Micellar Properties of Quillaja Saponin. 1. Effects of Temperature, Salt, and pH on Solution Properties

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We used surface tension and dye solubilization measurements to demonstrate that quillaja saponin molecules aggregate into micelles above a critical micelle concentration (cmc), whose value ranges between 0.5 and 0.8 g/L at 298 K. Below the cmc, this surfactant forms a saturated interfacial layer with 2 μmol/m^2. Increased temperature or pH increases the cmc, while increased salt concentration decreases this value. The size of saponin micelles was found to increase strongly with temperature but to have little dependence on salt concentration or pH. The effect of increased temperature on size is accompanied by a decrease in intrinsic viscosity, suggesting substantial dehydration of the micelles at higher temperatures. We also observed some differences between quillaja saponins obtained from various commercial sources. The knowledge gained from these studies of quillaja saponin solutions is useful in exploring their ability to extract solutes of biological significance and for understanding the functioning of these biological surfactants in foods and *in vivo*.

Keywords: *Micelles; critical micelle concentration; aggregation number; solubilization*

INTRODUCTION

Surfactants find wide application in the food industry because of their ability to enhance the solubility and/or dispersion stability of incompatible mixtures, such as those found in food emulsions and foams. In addition, edible surfactants have a number of applications in pharmaceutical, cosmetic, and detergent technology. Yet despite this extensive utilization, the colloidal properties of food-grade surfactants are often poorly characterized. In this study, we explore the micelle-forming properties of one such surfactant, quillaja saponin, a naturally occurring surfactant commonly used in the food industry.

Surfactants such as quillaja saponins are extensively used in the food industry to aid in forming stable oil/water mixtures (emulsions) and air/water mixtures (foams). During emulsion or foam formation, surfactants adsorb to the oil/water or air/water interface, lowering the energy of that surface and allowing the creation of smaller, more widely dispersed drops or bubbles. Once formed, the surfactants create a coating for the drop or bubble, therefore helping to keep that particle dispersed in solution. The ability of surfactants to help in emulsion and foam formation is directly dependent on the concentration of the surfactant at the oil/water or air/water interface, as well as the surface tension of that interface as a function of surfactant concentration. The first motivation for the present study was to determine the surface concentration and tension for quillaja saponin, in order to provide better information for the utilization of this surfactant as an emulsifier.

A second motivation for this research was the potential role of quillaja saponin in the development of a micellar-based extraction process for the removal of cholesterol from milk fat. Micellar-based extractions have gained wide attention in the chemical, environmental, and pharmaceutical fields due to their effectiveness and low cost (Scamehorn and Harwell, 1989; Calvert et al., 1994). However, very little exploration of the use of micelles for food extractions has been instigated. One possible exception is a recently developed technique employing quillaja saponin for the extraction of cholesterol (Sundfeld et al., 1993a,b). A high cholesterol extraction rate (greater than 90%) was achieved by using saponin to "bind" the cholesterol, followed by removal of the complex from aqueous solutions through adsorption on food-grade diatomaceous earth. It was speculated that the saponin's ability to extract cholesterol was due to its micelle-forming ability, whereby cholesterol solubilized within the saponin micelles.

It is well-known that the extent to which a surfactant solution can extract a solute is related to the micelle-forming properties of the surfactant. These properties include its critical micelle concentration (cmc) (concentration above which micelles form) and aggregation number (number of monomers in a micelle). Moreover, these micellar parameters are strongly affected by aqueous phase conditions such as temperature, salt concentration, pH, and sometimes even the presence of a hydrophobic solute. Hence, in order to achieve and optimize an extraction process employing surfactants, the micellar properties of the edible surfactant must be well characterized.

Finally, a third reason for our focus on quillaja saponin was the considerable interest these molecules have recently received in the medical literature. Saponins have been demonstrated to have a number of pharmaceutical effects, including enhanced vaccine effectiveness and antitumor and antimicrobial activity (Rouhi, 1995). In addition, saponins have exhibited an ability to reduce plasma cholesterol in a number of mammalian species including humans (Oakenfull and Sidhu, 1989). Bile acids and cholesterol, produced by the liver as bile to effect the digestion and absorption

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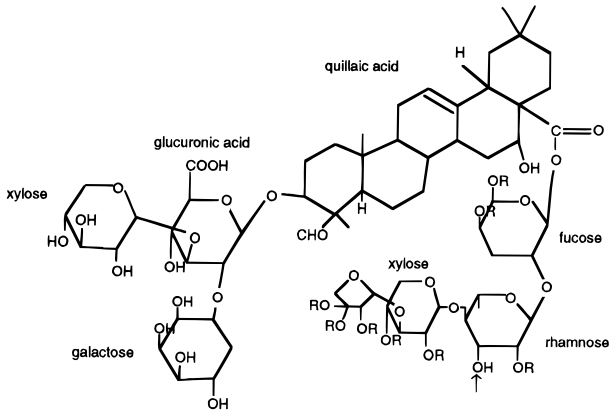

Figure 1. Representation of quillaja saponin molecule. Arrow denotes position at which glucose moiety is attached in one form of the molecule.

of dietary lipids, are very efficiently recycled. After completing their role as surfactants in lipid digestion and absorption, they return to the liver in the bloodstream. Saponins are believed to interrupt this cycle by forming mixed micelles with bile acids, thus inhibiting their ability to be reabsorbed, as well as by forming "complexes" with dietary cholesterol. The consequence is an increased fecal excretion of cholesterol and bile acids, resulting in lower plasma cholesterol levels. Recognizing the importance of the aggregation properties of saponins in this scenario, Oakenfull (1986) investigated aggregates of a variety of saponin types and suggested that many had micelle-forming capabilities in aqueous solution. In the present work, we build on this earlier study by Oakenfull and determine whether quillaja saponins indeed form aggregates at a critical surfactant concentration, consistent with a micelle-forming solution. In addition, we explore the effect of aqueous solution conditions on the aggregation properties of the saponin. Such information can be of crucial importance in understanding the functioning of these molecules in pharmaceutical applications.

