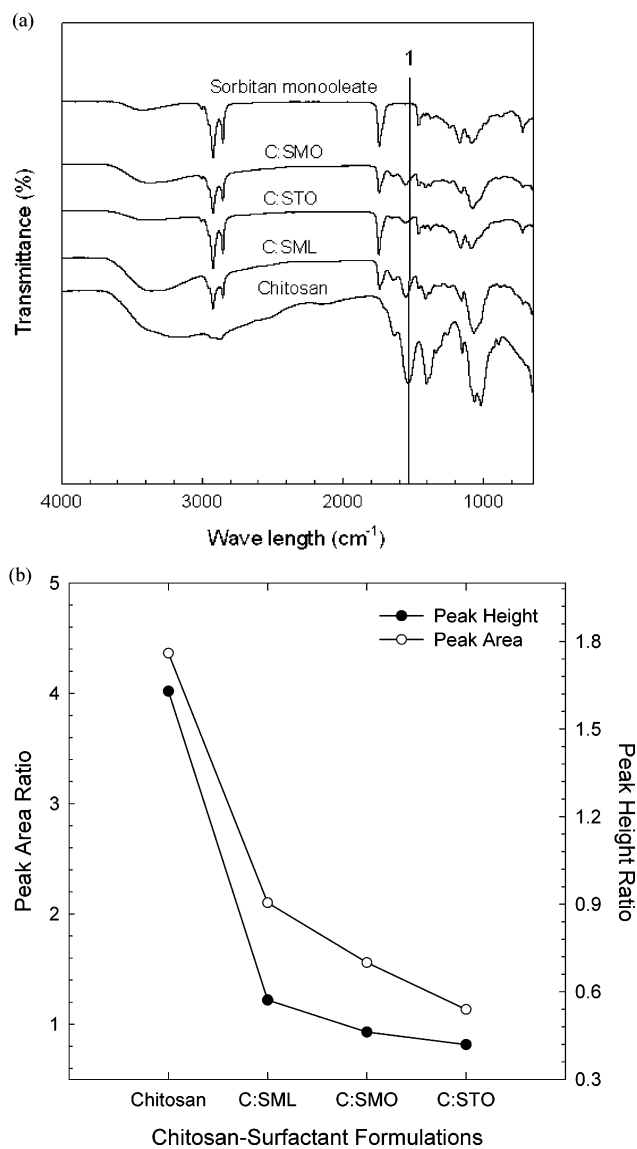


Figure 4. (a) FTIR spectra of sorbitan monooleate, chitosan–sorbitan monooleate (C:SMO), chitosan–sorbitan trioleate (C:STO), chitosan–sorbitan monolaurate (C:SML) (1:2 mol/mol), and chitosan. Note: all chitosan formulations were prepared in a 1% acetic acid solution. (b) The change in the peak area ratio and peak height ratio ($1533\text{ cm}^{-1}/1152\text{ cm}^{-1}$) for the 1:2 (mol/mol) chitosan–surfactant mixtures. Chitosan–sorbitan monolaurate (C:SML), chitosan–sorbitan monooleate (C:SMO), and chitosan–sorbitan trioleate (C:STO).

steady shear rate ($\dot{\gamma}$) approaches zero, η represents the zero-shear viscosity (η_0) and its slope on a log–log scale in the high-shear-rate regime is given by $n-1$. According to Carreau et al., the polymer has a liquidlike behavior when n is close to 1.³⁵ For the 2% (w/v) chitosan solution, having a degree of deacetylation of 85%, the zero shear viscosity was 3.26 Pa·s and the shear thinning power-index n value was 0.56 (Figure 7). Cho et al. reported a zero shear viscosity of 2.1 Pa·s and a power-index value of 0.70 for a 2% (w/v) chitosan solution (i.e., chitosan having a degree of deacetylation of 93%).³⁶ The chitosan concentration used in this study was much higher than the known entanglement concentration (C_e) of 0.75% (w/v) for chitosan.³⁶

(35) Carreau, P. J.; De Kee, D.; Chhabra, R. P. *Rheology of polymeric systems: principles and applications*; Hanser/Gardner Publications: Munich, New York, Cincinnati, 1997.

