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# Characterization of Molecular interactions between Chitosan and Sodium Dodecyl Sulfate (SDS)

**[1]G Geetha [2]C Suresh Kumar and [3]N Devanna**

[*1]Department of Biochemistry, Oil Technological Research Institute. Jawaharlal Nehru Technological University Anantapur, Anantapur 515001 India.
[2]Department of Biochemistry, Sri Krishnadevaraya University, Anantapur 515003, India.
[3]Department of Chemistry, Oil Technological Research Institute, Jawaharlal Nehru Technological University Anantapur, Anantapur 515001 India.

***Corresponding author:** Geetha G

Email: ggeetha552@gmail.com

Phone: +919731605363

**Abstract:**

Chitin is the next most abundant natural polysaccharide after cellulose. It can be found in shells of shrimps, crabs and crayfish, which are waste products in food industry. Chitosan is becoming popular in an increasing number of industrial applications due to its availability, low cost and biodegradability. Many of these properties depend on its ability to interact with anionic surface active molecules, such as phospholipids, surfactants, and bile acids. The purpose of this study was to characterize the interaction between chitosan and a model anionic surfactant (sodium dodecyl sulfate, SDS) using isothermal titration calorimetry (ITC), surfactant-selective electrode (SSE), and turbidity measurements. ITC and SSE indicated that SDS bound strongly to chitosan via a highly exothermic interaction. The turbidity measurements indicated that chitosan formed insoluble complexes with SDS that strongly scattered light. This study provides information about the origin and characteristics of molecular interactions between chitosan and anionic surface-active lipids that may be useful for the rational design of chitosan-based food ingredients with specific nutritional and functional characteristics, e.g., cholesterol lowering or fat replacement.

**Keywords: Chitosan, SDS, Interaction, Isothermal Titration Calorimetry, Surfactant-Selective Electrode**

## Introduction:

Chitosan is a cationic biopolymer that has many potential applications in the food, cosmetics, and pharmaceutical industries because of its unique nutritional and physiochemical properties (1-6). Previous research has shown that chitosan can be used for heavy metal chelation, wastewater treatment, cholesterol lowering, texture modification, emulsion stabiliza- tion, etc. (3-8). Many of these applications depend on interactions between chitosan and anionic surface-active

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materials, e.g., phospholipids, small molecule surfactants, or bile acids. The rational application of chitosan in the food industry depends on understanding the origin and nature of these interactions.

Chitosan is a (1-4)-linked 2-amino-2-deoxy-â-D-glucan derived from fully or partially deacetylated chitin (1). It has three types of reactive functional groups: an amino group at the C-2 position (pKa ≈ 6.3-7) and primary and secondary hydroxyl groups at the C-3 and C-6 positions, respectively (1-3). At relatively low pH (<6.5), chitosan is positively charged and tends to be soluble in dilute aqueous solutions, but at higher pH it tends to lose its charge and may precipitate from solution due to deprotonation of the amino groups (2, 3). The properties of chitosan in aqueous solution depend on its molecular weight and degree of deacetylation, as well as the prevailing solution pH and ionic strength (3, 4). Previous studies have shown that chitosan can interact with anionic surfactants to form either soluble or insoluble complexes (4, 9, 10). These complexes may be stabilized by electrostatic, ion-dipole, and hydrophobic interactions and can be formed even when the concentration of surfactant is below the critical micelle con- centration (CMC) (10, 11).

A wide variety of analytical methods have been used to provide information about polyelectrolyte-surfactant interactions, including surface tension, fluorescence spectroscopy, viscometry, conductivity, and isothermal titration calorimetry techniques (9-15). In this study, surfactant-selective electrode (SSE), isothermal titration calorimetry (ITC), and turbidity measurements are used to characterize the interactions between chitosan and sodium dodecyl sulfate (SDS). SDS was chosen as a model anionic surface-active compound because its prop- erties in aqueous solutions are well characterized. The SSE technique provides information about the amount of surfactant bound to the chitosan, the ITC technique provides information about the enthalpy changes associated with surfactant-chitosan interactions, and the turbidity technique provides information about the formation of insoluble complexes.

**Materials and methods:**

Medium molecular weight chitosan (75-85% deacety- lation) was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Analytical-grade sodium dodecyl sulfate (SDS), sodium acetate, and acetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Double-distilled water was used for the preparation of all solutions.