The structure of the surfactant is expected to play a role in determining its micellar parameters. Quillaja saponin, like all saponins, is a glycoside. The hydrophilic groups of the molecule consist of sugars such as rhamnose, xylose, arabinose, galactose, fucose, and glucuronic acid, while the hydrophobic portions of the saponin are comprised of quillaic acid and gypsogenic acid (Oakenfull, 1986). Figure 1 shows the structure of a quillaja saponin molecule (Oakenfull and Sidhu, 1989). Glucuronic acid is the only ionizable group of the molecule; the other acids are attached as ester bonds to the main structure. Quillaja saponin is believed to contain two different structures, depending on whether a glucose unit is attached to the rhamnose moiety or not (Oakenfull and Sidhu, 1989; Higuchi et al., 1987). The resulting different head group structures may well lead to different colloidal properties in solution. We anticipate that most sources of quillaja saponin contain a mixture of both of these molecules and may include minority components as well, with a composition depending on the methods of preparation and purification. In order to explore the effect of the heterogeneity of this natural extract, we investigated the micellar properties of different sources of quillaja saponin. The supposition that these molecules often exist as mixtures, with properties that can vary with the source of the surfactant, is an issue that must be borne in mind with many food-grade or "biological" surfactants.

As can be seen from the discussion above, quillaja saponin is unlike more typical alkane-containing surfactants in that it does not contain a "tail" consisting of a straight hydrocarbon chain. This fact makes it difficult to estimate the molecule's aggregation properties simply by its structure (Meguro et al., 1987). Further, the presence of an ionizable group in the hydrophilic portion of the molecule may well affect its properties in aqueous solution at different pH values. This complexity of structure (Rouhi, 1995) may affect saponin's behavior as a "typical" surfactant, making its micellar properties more difficult to predict, and will ultimately affect its ability to solubilize other molecules such as cholesterol or therapeutic agents. Knowing these micellar properties would be extremely valuable in processes such as removing undesirable flavors from food oils and other extraction/purification procedures within food systems, as well as aiding in understanding the functioning of this molecule as a food emulsifier or pharmaceutical agent.

EXPERIMENTAL PROCEDURES

Materials. Quillaja saponin was obtained from Sigma Chemical Co. (St. Louis, MO), Acros Organics (Fair Lawn, NJ), and Penco of Lyndhurst Inc. (Lyndhurst, NJ). A molecular weight of 1650 (Oakenfull and Sidhu, 1989) was assumed to represent this molecule. 2′,7′-Dichlorofluorescein (401.21 MW) was obtained from Sigma Chemical Co. and employed in dye solubilization studies. Sodium chloride, used for investigating the effect of salt concentration on the cmc of saponin, was purchased from Fisher Scientific (Fair Lawn, NJ). Glacial acetic acid at a concentration of 17.4 M, sodium acetate, sodium carbonate, and sodium bicarbonate were used for preparing buffer solutions, which were then employed in studying the influence of pH on the cmc of saponin. These components were all obtained from Fisher Scientific. Sodium dodecyl sulfate (Pierce, Rockford, IL) with a cmc value of 8.1 mM was used for comparison in solubilization measurements. All chemicals were used as received and all aqueous solutions were prepared with doubly distilled water.

Methods. Quillaja saponin absorbs ultraviolet light in the region of 280−306 nm with an absorption maximum at 281 nm. Its concentration was monitored through its absorbance at 281 nm by an ultraviolet-visible (UV-vis) Shimadzu spectrophotometer (UV-160, Shimadzu, Kyoto, Japan). An extinction coefficient of 1.54 ± 0.02 L g^{-1} cm^{-1} was obtained in aqueous solution. Quillaja saponin also exhibits fluorescence with an excitation maximum at 298 nm and an emission spectrum maximum at 388 nm.

The cmc values of quillaja saponin from various sources and under various aqueous phase conditions were determined using a Wilhelmy plate Krüss 10 ST (Krüss, Charlotte, NC) equipped with a water bath. The Wilhelmy plate was cleaned by gently rinsing with doubly distilled water and then was flame-heated until just glowing. The glass sample vessel was thoroughly rinsed with ethanol, then repeatedly rinsed with doubly distilled water, and baked to dryness. Care was also taken to accurately zero the instrument. As a check of its performance, the Wilhelmy plate was used to determine the surface tension of water between measurements and the deviation from the literature value was less than 0.5% at 298 K. For the most part, surface tension values equilibrated in 1 h, and measurements were always taken after equilibration.

Experimentally, the cmc is determined from a break in a plot of surface tension γ versus the natural logarithm of surfactant concentration c, since once micelles form in solution, no significant changes in surfactant adsorption or surface energy of the air/water interface will occur. A linear regression was performed on the data for γ as a function of ln c above and below the approximate location of the cmc, requiring the data above the cmc to fit to a line of zero slope. The cmc was

then evaluated from the intersection of these two lines. The error in the cmc values thus determined was estimated from the standard deviation, using propagation of errors from the various parameters used in the linear regressions. The number of data points used in each evaluation varied between 7 and 20.

The cmc of quillaja saponin (Sigma) at 298 K was verified using dye solubilization studies. Excess dichlorofluorescein was added to well-sealed amber bottles containing fixed volumes of various aqueous saponin concentrations, and the solutions were allowed to equilibrate for approximately 6 days in a water bath (Gyrotory Water Bath Shaker Model G76, New Brunswick Scientific Co. Inc., New Brunswick, NJ) at 298 K with constant gentle agitation (shaker speed 2). After this time, the solutions were transferred to centrifuge tubes and subjected to centrifugation for 15 min at 298 K and 15 000 rpm in a RC-5B refrigerated superspeed centrifuge (DuPont Instruments, Wilmington, DE). The supernatant from each centrifuge tube was carefully transferred to 1 mm cuvettes, and the absorbances at 503 nm were recorded for each concentration of saponin. The solubility of dichlorofluorescein was also measured in water in a manner similar to that described above and found to be 137 ± 9 μM at 298 K. The extinction coefficient of dichlorofluorescein at 503 nm and 298 K was measured as $5.64 \pm 0.06 \times 10^4$ L mol^{-1} cm^{-1}.

