Isothermal Titration Calorimetry Study of Pectin−Ionic Surfactant Interactions

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Isothermal titration calorimetry (ITC) was used to measure enthalpy changes resulting from injection of anionic (sodium dodecyl sulfate, SDS) or cationic (dodecyl trimethylammonium bromide, DTAB) surfactants into aqueous 1 wt % pectin solutions (30, 60, or 90% methoxylated). In the absence of pectin, the critical micelle concentrations (cmc) determined by ITC were 14.7 mM for DTAB and 7.7 mM for SDS. Binding of DTAB to pectin was endothermic and was attributed to electrostatic attraction between the cationic surfactant and anionic biopolymer. Binding of SDS to pectin was exothermic and was attributed to hydrophobic interactions. Pectin reduced the cmc of SDS, probably because of long-range electrostatic repulsion between the molecules. Above a particular concentration, which depended on pectin and surfactant type, both ionic surfactants promoted pectin aggregation (monitored by turbidity increase). This study demonstrates the potential of ITC for providing valuable information about interactions between polysaccharides and amphiphiles.

Keywords: *Isothermal titration calorimetry; pectin; SDS; DTAB; binding*

INTRODUCTION

Interactions between biopolymers and small amphiphilic molecules are important in many food processes and materials. The binding of bile acids to water-soluble dietary fibers in the small intestine has been proposed as one of the major mechanisms responsible for the ability of fibers to reduce cholesterol and colon cancer (Lairon, 1996; Potty, 1996; Dongowski, 1997; Jenkins et al., 1995, 1998; Holt, 1999). The conformation and aggregation of biopolymers in aqueous solutions are altered by surfactants, which leads to changes in the stability, rheology, and appearance of the solution (Goddard, 1993a−c; Biswas and Chattoraj, 1997a,b; Caram-Lelham et al., 1997; Merta and Stenius, 1997; Maulik et al., 1998; Waninge et al., 1998; Kastner and Zana, 1999). The physicochemical properties of protein-stabilized oil-in-water emulsions are strongly influenced by protein−surfactant interactions at the droplet surfaces (Dalgleish et al., 1995; Dickinson et al., 1996; Cornec et al., 1998; Demetriades and McClements, 1998; 2000; Dickinson, 1999; Dickinson and Ritzoulis, 2000). Biopolymers may also interact with other small amphiphilic molecules present in foods, such as flavors, antioxidants, pro-oxidants, and preservatives (Baines and Morris, 1989; Kinsella and Whitehead, 1989; Bakker, 1995; Gaonkar, 1995; Boudaud and Dumont, 1996; Guichard, 1996; Donnelly et al., 1998). An improved understanding of the origin and nature of these interactions would lead to the design of foods with improved nutritional, physicochemical, and sensory properties.

When amphiphilic molecules are mixed with a solution of polymer molecules, they may exist in either a free or a bound form (Goddard, 1993a,b; Lindman and Thalberg, 1993). In either of these forms the amphiphiles may be present as individual molecules or as molecular clusters (e.g., micelles). The partitioning of amphiphilic molecules between different locations depends on the concentration and molecular characteristics of the polymer and amphiphile, as well as on prevailing environmental conditions such as temperature, pressure, and solvent composition (Goddard and Ananthapadmanabhan, 1993). A variety of physicochemical mechanisms may either favor or oppose binding, including electrostatic interactions, hydrophobic interactions, hydrogen bonding, and configurational entropy (Israelachvili, 1992; Lindman and Thalberg, 1993; Bergethon, 1998; Singh and Caram-Lelham, 1998). The relative importance of these mechanisms depends on the precise nature of the polymer−amphiphile system and usually has to be established experimentally.

A wide variety of analytical techniques are available to study biopolymer−amphiphile interactions, including equilibrium dialysis, electrical conductivity, chromatography, electrophoresis, ultracentrifugation, calorimetric, surface tension, dye-binding, viscometry, surfactant specific electrode, spectroscopic, and scattering techniques (Goddard, 1993a,b; Lindman and Thalberg, 1993). In this study, the potential of isothermal titration calorimetry (ITC) for studying interactions between ionic surfactants and an anionic polysaccharide (pectin) was investigated. ITC measures the heat absorbed or evolved when one solution is titrated into another solution (Ladbury and Chowdry, 1998; Blandameer et al., 1998). Previous studies have shown that it is an extremely valuable tool for studying polymer−amphiphile interactions because it provides data about binding enthalpies, critical aggregation concentrations, and binding stoichemistries (Fox et al., 1998; Ghoreishi et al., 1999; Kevelam et al., 1996; Singh and Nilsson, 1999a,b; Wang et al., 1997, 1998). These data can be used to provide valuable insights into the origin and nature of polymer−amphiphile interactions and the factors that influence them.