(36) Cho, J.; Heuzey, M.-C.; Begin A.; Carreau P. J. *Food Eng.* **2006**, *74*, 500–515.

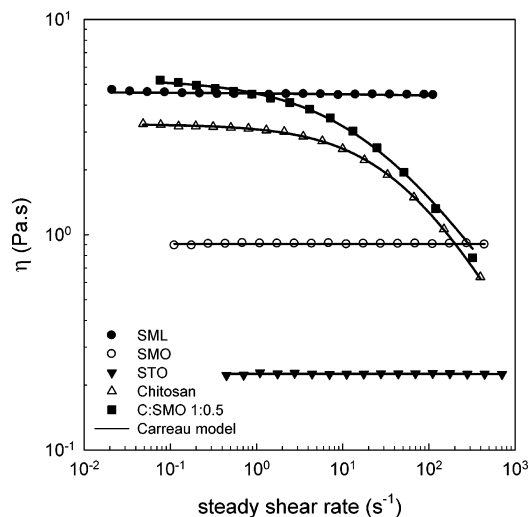


Figure 5. Viscosity of freshly prepared chitosan, sorbitan monooleate (SML), sorbitan monooleate (SMO), sorbitan trioleate (STO), and a 1:0.5 (mol/mol) chitosan–sorbitan monooleate mixture (C:SMO).

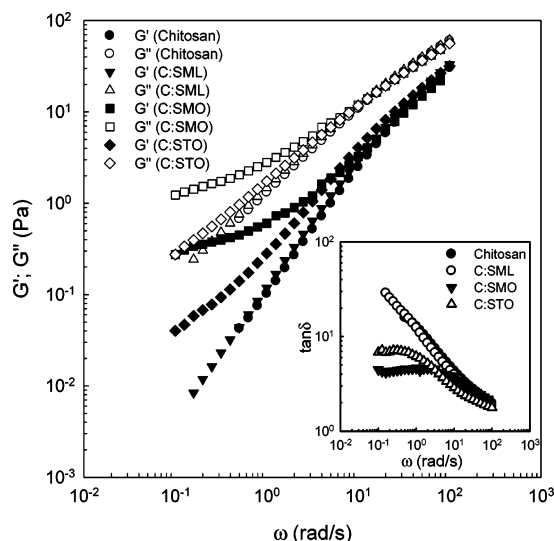


Figure 6. Effect of the sorbitan esters on the complex moduli G' and G'' as measured in LVE at 25 °C for the 1:0.3 (mol/mol) chitosan/sorbitan ester emulsions. Insert: Effect of $\tan \delta$ on frequency. Chitosan–sorbitan monolaurate (C:SML), chitosan–sorbitan monooleate (C:SMO), and chitosan–sorbitan trioleate (C:STO).

Thus, the chitosan chains can form entanglements between the macromolecules, resulting in enhanced rheological properties. Amiji et al. reported that chitosan could self-associate by intermolecular hydrophobic interactions.³⁷ A decrease in the degree of deacetylation of chitosan is accompanied by an increase in the number of hydrophobic groups and a decrease in the number of charged groups. Therefore, at higher degrees of deacetylation, it is more favorable for physical interactions to occur resulting in an enhancement of dynamic rheological properties. For chitosan having a degree of deacetylation of 85%, the lower n value can be attributed to the number of hydrophobic interactions. These interactions restrict the motion of individual chitosan chains, resulting in the requirement for longer times to form new entanglements and hydrophobic associations necessary to replace those disrupted by the flow at high shear rates.

7.2. Small Amplitude Oscillatory Shear Tests. The dynamic rheological properties of chitosan and surfactant mixtures (1:0.3

(37) Amiji, M. M. *Carbohydr. Polym.* **1995**, *26*, 211–213.