As in the case of surface tension measurements, a break in a plot of dye solubility versus surfactant concentration indicates the concentration required for micelle formation. Here it is expected that, for concentrations below the cmc, dye solubility will be comparable to that in aqueous solution in the absence of surfactant, while above the cmc an enhancement in solubility will be observed. The cmc was determined from dye solubilization studies by finding the intersection between a horizontal line fit to solubilities below the cmc and a linear regression of data above the cmc. Propagation of error in this procedure leads to an estimate of the error in the cmc value, as was the case for the surface tension measurements discussed above.

To investigate the effect of pH on the cmc of the different sources of saponin, we prepared buffer solutions at different pH values. Buffer solutions at pH 3.0 and 4.4 were prepared by using acetic acid and sodium acetate. The concentrations of the sodium acetate for the different buffer solutions were kept low, less than 2 mM, in order to avoid any salt effects on the micellar properties of saponin. To determine whether such salt effects were indeed absent, at a given pH the concentrations of acetic acid and sodium acetate were varied, keeping the ratio of acid to salt constant. Such changes affected the cmc of the saponin solution by less than 5%, within an acceptable range of error. Saponin solutions were prepared fresh for each set of measurements, and surface tension measurements were done immediately after solutions were prepared. Moreover, the solutions were placed in sealed vials to avoid any acidic gas like CO_2 from dissolving in the water. For pH measurements at 8.8, we used sodium carbonate and sodium bicarbonate at salt concentrations less than 1 mM.

Further characterization of the surfactant and its aggregates was carried out using light scattering and viscosity measurements. The sizes of saponin micellar aggregates from various sources and under different aqueous phase conditions were measured by dynamic light scattering with a Microtrac 9230 ultrafine particle analyzer (Leeds and Northrup, St. Petersburg, FL) equipped with a water bath. The particle analyzer uses the principle of the fiber-optic Doppler anemometer (Hunter, 1986) to measure the diffusion coefficient D of colloidal particles. From this value, the known solution temperature T and solvent viscosity η_o, the instrument calculates the particle size from the Stokes−Einstein relation:

$$r = kT/6\pi\eta_o D \quad (1)$$

Here k is the Boltzmann constant and r is the radius of the particle. Errors in the measured values of r were obtained from the standard deviation of six to eight measurements on the same solution under various aqueous phase conditions. The viscosities of aqueous saponin solutions were measured above the cmc with an A83 KMAX size 50 Canon Fenske viscometer (Industrial Research Glassware Limited, Roselle, NJ) immersed in a temperature-controlled water bath. Viscosities η were determined from the relation $\eta/\eta_o = \rho_s t_s/\rho_o t_o$, where subscripts s and o indicate the aqueous saponin solution and pure solvent (doubly distilled water), respectively, t is the time of flow, and ρ represents the densities of the fluids. Fluid densities were measured independently.

RESULTS AND DISCUSSION

Measurements of surface tension, solubility and size as a function of aqueous surfactant concentration yield two quantities of obvious importance in evaluating a surfactant's performance as an emulsifier. First, surface tension measurements can be used to determine the concentration of surfactant at the interface and, in particular, to find the maximum amount of surfactant that can be attained per unit area of interface. Surfactant concentrations leading to such packed or saturated interfaces will be most effective in aiding the formation and stabilization of emulsions and foams. Further, surface tension, solubility, and size measurements can be used to identify the cmc, if one exists for a particular surfactant. Because the cmc signals the onset of micelle formation, surfactant concentrations above the cmc will have no further impact in decreasing the interfacial tension, and this critical concentration thus indicates a concentration limit for emulsifier effectiveness.

An understanding of the solubilization capabilities of a surfactant also directly hinges on the determination of the cmc and size of the micelle. Because of the hydrophobicity of the interior of the micelle, hydrophobic solutes experience a significant enhancement in solubility at surfactant concentrations above the cmc. Hence, a surfactant with a lower cmc will solubilize hydrophobic solutes at lower surfactant concentrations than one with a higher cmc. A micelle with a larger aggregation number generally has a better capacity to solubilize hydrophobic solutes (Mackay, 1987); the size of the micelle often also dictates its propensity to extract solutes.

Determination of the cmc. There exist various methods for the determination of the cmc for a surfactant. In the present study, surface tension measurements and dye solubilization studies were used to obtain the cmc of quillaja saponin. It is useful to employ more than one technique to determine the cmc, in order to establish the reliability of the values obtained, and to identify systematic differences originating with the technique itself. For example, dye solubilization sometimes depresses the cmc of a surfactant because it alters the hydrophobic environment within the micelle (Mackay, 1987).