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Table 1. Properties of Pectin Samples Used in This Study (Provided by Sigma Chemical Co.)

	pectin (esterified, K salt)	pectin (esterified, K salt)	pectin (esterified)
product no.	P311	P9436	P9561
loss on drying (%)	18.5	11.9	11.9
galacturonic acid (%)	66	74	82
methoxy groups (%)	2.8	8.0	12
degree of esterification (%)	26	67	89

MATERIALS AND METHODS

Materials. Pectin samples with different degrees of methoxylation (~30, ~60, and ~90%) were obtained from Sigma Chemical Co. (St. Louis, MO). These samples are subsequently referred to as P30%, P60%, and P90%, respectively. The moisture content, galacturonic acid content, percentage methoxy groups, and degree of esterification (DE) of the pectin samples were determined by the manufacturer (Table 1). Analytical grade dodecyl trimethyl amonium bromide (DTAB) and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. Distilled and deionized water was used for the preparation of all solutions.

Solution Preparation. Pectin solutions (1 wt %) were prepared by dispersing powdered pectin into distilled water and stirring for at least 2 h. Surfactant solutions (SDS or DTAB) were prepared by dispersing powdered surfactant into distilled water and stirring for at least 2 h.

Isothermal Titration Calorimetry. An isothermal titration calorimeter (VP-ITC, Microcal Inc., Northampton, MA) was used to measure enthalpies of mixing at 30.0 °C. Twenty-nine 10 μL aliquots of surfactant solution were injected sequentially into a 1480 μL titration cell initially containing either distilled water or 1 wt % pectin solution. Each injection lasted 20 s, and there was an interval of 300 s between successive injections. The solution in the titration cell was stirred at a speed of 315 revolutions min^{-1} throughout the experiments.

Turbidity and pH Titrations. Aliquots (200 μL) of surfactant solution were injected at 3 min intervals into a glass vial initially containing 14.5 g of 1 wt % pectin solution. Pectin solutions were stirred throughout the experiment using a magnetic stirrer. Two minutes after each injection, the pH (pH meter 320, Corning Inc., Corning, NY) and turbidity at 600 nm (Spectronic 21D, Milton Roy, Rochester, NY) of the pectin solutions were measured. Measurements were carried out in duplicate.

RESULTS AND DISCUSSION

Determination of Surfactant Critical Micelle Concentration. The cmc of the surfactants was determined using ITC by measuring the enthalpy change resulting from their injection into water. Heat flow versus time profiles resulting from sequential injections of 10 μL aliquots of surfactant solution (90 mM SDS or 150 mM DTAB) into a 1480 μL titration cell initially containing water were measured. The measurements for the DTAB system are shown in Figure 1. The surfactant concentrations in the injector were appreciably above the cmc, so that the injector contained a mixture of micelles and monomers. Initially, a series of relatively large endothermic peaks was observed when the surfactant solutions were injected into the reaction cell. These enthalpy changes are the result of micelle dissociation because the surfactant concentration in the reaction cell was initially below the cmc (Bijma et al., 1997). The endothermic nature of these peaks ($\Delta H > 0$) indicates that demicellization must lead to an increase in the overall entropy of the system, because

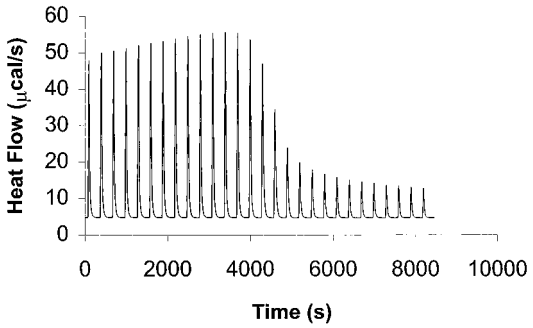

Figure 1. Heat flow versus time profiles resulting from injection of 10 μL aliquots of 150 mM DTAB into a 1480 μL titration cell initially containing water at 30.0 °C.