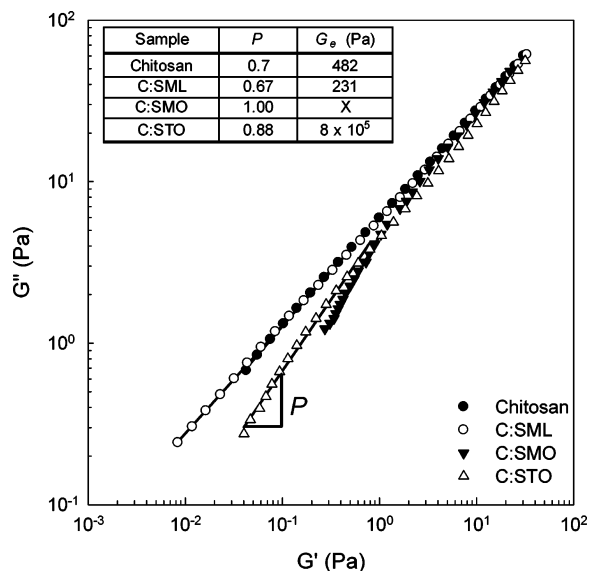


Figure 7. Cole–Cole plots for the chitosan–surfactant solutions at 25°C. Chitosan–sorbitan monolaurate (C:SML), chitosan–sorbitan monooleate (C:SMO), and chitosan–sorbitan trioleate (C:STO).

mol/mol) were investigated at 25 °C by small amplitude oscillatory shear tests in the linear viscoelastic zone. Figure 6 shows the effect of sorbitan esters on the complex moduli G' and G'' and $\tan \delta$.

In the absence of the surfactants, the chitosan solution followed typical solution behavior ($G' \ll G''$). The addition of sorbitan monolaurate had no effect on the physical properties of the chitosan solution. However, the complex moduli G' and G'' were distinctly enhanced when sorbitan monooleate and sorbitan trioleate were added, especially at the low-frequency zone. In addition, these mixtures were more elastic than chitosan–sorbitan monolaurate, as shown by a decrease in $\tan \delta$ (Figure 6). These effects may be attributed to the formation of emulsions, coalescence, and/or the interactions between chitosan and sorbitan esters, as supported by the observed morphologies. The viscoelastic properties of polymer systems may be presented in a Cole–Cole plot and the generalized expression is as follows^{38,39}

$$\log G''(\omega) = P \log G'(\omega) + (1 - P) \log(G_e - G'(\omega)) \quad (4)$$

where G_e represents the plateau storage modulus of G' at high frequency and P is the power-law index. Exponent P is equal to 0.5 for a one-mode Maxwell fluid model, which is described by the following equations:⁴⁰

$$G''(\omega) = \frac{G_e \omega^2 \tau^2}{1 + \omega^2 \tau^2} \quad (5)$$

$$G'(\omega) = \frac{G_e \omega \tau}{1 + \omega^2 \tau^2} \quad (6)$$

where τ is the relaxation time and ω is the angular frequency in rad/s. However, a P value larger than 0.5 indicates that the rheological properties of the polymeric systems cannot be predicted by the single-mode Maxwell model. The value of P is dependent on the structures within the polymer systems and

Table 4. Parameters Calculated Using the Three-Parameter Carreau Model

sample	η_0 (Pa·s)	t_1 (s)	n
sorbitan monolaurate	4.63	×	×
sorbitan monooleate	0.91	×	×
sorbitan trioleate	0.23	×	×
chitosan	3.26	0.02	0.56
chitosan–sorbitan monooleate 1:0.5 (mol/mol)	5.28	0.05	0.53

^a Where × represents values that were not calculated due to Newtonian behavior.

alternatively it may be explained by the complexity of the relaxation processes or by the structural evolution resulting from the physical and/or chemical interactions.^{36,38,39} Figure 7 includes the Cole–Cole plots for the mixtures of chitosan and sorbitan esters. For all solutions, P was between 0.67 and 1, indicating that the chitosan-based solutions deviate from the single Maxwell element behavior (Table 4). The P values were greatly dependent on the type of sorbitan ester. Similar to chitosan alone, a P value of 0.67 was obtained for the chitosan–sorbitan monolaurate mixture. However, the addition of sorbitan monooleate and sorbitan trioleate to the chitosan solution significantly increased the P value due to the evolution of structures formed from chitosan and the unsaturated sorbitan esters.