Figure 2 shows the effects of aqueous saponin concentrations on the surface tension of the solution at 298 K. These results were obtained for three different commercial sources of the saponin. From the results in Figure 2, we determined the cmc values tabulated in Table 1, with the reported errors representing the standard deviation for these values. It is interesting to note that at 298 K quillaja saponin from Sigma exhibits a cmc value that is slightly but significantly (at the 99% confidence level) lower than that for the two other sources of saponin. The variations in the ability of the different sources of saponin to form micelles may be due to differences in molecular structures contained within the source. For example, one source of saponin could contain a higher proportion of glucose attached to the hydrophilic head group than those from another

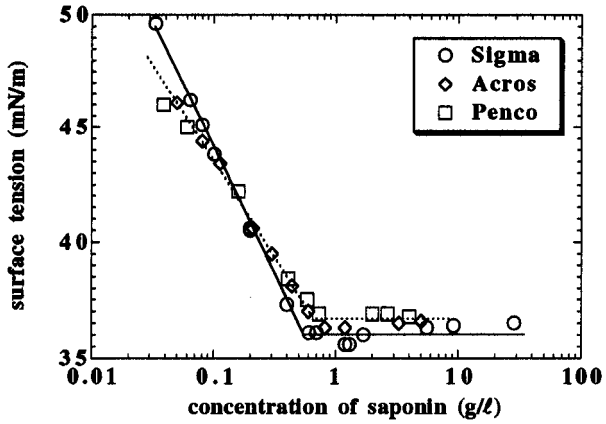

Figure 2. Effect of various sources of saponin concentration on the surface tension at 298 K.

Table 1. cmc's and Hydrodynamic Radii of Quillaja Saponin at Various Temperatures

	cmc (g/L)			hydrodyn radii (nm)	
T (K)	Sigma	Acros	Penco	Sigma	Acros
298	0.51 ± 0.04^a	0.70 ± 0.03^a	0.72 ± 0.05^a	3.6 ± 0.3	3.2 ± 0.3
	0.56 ± 0.04^b	0.74 ± 0.07^b	0.77 ± 0.04^b		
303				4.6 ± 0.3	3.5 ± 0.2
307[c]	0.76 ± 0.03	0.67 ± 0.03	0.35 ± 0.02	5.0 ± 0.3	3.7 ± 0.2
313	0.60 ± 0.03	1.25 ± 0.04	0.38 ± 0.03	5.8 ± 0.2	3.9 ± 0.1
325	0.80 ± 0.06	1.07 ± 0.04	0.85 ± 0.08		

[a] Determined from surface tension measurements. [b] Determined from dye solubilization measurements. [c] Hydrodynamic radii were obtained at 308 K.

source. This would change the hydrophobicity of the molecules and, thus, affect their tendency to form micelles. Industrial sources of quillaja saponin could also have impurities (not necessarily surface-active) such as salts, which would affect micelle-forming capabilities of the saponin molecules. Surface-active impurities are also a potential cause for differences in micelle-forming properties, and these if present would cause a minimum in surface tension curves. Figure 2 shows that the saponin obtained from Sigma demonstrates some influence of surface impurities. This latter effect is therefore the most likely explanation for the lower cmc value determined for this surfactant relative to the other two.

All three solutions clearly indicate a critical concentration above which aggregate formation is observed. These critical concentrations are comparable in magnitude and give us a good indication of the saponin concentrations required for micelle formation, regardless of source. Indeed, the clear break in surface tension values exhibited in Figure 2 supports the idea put forth by Oakenfull (1986) that quillaja saponins form *micelles*, as opposed to other types of aggregates which would not demonstrate such critical behavior.

The data for the air/water surface tension measured, using the Wilhelmy plate, as a function of surfactant concentration can be used to estimate the surfactant surface density (moles per area) at an air/water interface. Assuming a single solute in solution, the surface density Γ of surfactant can be determined from the Gibbs adsorption equation

$$\Gamma = -(1/RT)(\mathrm{d}\gamma/\mathrm{d} \ln c) \qquad (2)$$

where R is the gas constant (8.314 J/mol K), T is the absolute temperature (K), γ is the surface tension, and c is the surfactant concentration. Further, the area A per head group can be found from the surface density using the equation

$$A = 1/\Gamma N_{AV} \qquad (3)$$

where N_{AV} is the Avogadro constant (6.022×10^{23} mol^{-1}).

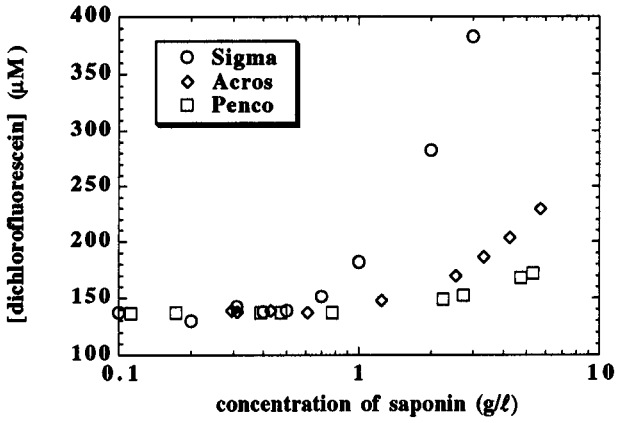

Figure 3. Solubility of 2′,7′-dichlorofluorescein as a function of saponin concentration at 298 K.

From the data in Figure 2 and eqs 2 and 3, the surface density for saponin from Sigma was found to be 0.2 nmol/cm^2. This value physically represents the amount of surfactant at the interface at saturation, since no more surfactant adsorbs to the surface above the cmc. This value for Γ yields for the area per head group a result of 83 Å^2. As a comparison, the area per head group of a typical nonionic surfactant like $C_{15}E_8$ is 45.2 Å^2 at 298 K (Meguro et al., 1987). Thus we see that the area per head group for quillaja saponin is significantly larger than that for more typical surfactants. Quillaja saponin has a number of sugar groups in its hydrophilic portion which may explain the reason for the anomalous size of the interfacial region occupied by its head group.

Figure 3 shows the solubility of dichlorofluorescein versus the logarithm of quillaja saponin concentration. The data in Figure 3 exhibit a break in the solubility at the cmc of the saponin, with enhancement of solubilization observed as micelles begin to form. The values of the cmc obtained from Figure 3 are reported in Table 1 and agree well with results obtained from surface tension measurements. This agreement suggests that incorporation of dichlorofluorescein within the micelle does not facilitate (or hinder) micelle formation. The agreement between these two very different methods for cmc determination, coupled with the enhanced dye solubility above the cmc, again provides strong evidence that quillaja saponin micelles form in solution above a well-defined critical concentration.