**Solution Preparation.** Biopolymer solutions and surfactant solutions were prepared by dispersing either chitosan (0.1 wt % ≡ 1 g/kg) or SDS into acetate buffer (100 mM acetic acid:sodium acetate, pH 3.0), followed by stirring overnight to ensure complete dissolution.

**Isothermal Titration Calorimetry (ITC).** An isothermal titration calorimeter (VP-ITC, Microcal Inc., Northampton, MA) was used to measure the enthalpy changes resulting from chitosan-SDS interactions. Aliquots of 10 µL each of SDS solution were injected sequentially into a 1.48-mL titration cell initially containing either acetate buffer solution or 0.1 wt % chitosan in acetate buffer. Each injection lasted 20 s, and there was an interval of 300 s between successive injections. The temperature of the

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solution in the titration cell was 30 $^0$ C, and the solution was stirred at 315 rpm throughout the experiments. Measurements were carried out in duplicate, and the results are reported as the mean, with the standard deviation being less than 5%.

**Surfactant-Selective Electrode (SSE).** SSE was used to follow the binding interaction between SDS and chitosan by measuring changes in the electromotive force (EMF) due to changes in the free SDS concentration in solution. The SSE cell consisted of the surfactant- selective electrode (Thermo Orion, Beverly, MA), a double-junction reference electrode (Thermo Orion), and a pH meter (420A+, Thermo Orion). Aliquots of 100 µL each of surfactant solution were injected at 3-min intervals into a beaker initially containing 14.8 mL of either acetate buffer or 0.1 wt % chitosan in acetate buffer. Buffer or chitosan solutions were stirred throughout the experiment using a magnetic stirrer, and the EMF was measured 2 min after each injection. Measurements were carried out in duplicate, and the results are reported as the mean, with the standard deviation being less than 2%. The EMF signal was linearly related to the logarithm of the SDS concentration below the CMC of the surfactant. The concentration of free SDS in the aqueous chitosan solutions was therefore determined from a calibration curve of EMF signal versus SDS concentration measured in the absence of chitosan under the same solution conditions.

**Turbidity Measurements.** Aliquots of 100 µL each of surfactant solution were injected at 3-min intervals into a beaker initially containing 14.8 mL of either acetate buffer or 0.1 wt % chitosan in acetate buffer. Chitosan solutions were stirred throughout the experiment using a magnetic stirrer, and the turbidity was measured 2 min after each injection at 600 nm (Spectronic 21D, Milton Roy, and Rochester, NY). Measurements were carried out in duplicate, and the results are reported as the mean, with the standard deviation being less than 6%. Turbidity measurements were normalized with respect to the chitosan concentration in the solution ($\tau$/c chitosan) to take into account dilution effects associated with titration of surfactant solution into the chitosan solution.

**Results and discussion:**

**Isothermal Titration Calorimetry.** SDS-chitosan interactions were characterized at 30.0 C by ITC (0 mM NaCl, 100 mM acetate buffer, pH 3.0). Heat flow versus time profiles resulting from sequential injection of 10-µL aliquots of SDS solution into a reaction cell containing either buffer or 0.1 wt % chitosan in buffer were measured. These curves were integrated to give the total enthalpy change per mole of surfactant ($\Delta$H) injected into the reaction cell versus the total surfactant concentration present in the reaction cell. In the absence of chitosan, the enthalpy change associated with each injection was highly endothermic for the first few injections, decreased appreciably after a certain number of injections, and then reached a relatively small endothermic value for the later injections. The large endothermic peaks at relatively low surfactant concentrations in the reaction cell can be attributed to micelle dissociation (18-21). The SDS concentration in the injector (100 mM) was appreciably greater than the critical micelle concentration of the surfactant under these solution conditions (CMC $\approx$ 6.6 mM, see below), so that the

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injector contained a mixture of micelles (~93%) and monomers (~7%). Initially, when the micelles were injected into the buffer solution, they dissociated because the SDS concentration in the reaction cell was below the CMC. However, after a certain number of injections, the SDS concentration in the reaction cell exceeded the CMC, and so the micelles no longer dissociated upon injection. The relatively small endothermic peaks observed at higher surfactant concentrations can be attributed to micelle dilution effects (18-21).