7.3. Effect of Surfactant Concentration on Viscosity. The viscosity was measured as a function of the ratio of chitosan to sorbitan ester by steady shear tests. For the chitosan–sorbitan monolaurate mixtures, no significant increase in viscosity was observed for concentrations ranging from 1:0.5 to 1:5 chitosan/sorbitan ester (mol/mol) as a function of shear stress (data not shown). However, for the 1:0.5 chitosan–sorbitan monooleate mixture, a slight increase in viscosity was observed when compared to chitosan alone. Interestingly, a 5–6-fold increase in viscosity to approximately 1×10^6 Pa·s was observed for the chitosan–sorbitan monooleate formulations when the concentration was increased from 1:1 to 1:5 (mol/mol) (Figure 8a). Specifically, high viscosity and yield stress values were obtained for the 1:1 to 1:3 (mol/mol) mixtures of chitosan and sorbitan monooleate. However, as the amount of sorbitan monooleate was increased above the 1:3 (mol/mol) concentration, a slight decrease in the viscosity and yield stress values were observed. This may provide an indication that phase inversion occurs in the mixtures of chitosan–sorbitan monooleate above the 1:3 ratio. Alternatively, the decreased rheological properties may be attributed to an excess of sorbitan monooleate molecules diffusing from chitosan's aqueous phase. For the 1:2 (mol/mol) chitosan–sorbitan trioleate cream, a significant decrease in viscosity and yield stress was observed in comparison to the 1:2 (mol/mol) chitosan–sorbitan monooleate cream (Figure 8b). Overall, among the chitosan–surfactant mixtures investigated, the greatest enhancement in rheological properties was observed for chitosan–sorbitan monooleate.

The stability of the chitosan–sorbitan monooleate 1:2 and 1:3 (mol/mol) formulations were investigated in terms of viscosity over a four month period while stored at 4 °C. From the results, the viscosity remained constant at approximately 1×10^6 Pa over the four month period (data not shown). This confirms a strong interaction between chitosan and sorbitan monooleate.

Discussion

Mixtures of the cationic biopolymer chitosan and the nonionic surfactants, sorbitan monolaurate, sorbitan monooleate, and sorbitan trioleate formed polysaccharide–surfactant complexes with unique properties. The polymer–surfactant complexes were

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(39) Thuresson, K.; Lindman, B.; Nystrom, B. *J. Phys. Chem. B* **1997**, *101*, 6450–6459.