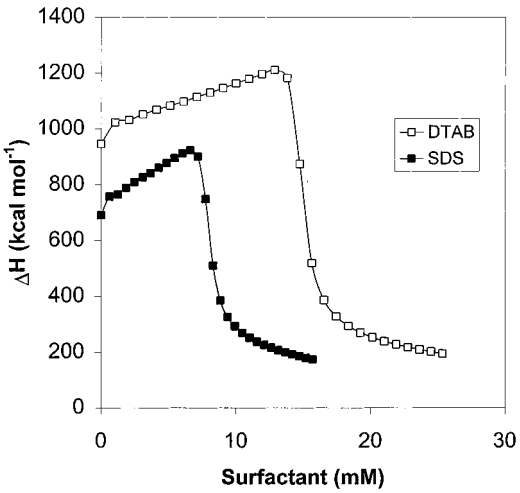

Figure 2. Dependence of enthalpy change per mole of surfactant on the surfactant concentration in the reaction cell for DTAB and SDS injected into water.

micelle dissociation is thermodynamically favorable below the cmc ($\Delta G < 0$); therefore, $T\Delta S > \Delta H$. This entropy increase has been attributed to the release of counterions associated with the surfactant headgroups when micelles break down to monomers (Bijima et al., 1997). After a certain number of injections, there was an appreciable decrease in peak height because the surfactant concentration in the reaction cell exceeded the cmc and so the micelles titrated into the reaction cell no longer dissociated. Above the cmc the enthalpy change is therefore solely the result of micelle dilution effects (Bijma et al., 1997). The dependence of the enthalpy change per mole of surfactant (ΔH_i) injected into the reaction cell on the surfactant concentration in the reaction cell was calculated by integration of the heat flow versus time profiles (Figure 2). The cmc of the surfactants was determined from the inflection point in the ΔH_i versus surfactant concentration curves as 14.7 $\pm$ 0.9 mM for DTAB and 7.7 $\pm$ 0.6 mM for SDS. These values are in excellent agreement with those determined for the same surfactants by other researchers. For example, previous studies have shown that at 30 °C the cmc of SDS falls in the range 7.4−8.4 mM (Ananthapadmanabhan, 1993; Singh and Nilsson, 1999a), whereas that of DTAB has been measured as 15 mM (Kastner and Zana, 1999) and as 15.1 mM (Meagher et al., 1998).

Influence of DTAB—Pectin Interactions on Enthalpy Changes. The enthalpy change resulting from injection of 150 mM DTAB solution into 1 wt % pectin solutions was measured using ITC. Pectin molecules

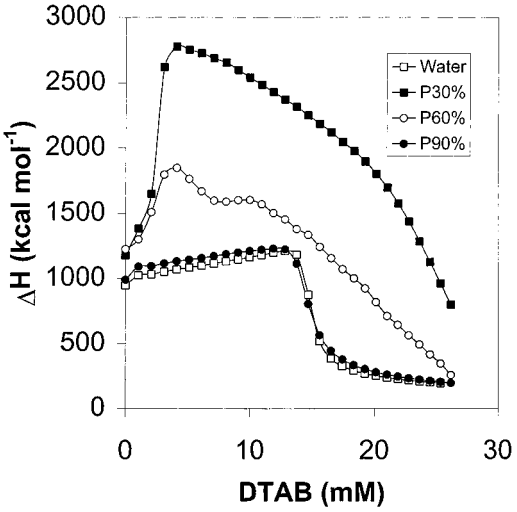

Figure 3. Dependence of enthalpy change per mole of surfactant on the surfactant concentration in the reaction cell for 150 mM DTAB injected into water and 1 wt % pectin solutions. The concentration of surfactant in the injector was initially above the cmc.