The reason that the observed enthalpy change associated with micelle dissociation was endothermic can be attributed to the hydrophobic effect (22-24). When micelles dissociate, there is an increase in contact area between water and the nonpolar tails of the surfactant molecules (25). The enthalpy change associated with these hydrophobic interactions is endothermic at relatively high temperatures (>25 C) but exothermic at lower temperatures (22, 26). Our experiments were carried out at 30 C; hence, the exposure of nonpolar groups to water would be expected to be endothermic. Nevertheless, it should be noted that there are also contributions to the enthalpy change associated with other molecular events occurring during micelle dissociation, such as the release of bound counter ions or the increase in separation between surfactant head groups (24). The CMC of the SDS was calculated from the inflection point of the $\Delta H$ versus [SDS] profiles, as described previously (18, 19), to be $6.6 \pm 0.2$ mM.

The form of the isothermal titration calorimetry profiles was qualitatively different in the absence and in the presence of chitosan in the reaction cell. In the presence of 0.1 wt % chitosan, the peaks were strongly exothermic at relatively low SDS concentrations (<4 mM), became strongly endothermic at intermediate SDS concentrations (~4-10 mM), became increasingly less endothermic at higher SDS concentrations (~10-13 mM), and became similar in magnitude to those measured in the absence of chitosan at still higher SDS concentrations. The form of the ITC profiles suggests that there is a strong exothermic reaction between the SDS and the chitosan at relatively low SDS concentrations. Once a critical SDS concentration was exceeded (~4 mM), the enthalpy change switched from exothermic to endothermic, which suggested that there was no further interaction because the chitosan had become saturated with SDS. Any additional SDS injected into the reaction cell would then be located exclusively in the aqueous phase. Relatively large endothermic peaks were observed at SDS concentrations slightly higher than the saturation concentration because of micelle dissociation, as described above in the absence of chitosan. These endothermic peaks decreased in magnitude once the SDS concentration exceeded a certain value (~11 mM) since the free surfactant concentration in the reaction cell then exceeded the CMC. The change in SDS concentration from the concentration where the chitosan was saturated with surfactant to the concentration where no more demicellization was observed in the aqueous phase (~6-7 mM) was similar to the CMC of the surfactant measured in the absence of chitosan (~6.6 mM). This suggests that the presence of the chitosan-SDS complex did not strongly influence the micellization behavior of free SDS in the aqueous phase. On the other hand, the endothermic enthalpy change associated with demicellization was appreciably

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smaller in the presence of the chitosan-SDS complex than in its absence, which suggests that the complex somehow influenced the enthalpy of the process.

The above experiments were repeated in the presence of 100 mM NaCl in order to obtain information about the role of electrostatic screening on the interactions. In the absence of chitosan, the relatively large endothermic enthalpy change associated with micelle dissociation occurred over a much lower surfactant concentration range at 100 mM than at 0 mM NaCl, which can be attributed to the ability of salts to depress the CMC of ionic surfactants (25). The CMC of the surfactant in the 100 mM NaCl buffer solution was calculated from the inflection point of the ΔH versus [SDS] profiles to be 1.5 ( 0.1 mM, which is about 4-fold smaller than in the absence of salt. In the presence of chitosan, there was a large exothermic enthalpy change at SDS concentrations less than 4 mM, which could be attributed to SDS-chitosan interactions. A small endothermic peak was observed at surfactant concentrations between 5 and 7 mM SDS, which can be attributed to demicellization of the surfactant micelles in the aqueous phase. The width of this peak was approximately equal to the width of the endothermic peak associated with demicellization measured in the absence of chitosan, which again suggests that the presence of the chitosan-SDS complex did not appreciably alter the micellization behavior of the free SDS in the aqueous phase. Nevertheless, the peak height was appreciably smaller than that observed in the absence of chitosan, which suggests that the chitosan-SDS complex somehow depressed the enthalpy change associated with demicellization in the aqueous phase. At >8 mM SDS, the enthalpy change was similar in the presence and in the absence of chitosan, and can be attributed to the enthalpy of sample dilution.