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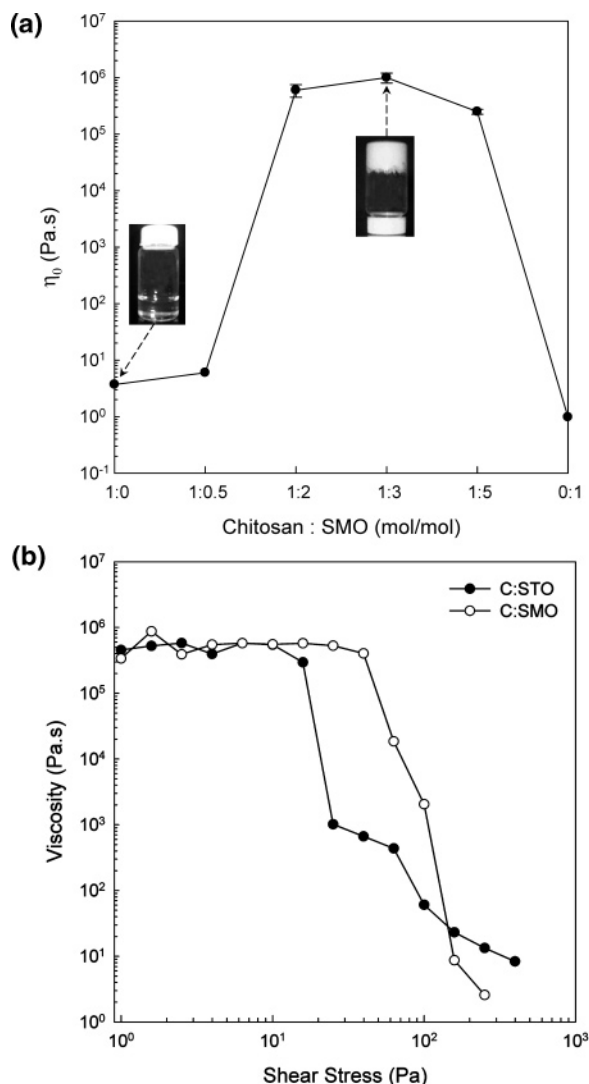


Figure 8. (a) Evolution of the viscosity in terms of solution concentration for chitosan–sorbitan monooleate (C:SMO). Photographs of the chitosan solution and an inverted vial containing C:SMO. (b) Comparison of the viscosity as a function of shear stress for chitosan–sorbitan monooleate (C:SMO) and chitosan–sorbitan triooleate (C:STO) at a molar concentration of 1:2.

evidenced by the desorption of chitosan from the solution surface with the addition of the sorbitan esters (Figure 2b). Furthermore, FTIR, morphological, and rheological analyses provided support for the presence of interactions between chitosan and sorbitan esters. From the FTIR results, the strongest interaction was found for mixtures of chitosan and sorbitan triooleate and the weakest interaction was observed for chitosan–sorbitan monooleate (Figure 4b). The ratio of sorbitan headgroup to carbon atoms in the hydrophobic tail of the various sorbitan esters was found to be directly related to the peak area ratio of NH_3^+ groups to ether groups of the sorbitan esters from the FTIR study (data not shown). Thus, the interaction between the sorbitan esters and chitosan can be related to the number of sorbitan headgroups and hydrophobic interactions.

The differences in the degree of interaction between chitosan and the sorbitan esters can also be attributed to the variations in chemical structure for each surfactant molecule. As shown in Figure 1, sorbitan monooleate consists of a lauric acid side chain on a sorbitan ring. The small unimodal droplets that were observed for the chitosan–sorbitan monooleate emulsion were expected, as sorbitan monooleate is a small saturated polar

molecule (Figure 3, Table 1). Furthermore, smaller-sized droplets have larger surface areas, indicating that a higher number of sorbitan headgroups are present for the sorbitan monooleate droplets in comparison to the other surfactants. The structural and nonionic nature of the surfactant also allows for a high number of sorbitan headgroups to tightly pack and form micelle-like structures. The high population of headgroups on the droplet's surface is an unfavorable environment to form interactions between chitosan and sorbitan monooleate, possibly due to the electrostatic repulsion exerted by the protonated amine groups of chitosan and the decreased hydrophobic regions. Therefore, an optimal distance between the sorbitan headgroups may be required to form interactions between chitosan and sorbitan esters. This was further supported by comparing sorbitan monooleate to sorbitan monooleate and then sorbitan monooleate to sorbitan triooleate.

The difference between sorbitan monooleate and sorbitan monooleate is that the latter contains a longer unsaturated carbon chain (i.e., an oleic acid side chain). The double bond allows for a looser packing of the sorbitan monooleate molecules, which may result in interactions between a higher number of chitosan chains. The loose packing of the sorbitan monooleate molecules within the droplets and higher molecular weight for this sorbitan ester may account for the larger-sized droplets that are obtained for this surfactant and chitosan (Tables 1 and 3). In addition, repulsion between the sorbitan headgroups is lowest for sorbitan monooleate, as indicated by its low CMC value in comparison to the other sorbitan esters. The aggregation of the sorbitan monooleate droplets indicates that the organization of the sorbitan monooleate molecules allows for hydrophobic interactions to occur between the droplets.