The results in Figure 3 indicate that the dye solubility increases monotonically above the cmc. By replotting the dye solubility above the cmc versus surfactant concentration on a linear scale, we find that the solubility increases linearly with surfactant concentration above the cmc, with different slopes for the three sources of saponin. Saponin obtained from Sigma solubilized ~40 mg of dye/g of saponin above the cmc, corresponding to about one molecule of dye for every six molecules of saponin. Saponin from Acros or Penco, on the other hand, incorporated only 1 molecule of dye for every 33 and 77 saponin molecules, respectively.

To compare the solubilization capacity of quillaja saponin with another, better characterized surfactant,

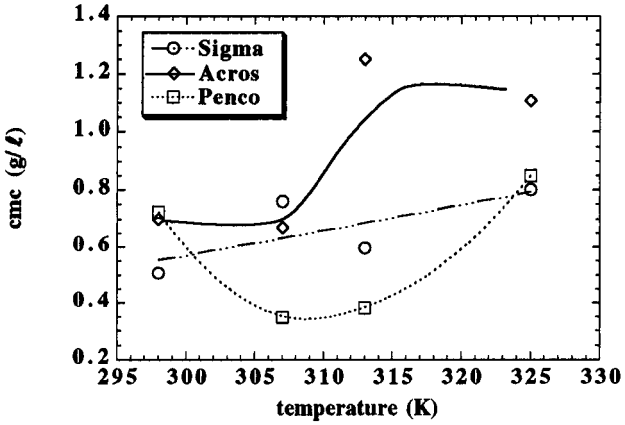

Figure 4. Effect of temperature on the cmc of aqueous saponin solutions.

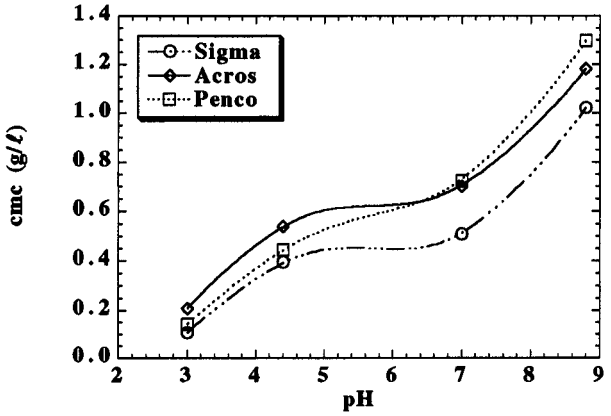

Figure 5. Effect of the pH of the aqueous phase on the cmc of saponin solutions at 298 K.

we also studied the solubilization of dichlorofluorescein in sodium dodecyl sulfate (SDS). As expected, the solubility of dichlorofluorescein in SDS below its cmc is comparable to that in water. After micelles form, it takes ~90 SDS molecules to solubilize 1 molecule of the dye. On a weight basis, this translates to ~15 mg of dye solubilized per gram of SDS added beyond its cmc. Thus, we see that dichlorofluorescein solubility in Acros or Penco saponin solutions is comparable to that in conventional SDS micellar solutions. Saponin from Sigma, however, has solubilization properties that clearly surpass these other surfactants.

Effect of Aqueous Phase Conditions on the cmc of Quillaja Saponin. Temperature usually plays a weak role in altering the cmc of a surfactant (Hunter, 1986). The cmc values of nonionic surfactants like C_nE_8 (n = 10−15) decrease with increased temperature (Meguro et al., 1987), while those of ionic surfactants usually increase with increased temperature. SDS actually exhibits a minimum near room temperature (Muller, 1993). The effect of temperature on the cmc of pure aqueous saponin solutions from various sources is shown in Figure 4, with cmc values of these sources at different temperatures tabulated in Table 1. The cmc values of saponin from Sigma and Acros tend to marginally increase with temperature. Consistent with the cmc behavior of some ionic surfactants (Muller, 1993), the cmc of saponin from Penco clearly exhibits a minimum with respect to temperature.

It follows from the presence of glucuronic acid in the quillaja saponin molecule that conditions for micelle formation could depend on the aqueous pH. As one varies the pH relative to the pK value(s) of the acid group(s) on a surfactant, the net charge on the head group will vary. The presence of charge then causes electrostatic repulsion between head groups, which tends to increase the cmc. The pK of glucuronic acid is 3.18 (Kortüm et al., 1961), and therefore, we expected that as the pH of the aqueous phase was increased above this value the cmc would increase also. From Figure 5, we find the cmc values of all sources of quillaja saponin increase monotonically with aqueous phase pH in the pH range of 3.0−8.8.

The increase in cmc was most significant at pH 8.8, the highest pH studied. Given the low pK for glucuronic acid in solution, this was a surprising observation, since the effect of pH on molecular charge should be strongest around the pK. Surfactants containing acid or base groups that exhibit such effects of pH on their micellization properties have received significantly less attention in the literature than the behavior of surfactant salts. However, studies indicate the influence of pH on micellar properties may be more complex than that predicted by simple variation of charge above the solution pK. First, the presence of charged surfactant within the micelle tends to promote counterion binding in order to reduce surfactant headgroup interactions (Lucassen-Reynders, 1981; Brackman and Engberts, 1989). As a result, the pK of the saponin micellar aggregate could be several units higher than the value for glucuronic acid in solution. Second, the fact that partial deprotonation occurs for most pH values means that we are really dealing with a mixture of surfactants, some charged and others uncharged, and those species may not participate equally in mixed micelle formation. The cmc of the surfactant dodecyldimethylamine oxide, which develops a positive charge as the pH is lowered, is most strongly affected at very low pH values, only as the molecules approach complete protonation (Brackman and Engberts, 1992; Chang et al., 1985; Herrmann, 1962; Ikeda et al., 1978, 1979). Finally, in the case of a natural mixture, such as quillaja saponin, the presence of acidic or basic impurities within the mixture could also influence the results. Interestingly, however, the results shown in Figure 5 differ very little between the three sources of surfactant, suggesting this latter effect is not a substantial one.