**Surfactant-Selective Electrode Measurements.** The change in EMF measured using a surfactant-selective electrode when SDS was titrated into buffer and into chitosan in buffer (pH 3.0, 0 mM NaCl, and 100 mM acetate buffer) at room temperature was measured. In the absence of chitosan, there was an approximately linear decrease in EMF with increasing logarithm of SDS concentration, until the CMC was reached (SDS ~6 mM), and there was a slight break in the curve. In the presence of chitosan, the EMF was much higher at lower SDS concentrations, which indicated that the concentration of free SDS in the aqueous phase was reduced, presumably because of SDS-chitosan binding. When the SDS concentration exceeded about 4 mM, there was a rapid decrease in the EMF, which can be attributed to the fact that the chitosan became saturated with surfactant and any additional SDS injected into the solution was present in the aqueous phase.

The concentration of free SDS in the solution containing chitosan was determined from a calibration curve of EMF versus SDS concentration. The free SDS concentration remained close to zero when the total surfactant concentration in the solutions was increased from 0 to 4 mM SDS, which suggested that all of the added surfactant was strongly bound to the chitosan. At higher total SDS concentrations, the increase in free SDS was approximately equal to the increase in total SDS, which suggested that the chitosan had become saturated with surfactant. The above experiment was repeated in the presence of 100 mM NaCl.

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In this case, there was a small increase in free SDS as the total SDS concentration was increased from 0 to 3.4 mM SDS, followed by a steeper increase at higher surfactant concentrations. These results suggest that SDS was bound less strongly to the chitosan in the presence of NaCl, presumably because of screening of the electrostatic attraction between the anionic surfactant and cationic biopolymer.

**Turbidity Measurements.** At relatively low surfactant concentrations (<5 mM SDS), there was an approximately linear increase in the normalized turbidity of the chitosan solutions with increasing SDS concentration, which suggested that progressively more insoluble complexes were formed. Eventually, the normalized turbidity reached a maximum value, which probably occurred because the chitosan became saturated with SDS. At the same surfactant concentration, the normalized turbidity of the solutions was considerably lower in the absence of 100 mM NaCl than in its presence, which suggested that either the concentration of insoluble SDS-chitosan aggregates formed was reduced or the size of the aggregates changed in the presence of salt. Since our SSE studies suggested that the SDS-chitosan interaction was stronger in the absence of salt, we believe that the differences in turbidity are mainly due to changes in the size of the SDS-chitosan complexes.

**Implications of Anionic Surfactant-Chitosan Interactions.** Our results clearly show that a model anionic surfactant (SDS) strongly binds to cationic chitosan and forms insoluble com- plexes, probably through a mechanism involving electrostatic attraction. Previous structural studies suggest that cationic chitosan molecules wrap around anionic SDS micelles through electrostatic attraction to form nano structured chitosan-SDS complexes (9, 27). The formation of these complexes may be particularly useful for the encapsulation or controlled release of certain food components. For example, nonpolar molecules (such as flavors, antioxidants, antimicrobials, vitamins, and bioactive lipids) could be solubilized within surfactant micelles, and then chitosan could be added to form nanostructured complexes. These complexes could then be added to food systems as functional ingredients, which may have novel encapsulation or release properties. This research is also useful for understanding the molecular basis for the ability of chitosan to reduce cholesterol levels (1,5). It seems likely that chitosan molecules wrap themselves around bile acid micelles via strong electrostatic interactions, which leads to the formation of insoluble complexes, thus increasing the amount of bile acids excreted in the feces. In a future study, we intend to use the ITC methodology developed in this study to provide further insights into the origin and nature of bile acid-chitosan interactions. The ability of chitosan to bind strongly to anionic surfactants (as well as other anionic molecules) and form insoluble complexes may also prove to be a useful means of purifying aqueous waste matter. Finally, the ability of chitosan to strongly bind anionic surfactants may be undesirable in some food products, since the surfactants may lose their functionality after binding to the biopolymer, e.g., in surfactant-stabilized emulsions.

**Conclusions.** The results from the ITC, SSE, and turbidity measurements are complementary and provide valuable insights into the nature of SDS-chitosan interactions. ITC and SSE indicated that SDS bound strongly to chitosan via a highly exothermic interaction, while the turbidity measurements indicated that the resulting complex formed was insoluble. The chitosan used in our experiments

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could adsorb approximately 4 mM of SDS per 0.1 wt % chitosan before becoming saturated with surfactant. The SDS-chitosan interaction was appreciably weakened by the presence of 100 mM salt, which suggested that it was predominantly electrostatic in origin. This study provides information that may lead to the rational design of chitosan-based food ingredients with specific nutritional and functional characteristics, e.g., cholesterol lowering, fat replacement, or ingredient binding.

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