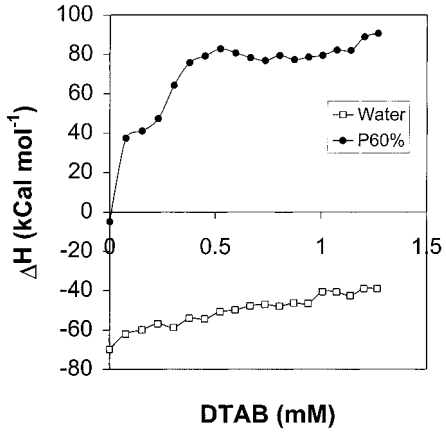

Figure 4. Dependence of enthalpy change per mole of surfactant on the surfactant concentration in the reaction cell for 7.5 mM DTAB injected into water and a 1 wt % pectin solution. The concentration of surfactant in the injector was initially below the cmc.

with different degrees of methoxylation (~30, 60, and 90%) were used to facilitate identification of the origin of the surfactant−pectin interactions. The dependence of the enthalpy change per mole of surfactant injected into the reaction cell on the surfactant concentration in the reaction cell was determined by integration of the measured heat flow versus time profiles (Figure 3). The presence of pectin in the reaction cell caused an appreciable change in the enthalpy−surfactant concentration profile. The enthalpy change when DTAB was injected into the reaction cell was considerably more endothermic when pectin was present. The magnitude of this effect increased as the degree of methoxylation of pectin decreased, that is, as the pectin molecules became more negatively charged. The most likely origin of this interaction is electrostatic attraction between the positively charged DTAB molecules and the negatively charged carboxyl groups on the pectin molecules (Goddard, 1993b; Lindman and Thalberg, 1993). P90% has relatively few negatively charged groups, and therefore there was little electrostatic attraction between the DTAB and pectin molecules. There was a significant increase in ΔH_i for P30% and P60% solutions when the DTAB concentration increased from 0 to 4 mM, after which ΔH_i decreased toward the value in the absence of pectin. A number of different physicochemical mechanisms may contribute to the observed enthalpy changes, including (a) dissociation of micelles, (b) changes in surfactant−surfactant, polymer−polymer, or surfactant−polymer interactions, (c) changes in polymer conformation, and (d) changes in hydration or counterion distribution. One of the major limitations of the ITC technique is that it measures only the overall enthalpy change of a system and it is not possible to directly isolate the contribution of different mechanisms. Ideally, the technique should therefore be used in combination with techniques that provide complementary information about the system, for example, the number, location, and organization of surfactant and counterions bound to the polymer and the conformation and association of the polymer molecules. Nevertheless, it is often possible to manipulate the experimental conditions to isolate one or more contributions. For example, the contribution of micelle dissociation to the enthalpy can be removed by injecting surfactant monomers (rather than micelles) into a polymer solution. This was achieved by injecting a 7.5 mM DTAB solution (i.e., [surfactant] < cmc) into a 1 wt % P60% solution. The ΔH_i for injection of DTAB monomers into pure water was exothermic, but the ΔH_i for injection into the pectin solution was endothermic (Figure 4). This observation is in agreement with the net increase in enthalpy observed when DTAB micelles were injected into pectin solutions rather than water (Figure 3). The fact that the binding reaction was endothermic indicates that there must be an increase in the overall entropy of the system so that the overall free energy change is favorable ($\Delta G < 0$, so $T\Delta S > \Delta H$). This entropy increase may come from the release of counterions originally associated with the headgroups of the surfactant molecules and the carboxyl groups of the pectin.

The fact that the endothermic enthalpy change resulting from the first injection of DTAB into the pectin solution was greater than that resulting from the first injection of DTAB into water suggests that the critical aggregation concentration (cac) was below the lowest surfactant concentration studied (i.e., <0.1 mM DTAB). This finding is in agreement with previous studies that have found that the cac of a surfactant may be many orders of magnitude below its cmc (Lindman and Thalberg, 1993). The higher ΔH_i for injection of DTAB into P30% and P60% solutions than for injection of DTAB into water (Figure 3) suggests that the surfactant binds to the biopolymer across the whole range of DTAB concentrations used (0−26 mM). However, the sharp increase in ΔH_i from 0 to 4 mM DTAB, followed by the decrease at higher surfactant concentrations, suggests that the nature of the binding is different above and below 4 mM DTAB. At relatively low surfactant concentrations it is likely that a few isolated monomers or micelles bind to the polymer without changing its overall conformation significantly. At relatively high surfactant concentrations the pectin molecules may change their conformation so that the polymer chains wrap around surfactant micelles, thus optimizing the electrostatic interactions between the positively charged headgroups and the negatively charged carboxyl groups (Figure 5). The polymer chains surrounding a micelle may be from a single pectin molecule or they might be from two different pectin molecules so that the micelle forms a "salt-bridge" between them. The formation of