As with the pH effect, addition of a salt such as NaCl will significantly influence electrostatic interactions for charged surfactants, in this case by partially screening the electrostatic repulsion between head groups. As a result, cmc values for ionic surfactants are appreciably reduced in the presence of salt. For nonionics, higher salt concentrations increase the hydrophobicity of the surfactant, also resulting in a decreased cmc. In the case of quillaja saponin, we find that the cmc's of all the sources of saponin decrease notably with increasing NaCl concentration (Figure 6). The strength of the dependence on salt is somewhat weaker for all of the saponins than for typical ionic alkyl surfactants such as SDS or dodecyldimethylammonium chloride (Ikeda, 1984), particularly at ionic strengths below 0.2 M. Consistent with the discussion above, this observation most likely reflects a significantly lower percentage of charge in the micelle at this pH, relative to typical surfactant salt dissociation values.

Determination of the Size and Intrinsic Viscosity of Saponin Micelles. The effective hydrodynamic radii of the quillaja saponin aggregates were determined through dynamic light scattering. This technique measures the diffusion coefficient of the aggregate, which

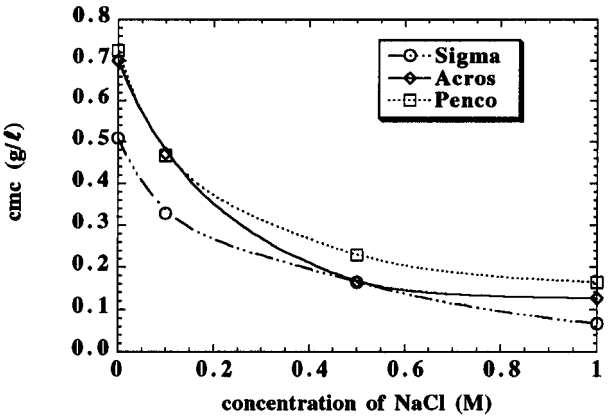

Figure 6. Effect of sodium chloride concentration on the cmc of saponin solutions at 298 K.

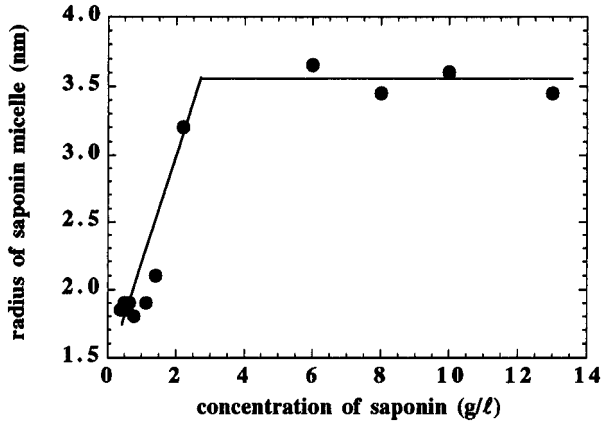

Figure 7. Effect of quillaja saponin (Sigma) concentration on the hydrodynamic radius at 298 K.

is then related to the micellar size through the Stokes–Einstein equation (eq 1). In reporting effective radii for the aggregates, it is necessary to make the assumption that the diffusion coefficients we measured represent values for isolated, spherical aggregates. In assuming that the aggregates are isolated, we are neglecting concentration effects on the diffusion coefficient. For hard-sphere or other repulsive interactions between particles, this would result in an overestimation of the micellar size. In addition, in assuming a spherical geometry for the micelle, the radius we report represents an "effective" hydrodynamic radius for the diffusing aggregate—a value that may differ from the geometric dimensions characterizing the particle. Despite these approximations, however, the measurements described below are useful for determining trends in aggregate size variations with surfactant concentration and aqueous solution conditions.

Figure 7 shows the effect of quillaja saponin concentration on the hydrodynamic radius at 298 K. At concentrations below 1 g/L of saponin, the radius of the particles detected is less than 2 nm, a value not distinguishable from the lower limit (1.9 nm) of the instrument. Thus, at these concentrations we conclude that any particles present are of a size we cannot detect. As we increased the concentration, we observed a clear increase in particle size, until we reached a radius between 3.4 and 3.7 nm at concentrations above 2.5 g/L. This increase can be attributed to the increasing presence of micellar aggregates as the concentration moves above the cmc. For comparison, Oakenfull (1986) reported a micellar radius of ~3 nm, although the concentration and temperature at which this value was obtained were not reported. Interestingly, the results shown in Figure 7 do not demonstrate any discernible concentration dependence of the size for concentrations above 2.5 g/L. This result indicates that there is no obvious increase in the reported size due to concentration effects, suggesting that the concentration effects discussed in the previous paragraph do not affect our results within experimental error. Average values for the aggregates' hydrodynamic radii at 298 K for concentrations above the cmc are reported in Table 1.

The aggregation number of the micelles can be estimated from the information gained from dynamic light scattering measurements. By modeling our solution as a dilute suspension of hard spheres, we may utilize Einstein's equation for the viscosity η of such a suspension:

$$\eta = \eta_o(1 + 2.5\phi) \tag{4}$$

where η_o is the viscosity of pure water and ϕ is the volume fraction of the particles. This model may be compared with the experimental measurement of the intrinsic viscosity $[\eta]$ of the solution, which represents the rate of change of viscosity with saponin concentration c in the dilute limit as

$$\eta = \lim_{c \to 0} \eta_o(1 + [\eta]c) \tag{5}$$

Recognizing the relation between micelle volume fraction ϕ and saponin concentration c, we obtain the following expression for the aggregation number N as a function of the measured hydrodynamic radius r and the intrinsic viscosity:

$$N = 10\pi N_{AV} r^3/(3[\eta]MW_s) \tag{6}$$

In eq 6, MW_s is the molecular weight of the individual surfactant molecule.