Among the sorbitan esters employed in this study, sorbitan triooleate has the largest molecular weight and is the most hydrophobic, as it contains three oleic acid side chains on the sorbitan ring (Table 1, Figure 1). The sorbitan triooleate molecules formed the largest-sized droplets when mixed with chitosan, which is likely due to sorbitan triooleate's bulky chemical structure. The larger droplet size or small surface area indicates that fewer sorbitan headgroups are present at the surface of the droplet when compared to droplets formed from the other sorbitan esters. These properties allow for the largest distance between the sorbitan headgroups within the chitosan–sorbitan triooleate droplet. Furthermore, sorbitan triooleate alone had the lowest viscosity value followed by sorbitan monooleate and then sorbitan monooleate in response to shear rate (Figure 6). Thus, sorbitan triooleate molecules have a higher rate of diffusion in the chitosan phase in comparison to the other surfactants. From the literature, the stability of emulsions generally depends on the viscosity of the continuous phase as the viscosity influences the diffusion of the droplets.² Overall, a surfactant solution having a low viscosity and an optimal distance between sorbitan headgroups is required to compensate for the electrostatic repulsions and hydrophobic interactions of the chitosan chains. A proposed schematic illustrating the differences between the chitosan–surfactant complexes is shown in Figure 9 (not drawn to scale). “A necklace of micelle pearls on a polymer backbone” is often used to describe polymer–surfactant complexes.⁴¹

The observed morphologies for the chitosan–surfactant mixtures may be related to their rheological properties. The viscosity ratios of sorbitan esters and chitosan ($\eta_{0,\text{sorbitan esters}}/\eta_{0,\text{chitosan}}$ at 25 °C) were determined from the three-parameter Carreau model (Table 4). The viscosity ratios were 1.42 for sorbitan monooleate, 0.28 for sorbitan monooleate, and 0.08 for

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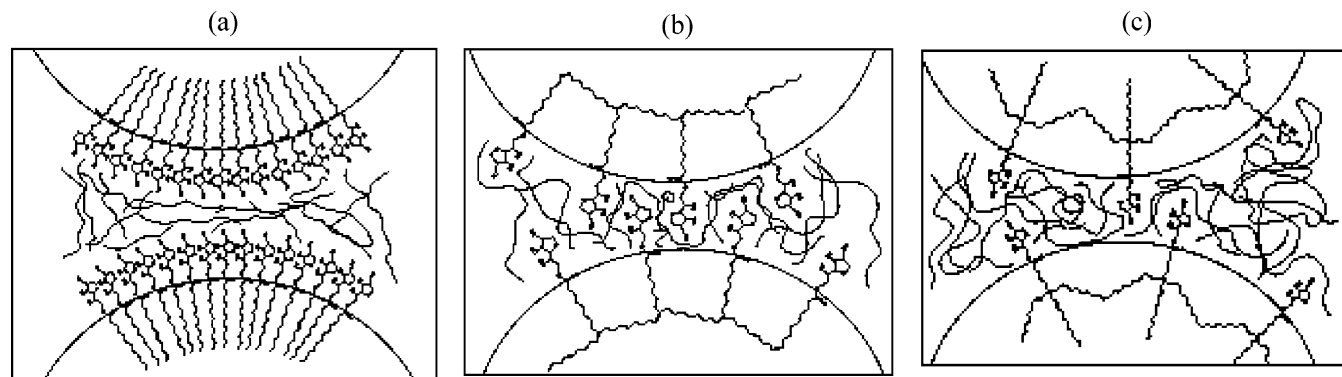


Figure 9. Schematics that may represent the structural organization of droplets formed from sorbitan monolaurate (a), sorbitan monooleate (b), and sorbitan trioleate (c) in a chitosan solution (not drawn to scale).