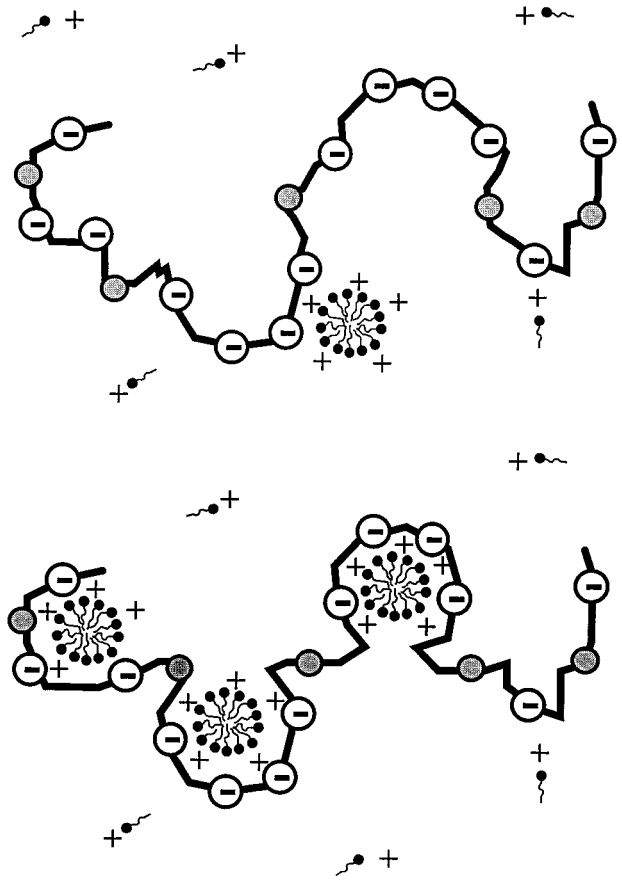

Figure 5. Schematic representation of the interaction between DTAB micelles and anionic polysaccharide.

this type of surfactant−polymer complex is supported by experimental evidence from a variety of analytical techniques (Goddard, 1993b; Lindman and Thalberg, 1993). The molar ratio of DTAB to sugar monomers in the pectin varied from 0 to 0.57 during the titration experiment, which corresponds to final molar ratios of DTAB/carboxyl groups of 0.77, 1.7, and 5.2 for P30%, P60%, and P90%, respectively. At the highest surfactant concentrations, the ΔH_i for injection of DTAB into P30% and P60% solutions tended toward the value for injection of DTAB into water (Figure 3), which suggests that the negative groups on the pectin molecules may have become saturated with DTAB around a molar ratio of 1:1. If the surfactant/carboxyl group ratio is 1:1, then ITC may prove to be a useful method of quantifying the number of carboxyl groups on pectin molecules, provided the solution conditions are controlled so that all of the carboxyl groups are ionized.

Influence of DTAB on pH and Turbidity of Pectin Solutions. The pH and turbidity of 1 wt % pectin solutions (P30%, P60%, and P90%) were measured as a 150 mM DTAB solution was titrated into them (Figures 6 and 7). The initial pH of the pectin solutions decreased as the degree of methoxylation increased, being about 5.7, 4.8, and 2.9 for the P30%, P60%, and P90%, respectively. The pH of the P90% solution was relatively insensitive to DTAB concentration, but there was a slight decrease in the pH of the P30% and P60% solutions with increasing surfactant concentration (Figure 6). The p*K* value for pectin is 3.5, and therefore the pH decreases may have caused some protonation of the charged groups on the pectin molecules as the DTAB concentration increased. Nevertheless, the pH was always >1 above the p*K* value, which suggests that any changes in protonation would be small, especially for P30%.

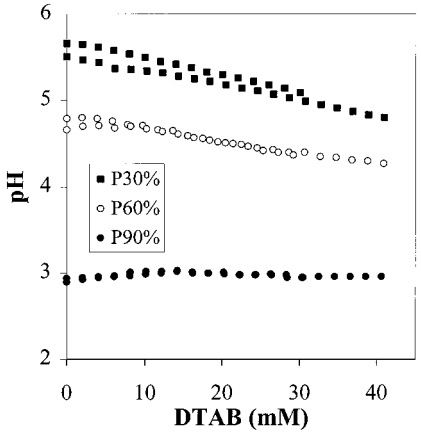

Figure 6. Dependence of pH on surfactant concentration for 150 mM DTAB titrated into 1 wt % pectin solutions.