By measuring the viscosity of a series of dilute quillaja saponin solutions above the cmc at 298 K, we determined the intrinsic viscosity of our micellar solution at 298 K to be 3.67 ± 0.15 and 3.76 ± 0.11 mL/g for saponin from Sigma and Acros, respectively. Our measurements yield from eq 6 values of 49 (Sigma) and 33 (Acros) for the aggregation number of the saponin micelles, comparable to the value of ~50 given by Oakenfull (1986).

Effect of Aqueous Phase Conditions on the Size of the Saponin Micelles. Temperature plays an important role in the size of micelles. Micelles usually become larger as the temperature is increased (Ribeiro and Dennis, 1987), for reasons not well understood (Nishikido, 1990; Lindman and Wennerström, 1991). Figure 8 shows the effect of temperature on the micellar radius of quillaja saponin from Sigma and Acros, with results tabulated in Table 1. Both sources of saponin exhibited a systematic increase in size with temperature, consistent with trends generally found for other surfactants. It is important to recognize that these increases may also be attributed to a change in shape of the micellar aggregate, since in our measurements a nonspherical particle can appear to have a larger hydrodynamic radius than a sphere of equal volume.

Values for micellar radii for solutions prepared from quillaja saponin from Penco are not shown in Figure 8. Dynamic light scattering results on these solutions showed a broad distribution of particle sizes, including some particles in the nanometer-size range, but also

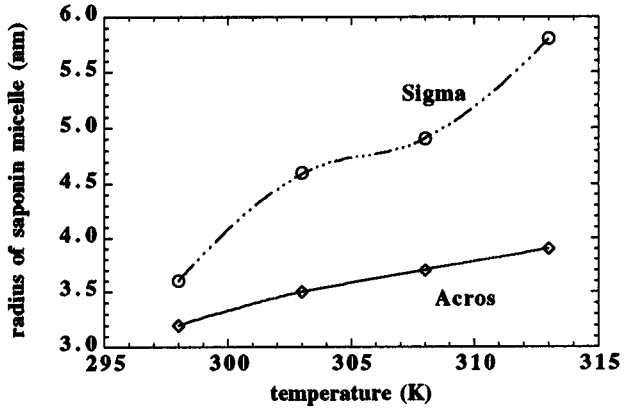

Figure 8. Micellar radii of saponins as a function of temperature.

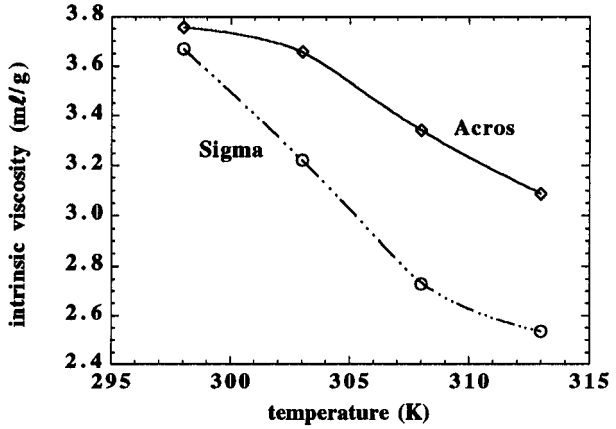

Figure 9. Effect of temperature on the intrinsic viscosity of saponin micelles.

indicated the presence of particles with a very large mean size, on the order of 0.1 μm. Although the detection of aggregates with sizes on the order of a few nanometers may indicate the presence of micelles within the Penco saponin solution, we believe the much larger submicrometer particles are unlikely to represent micellar assemblages. These latter particles may be due to the presence of insoluble components within this industrial-grade material, which coexist as large aggregates with the soluble micelle-forming quillaja saponin. However, given the strong scattering resulting from these larger aggregates, it was difficult to obtain quantitative sizes for micelles within the Penco saponin solutions. We note that such difficulties were completely absent in the scientific-grade saponin solutions from Sigma and Acros, in which the particle sizes were strikingly monodisperse and on the order of nanometers.

As seen in Figure 8, the effect of temperature was significantly larger for saponin obtained from Sigma than for the compound from Acros. This contrast is particularly interesting in light of the different solubilization properties of the two surfactants. It is possible that such differences may be traced to the presence of surface-active impurities, which were more prominent for the surfactant from Sigma. It has been noted that the addition of bile salts, which themselves form micelles, to saponin solutions enable saponin micelles to grow into rodlike micellar structures (Oakenfull, 1986). The bile salt surfactants are believed to reduce steric constraints present in the unmixed saponin micelles, allowing the formation of larger and more nonspherical mixed micelles. Additional surfactant present in the quillaja saponin from Sigma may give these micelles more freedom to grow in size with temperature and to incorporate solutes such as dichlorofluorescein. On the other hand, the Acros (and Penco) may contain nonsurface-active impurities which act to repress micelle growth and solute incorporation. We saw evidence of such impurities in the Penco samples, as noted above.