sorbitan trioleate. Similar to previous studies, a low viscosity ratio (dispersed phase to continuous phase) resulted in a larger individual particle size.^{42,43} As shown in Figure 6, the evolution of structures for the polymer–surfactant complexes can be seen at the low-frequency zone for chitosan–sorbitan monooleate and chitosan–sorbitan trioleate. Interestingly, the chitosan–sorbitan monooleate mixture had a greater effect on the complex moduli G' and G'' for chitosan in comparison to the chitosan–sorbitan trioleate mixture. The large aggregates of chitosan–sorbitan monooleate hindered the flow of the solution. For the chitosan–sorbitan monolaurate mixture, no changes in the complex moduli G' and G'' were observed in comparison to chitosan alone due to the small particle size, low density, and decreased hydrogen-bonding interactions (Figure 4, Table 1). Therefore, the aggregation and/or coalescence of the droplets was required to achieve a highly viscous cream formulation as obtained for chitosan–sorbitan monooleate and chitosan–sorbitan trioleate and not for chitosan–sorbitan monolaurate. The highly viscous chitosan–sorbitan monooleate cream also had greater stability in terms of its yield stress in comparison to the chitosan–sorbitan trioleate cream (Figure 8b). Furthermore, the onset of the creaming effect occurred at a lower concentration for chitosan–sorbitan monooleate. The larger droplet size or increased volume fraction of the chitosan–sorbitan monooleate droplets may account for the differences between the chitosan–sorbitan monooleate and sorbitan trioleate viscous creams.

According to Groot, the mechanism of interaction between polymer and surfactant may be deduced by consideration of parameters such as polymer size, mode of surfactant adsorption (continuous or discrete micelles), and possible site of interaction (headgroup or tail).⁴¹ For the mechanism of interaction between chitosan and sorbitan esters, the concentration (dilute, semidilute, and concentrated) of the formulation must also be considered, as different structural conformations occur at the various concentrations. In the dilute formulations, small localized chitosan–sorbitan ester droplets that do not interact with each other are present along the polymer chains. As shown in Table 2, a decrease in the molecular area in the dilute state suggests that the surfactant molecules adsorb to specific binding sites on

chitosan, resulting in a more-compact structure which Groot refers to as a polymer–surfactant complex.⁴¹ It may be postulated that the formation of the polymer–surfactant complex occurs in two stages. First, sorbitan esters interact with chitosan by hydrogen bonding. This interaction may occur between the amine, ammonium ions, and hydroxyl groups of chitosan and the hydroxyl and carbonyl groups of the sorbitan ester headgroup. Second, hydrophobic interactions between the sorbitan ester tails (tail attraction) allow for the formation of the complex structures. For the semidilute formulations (above the overlap concentration), the droplets can expand in size with increasing concentrations of the chitosan–sorbitan ester mixtures. In this way, the localized droplets are closer in proximity or superimposed and can form globalized polymer–surfactant complexes. The hydrophobic interactions between the droplets enhance the stability of the complex structures. For the concentrated state, the chitosan chains are entangled, which restricts the expansion of the droplets. Therefore, depending on the diffusivity of the sorbitan ester within the chitosan matrix and the degree of interaction between chitosan and sorbitan esters, enhanced globalized complexes (i.e., system) can develop.

Conclusions

The length and degree of saturation of the surfactant hydrocarbon chains and chemical architecture have a significant impact on the development of chitosan–surfactant complexes. An optimal distance between chitosan's protonated amine groups is required for interactions to occur between chitosan and sorbitan esters. The chitosan–sorbitan monooleate cream may be used for the development of stable emulsions for applications in the food and drug delivery industries. The biocompatibility and toxicity of the chitosan–surfactant cream is currently being evaluated *in vitro* and *in vivo*. Future studies will also focus on the mechanism and organization of the polymer and surfactant molecules within the emulsion system.

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