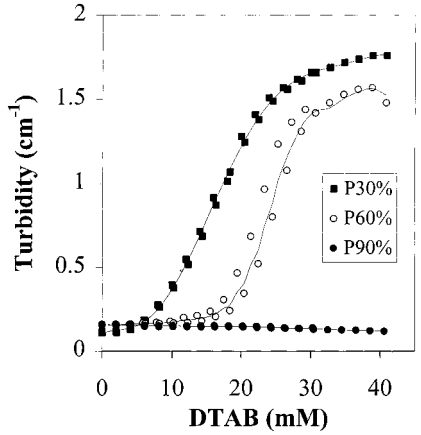

Figure 7. Dependence of turbidity on surfactant concentration for 150 mM DTAB titrated into 1 wt % pectin solutions.

The change in turbidity of the pectin solutions with DTAB concentration depended strongly on the degree of pectin methoxylation (Figure 7). The turbidity of the P90% solution did not change throughout the course of the titration, indicating that DTAB had little influence on the aggregation of the pectin molecules. This was to be expected because the ITC experiments showed that there was little interaction between DTAB and P90% (Figure 3). On the other hand, there was an appreciable increase in turbidity for the P30% and P60% solutions once the DTAB concentration exceeded a particular level (Figure 7). The concentration at which the turbidity increase occurred was lower for the pectin with the highest negative charge (P30%), which suggests that the origin of the polymer aggregation was not charge neutralization. We suspect that the observed increase in turbidity is due to formation of positively charged surfactant micelles that act as salt bridges between negatively charged pectin molecules. As the charge density of the pectin increases, the amount of DTAB required to form these salt bridges decreases.

Influence of SDS−Pectin Interactions on Enthalpy Changes. The enthalpy changes resulting from the injection of a 90 mM SDS solution into 1 wt % pectin solutions (P30%, P60%, and P90%) was measured using ITC (Figure 8). The presence of pectin in the reaction cell had a pronounced influence on the enthalpy−surfactant profiles, with the magnitude of the effect

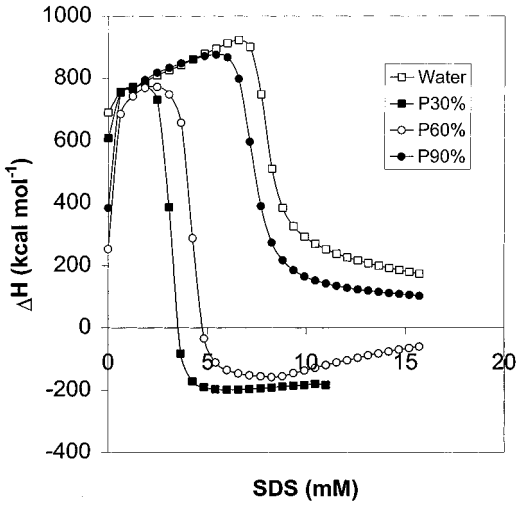

Figure 8. Dependence of enthalpy change per mole of surfactant on the surfactant concentration in the reaction cell for 90 mM SDS injected into water and 1 wt % pectin solutions. The concentration of surfactant in the injector was initially above the cmc.

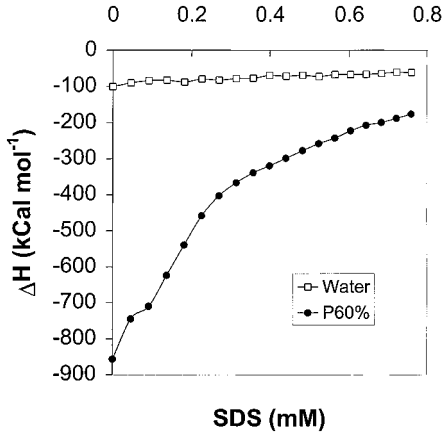

Figure 9. Dependence of enthalpy change per mole of surfactant on the surfactant concentration in the reaction cell for 4.5 mM SDS injected into water and a 1 wt % pectin solution. The concentration of surfactant in the injector was initially below the cmc.