The intrinsic viscosity of dilute aqueous surfactant solutions has been observed to change with temperature, possibly due to the influence of temperature on both the shape and hydration of the micelle (Mandal et al., 1980; 1985). The intrinsic viscosity $[\eta]$ can be related to the "shape factor" ν and the hydration factor δ (grams of water bound per gram of micelle) as

$$[\eta] = \nu(\bar{v}_s + \delta\bar{v}_w) \tag{7}$$

where $\bar{v}_s$ and $\bar{v}_w$ are the partial specific volumes of surfactant and water, respectively. For a spherical particle, $\nu = 2.5$ (cf. eq 6). For a nonspherical particle, the shape factor is larger than 2.5 and will be a function of the axial ratio. Consistent with results for other surfactants (Mandal et al., 1980, 1985), Figure 9 shows that, for quillaja saponin solutions, the intrinsic viscosity does indeed demonstrate an influence of temperature, exhibiting a monotonic decrease in $[\eta]$ over the temperature range studied. As discussed below, such a decrease could be due to changes in values for ν and δ in eq 7. However, again the complex nature of the saponin mixture could also play a role, if the composition of the micelle changed significantly with temperature.

Assuming for the moment that the change in shape factor or composition of saponin micelles over the temperature range for our measurements is much less than the change in hydration of the bulk hydrophilic micellar head group with temperature, we take the derivative of eq 7 and obtain

$$\mathrm{d}[\eta]/\mathrm{d}T = \nu\bar{v}_w\mathrm{d}\delta/\mathrm{d}T \tag{8}$$

This equation requires the assumption that the partial specific volumes are independent of temperature, which is reasonable since the solutions are virtually incompressible. Thus, dehydration of micellar head groups with increasing temperature, corresponding to negative values of $\mathrm{d}\delta/\mathrm{d}T$, translates qualitatively to decreasing intrinsic viscosities with temperature. If the decrease in $[\eta]$ were attributable solely to dehydration with increasing temperature of spherical micellar aggregates, the data shown in Figure 9 for Sigma would predict well over a 50% decrease in the amount of water hydrating the micelle over the temperature range studied. Such a decrease in hydration is comparable to—although somewhat larger than—similar decreases reported for "pure" surfactants (Mandal et al., 1980, 1985). The extent of dehydration is less (~30%) for saponin obtained from Acros.

Alternatively, the decrease in $[\eta]$ with temperature shown in Figure 9 could be attributable to a decrease in the shape factor with increasing temperature. However, surfactant aggregates typically become less spherical as temperature increases, leading to an increase in shape factor as temperature goes up. Thus, assignment of the temperature effects shown in Figure 9 to a *decrease* in the shape factor seems unlikely.

In Figure 10 we used eq 6 to combine our information on the temperature dependence of the micellar radius and the intrinsic viscosity, enabling us to calculate the aggregation number of saponin micelles as a function

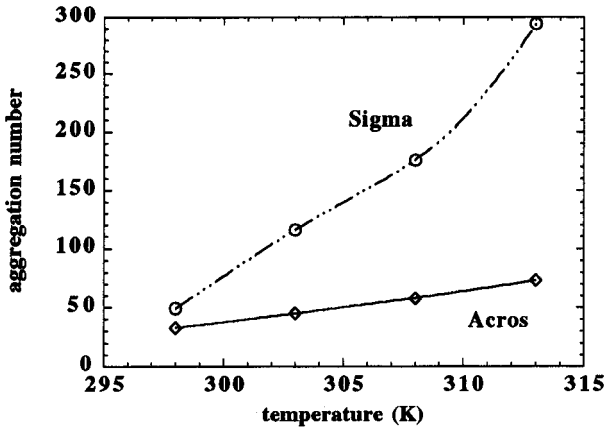

Figure 10. Change in aggregation number of saponin micelles with temperature.

of temperature. As seen from the figure, the aggregation number of quillaja saponin micelles increases monotonically in the temperature range of 298−313 K. The observed increase is a considerable one, but well within the range of increases observed for other surfactants such as alkyl poly(oxyethylene) nonionics (Balmbra et al., 1964). The observed increase in aggregation number with increasing temperature may be a direct result of the presence of more surfactant molecules in a micelle. It could also reflect a change in shape of the micelle: as mentioned above, a different shape would affect the "effective" hydrodynamic radius and, therefore, our calculated aggregation numbers. Such a change in micellar geometry, however, would also have to contribute to the observed changes in intrinsic viscosity, as discussed above.

Finally, we measured the hydrodynamic radii of saponin micelles as a function of salt concentration and pH. Interestingly, we observed that varying salt and pH values of the aqueous medium did not appear to significantly affect the micelle sizes of saponin from any of the sources. This result is in contrast to that observed for the cmc values of these surfactants, which showed a marked dependence on salt concentration and pH. Thus, the ease with which micelles form, as reflected through cmc values, is influenced by the presence of ions within solution and/or within the micelles, while the radii of the aggregates formed is relatively independent of these effects.

Understanding the micelle-forming properties of quillaja saponin is essential for predicting the functionality of this surfactant in stabilizing emulsions and foams and for interacting with other food components. Below the cmc, addition of this surfactant helps to reduce interfacial tensions in foods and thus promotes the formation of emulsions and foams. Like all surfactants, the efficacy of quillaja saponin in this regard is limited by the amount of surfactant that can be adsorbed to a saturated interface. This saturation concentration can be estimated from surface tension measurements as discussed above.

Above the cmc, little effect of additional surfactant on interfacial energies will be observed, while the presence of aqueous micelles can have complex influences on emulsion and foam stability (Nikolov and Wasan, 1989; Nikolov et al., 1989; McClements and Dungan, 1993, 1995, 1997). In addition, the micellar properties of these saponins are closely tied to the ability to solubilize, and hence extract, hydrophobic solutes such as cholesterol. Factors that lower the cmc, such as lower temperatures, higher salt concentrations, and lower pH values, make it easier to form micelles with less of the surfactant. Factors that increase the aggregation number of the micelles, on the other hand, tend to increase the capacity of the micellar solution for solute extraction. Increasing temperature or salt concentration would have this latter effect. Hence, the knowledge gained from these studies of quillaja saponin solutions will be very useful for developing various micellar extraction and purification processes in food and other technologies and for understanding the functioning of these biological surfactants *in vivo*.

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