depending on the degree of pectin methoxylation. Initially, this seemed to be surprising because both SDS and pectin are negatively charged, and therefore one might not have expected surfactant binding due to electrostatic repulsion. Compared to the titration of SDS into water, the presence of pectin in the reaction cell caused a reduction in ΔH_i at low surfactant concentrations (<1 mM SDS). In addition, the sharp decrease in enthalpy observed at the cmc of SDS when it was injected into water occurred at lower surfactant concentrations when pectin was present. This effect increased as the degree of methoxylation of the pectin molecules decreased, that is, as they became more negative. It was postulated that this effect was due to a lowering of the cmc of SDS in the presence of pectin. There are a number of possible reasons for this reduction in cmc. First, salts are known to lower the cmc of ionic surfactants (Flockhart, 1961), and therefore any counterions associated with the pectin molecules may have caused a reduction of the cmc. This effect would be expected to increase as the degree of pectin methoxylation decreased because the concentration of counterions associated with the pectin molecules would increase. A second possible mechanism for the lowering of the cmc is the change in counterion type associated with the surfactant micelles. The potassium salt of dodecyl sulfate has a lower cmc than the sodium salt (Ananthapadmanabhan, 1993), and therefore the cmc of the surfactant may have been reduced because potassium ions associated with the pectin exchanged with sodium ions associated with the SDS micelles. A third possible mechanism for the lowering of the cmc is the ability of highly charged polyelectrolytes to greatly reduce the Debye screening length of aqueous solutions (Waninge et al., 1998). When SDS is injected into a solution containing pectin, there is an electrostatic repulsion between the negatively charged surfactant molecules and the negatively charged pectin molecules. By forming micelles, the surfactant molecules are able to reduce the average distance between the pectin and surfactant molecules, which makes micelle formation more thermodynamically favorable, that is, lowers the cmc. Further studies are needed to establish the precise mechanism responsible for the lowering of the surfactant's cmc by pectin.

The ΔH_i at low surfactant concentrations (<1 mM SDS) was lower for SDS injected into pectin solutions than into water, which suggests that an exothermic process partly compensated for the endothermic dissociation of micelles. It was postulated that this exothermic process was due to binding of surfactant to pectin. To examine this effect, the enthalpy change resulting from injection of surfactant monomers (4.5 mM SDS) into a 1 wt % P60% solution was measured. Injection of monomers was achieved by using a surfactant concentration in the injector that was below the cmc of SDS. The ΔH_i for injection of SDS monomers into the pectin solution was considerably more exothermic than for injection into water (Figure 9), which suggests that the monomers interacted with the biopolymer molecules. The exothermic nature of the enthalpy change suggests that the SDS molecules may have formed micelle-like clusters upon binding to the pectin, because cluster formation is the process opposite to micelle dissociation, which is endothermic (Figure 2). This observation is consistent with studies of the binding of ionic surfactants to various types of uncharged polymer, where it is proposed that the nonpolar tails of the surfactant molecules bind cooperatively to hydrophobic regions of the polymer (Wang and Olofsson, 1998; Kevelam et al., 1996; Singh and Nilsson, 1999a,b). A number of studies have shown that anionic surfactants bind more strongly to uncharged polymers than cationic surfactants (Wang and Olofsson, 1998). This may explain why SDS interacted more strongly than DTAB with the pectin with the lowest negative charge (P90%) (Figures 3 and 8).

Influence of SDS on pH and Turbidity of Pectin Solutions. The pH and turbidity of 1 wt % pectin solutions (P30%, P60%, and P90%) were measured as a 90 mM SDS solution was titrated into them (Figures 10 and 11). There was little change in the pH of any of the pectin solutions as the SDS concentration was increased (Figure 10), which suggests that protonation/deprotonation of the carboxyl groups would not have been significant during the ITC experiments. The change in turbidity of the pectin solutions with SDS concentration depended strongly on the degree of pectin methoxylation (Figure 11). The turbidity of the P90% solution did not change throughout the course of the

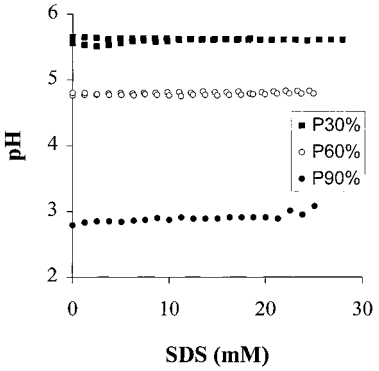

Figure 10. Dependence of pH on surfactant concentration for 90 mM SDS titrated into 1 wt % pectin solutions.

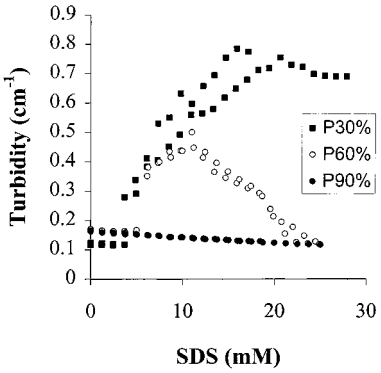

Figure 11. Dependence of turbidity on surfactant concentration for 90 mM SDS titrated into 1 wt % pectin solutions.

titration, indicating that SDS did not promote significant pectin aggregation. This was to be expected because the ITC experiments showed that there was little interaction between SDS and P90% (Figure 8). On the other hand, there was an appreciable increase in turbidity for the P30% and P60% solutions once the SDS concentration exceeded a particular level (Figure 11). The surfactant concentration at which the turbidity increase first occurred was lower for P30% (~4 mM SDS) than for P60% (~6 mM SDS). These surfactant concentrations are slightly higher than the concentrations at which micelles formed in the aqueous phase: ~3 mM SDS for P30% and ~4 mM SDS for P60% (Figure 8). This observation suggests that surfactant micelles are required in the aqueous phase to promote pectin aggregation. It was interesting that the turbidity of the P60% solution increased up to a maximum value around 10 mM SDS, after which it decreased, whereas the turbidity of the P30% solution continued to increase up to ~20 mM SDS and then remained relatively constant (Figure 11). The turbid pectin−SDS solutions had a silvery white anisotropic appearance similar to certain kinds of shampoo. These results indicate that SDS promoted the formation of microphases in the pectin solutions that were large enough to scatter light.

It is postulated that the formation of the microphases was due to phase separation of the similarly charged polymer molecules and surfactant micelles, in agreement with the mechanism proposed by other researchers (Lindman and Thalberg, 1993). The origin of this effect is that micelle−polymer interactions are less favorable than the average of the micelle−micelle and polymer−polymer interactions, so the system adopts a phase-separated configuration that minimizes the unfavorable interactions (Tirrell, 1993). It should be noted that the microphases form as the result of separation of the system into "polymer-rich" and "polymer-depleted" phases, rather than due to formation of insoluble polysaccharide−surfactant complexes (as is the case for oppositely charged species, such as DTAB and pectin). When the SDS concentration is increased, the Debye screening length of the aqueous phase is reduced because the micelles act as strong polyions (Tirrell, 1993). As a result, the electrostatic repulsion between the pectin and SDS micelles is screened. In the case of the P60% solution, the reduction in electrostatic repulsion between polymer and surfactant is large enough to prevent phase separation from occurring. On the other hand, the charge density of the P30% molecules is appreciably greater than that on the P60% molecules, and therefore a higher SDS concentration would be required to reduce the Debye screening length to a point at which electrostatic repulsion interactions were insufficient to promote phase separation.

Conclusions. This study has shown that ITC can provide valuable information about interactions between pectin and ionic surfactants. The technique was able to detect enthalpy changes associated with micelle formation and with binding of surfactant molecules to biopolymers. Binding of DTAB to pectin was attributed to electrostatic attraction between the cationic surfactant and the anionic pectin, whereas binding of SDS to pectin was attributed to hydrophobic interactions between the nonpolar tail of the surfactant and methoxyl groups. Both types of surfactant promoted aggregation of pectin molecules above a particular concentration, probably through charge neutralization and cross-linking via surfactant micelles.

A more thorough understanding of the origin and nature of pectin−surfactant interactions would require combining data obtained using ITC with that obtained using complementary analytical techniques. Ideally, one would like to know the number of surfactant molecules and counterions bound per polymer, whether the surfactant binds as monomers or micelles, the binding strength, and changes in the polymer conformation and aggregation. This type of information would require the use of a wide variety of analytical techniques, such as equilibrium dialysis, surfactant specific electrodes, NMR, scattering, spectroscopy, chromatography, electrophoresis, and viscometry techniques.

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