Sodium Caseinate-Stabilized Emulsions: Factors Affecting Coverage and Composition of Surface Proteins

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As the concentration of caseinate in sodium caseinate-stabilized emulsions increased, the protein surface concentration increased. It plateaued at 1.36 ± 0.05 mg/m^2 at a caseinate concentration between 2 and 4% (w/w), but the surface protein concentration markedly increased as caseinate concentration rose from 5 to 7.5%. At caseinate concentration below 2%, β-casein adsorbed at the surface of oil droplets in preference to other caseins. Increasing the oil concentration from 10 to 30% (w/w) decreased the surface protein concentration from 3.7 ± 0.3 to 1.4 ± 0.04 mg/m^2, but further increases in oil concentration had much less effect. A decrease in surface protein concentration was observed as the homogenization pressure increased from 34 to 136 bar, but higher pressures had no further effect. β-Casein was adsorbed preferentially at the droplet surface in emulsions homogenized at pressures above 204 bar. Addition of calcium chloride to sodium caseinate solutions above 0.08% w/w resulted in formation of large casein particles/aggregates which adsorbed on to the droplet surface causing higher surface protein concentration.

Keywords: *Sodium caseinate; oil-in-water emulsions; protein adsorption; surface composition*

INTRODUCTION

Sodium caseinate is an excellent emulsifying agent and is a common ingredient in a wide range of emulsions, e.g., coffee whitener, cream liqueur, and whipped toppings. Caseinate is a composite of four different proteins, α_{s1}-, α_{s2}-, β-, and κ-caseins in weight proportions of approximately 4:1:4:1. All caseins are amphipathic proteins with a strong tendency to adsorb at oil−water interfaces during emulsion formation, reducing interfacial tension (Mulvihill and Fox, 1989; Tornberg et al., 1990). This produces an adsorbed layer of protein around the oil droplets which protect them against subsequent coalescence and flocculation.

Being the more surface active, β-casein has been shown to be adsorbed in preference to α_{s1}-casein in emulsions stabilized by a mixture of α_{s1}- and β-caseins (Dickinson et al., 1988b). However, Robson and Dalgleish (1987) demonstrated no preferential adsorption of β-casein in sodium caseinate-stabilized emulsions immediately after homogenization. However, after aging these emulsions, β-casein displaced some of the α_{s1}-casein. Hunt and Dalgleish (1994) found no preference for β-casein or α_s-casein in sodium caseinate-stabilized emulsions. Most investigations to date have concentrated on the adsorption of pure caseins and mixtures of isolated proteins on to a planar oil−water interface and in emulsion systems (Benjamins et al., 1975; Dickinson et al., 1988a,b). Such studies have provided valuable fundamental information on the composition of stabilizing adsorbed layer around oil droplets. However, there is less information available on the adsorption behavior of commercially produced sodium caseinates (Robson and Dalgleish, 1987) that are used by the food industry in real food emulsions. It is likely that these caseinates would behave differently because of the structural modifications to the protein that may occur during processing. The effects of wide range of processing and solution conditions (oil concentration, protein concentration, and salt concentration) on adsorption have not been explored.

In this report we describe the effects of some compositional and processing variables on the adsorption behavior of sodium caseinate in soya oil-in-water emulsions.

MATERIALS AND METHODS

Materials. Sodium caseinate (Alanate 180) was obtained from the New Zealand Dairy Board, Wellington, New Zealand. Soya oil was purchased from Davis Trading Company, Palmerston North, New Zealand. All of the chemicals used were of analytical grade obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Emulsion Preparation. Emulsions were prepared from 2.5% (w/w) sodium caseinate solution and 30% (w/w) soya oil. In some cases, emulsions were made using varying concentrations of caseinate or soya oil. The mixture was adjusted to pH 7.0 and heated to 55 °C, and then passed through a two stage valve homogenizer without applying any pressure (Rannie a/s, Roholmsvej 8, DK 2620 Albertslund, Denmark). This produced a temporary oil-in-water emulsion. The mixture was then homogenized at the desired pressure, usually 102 bar for the first stage and 34 bar for the second stage. The resulting homogenized emulsion was stored at 20 °C. At least two separate emulsions were prepared for each treatment.

A Malvern MasterSizer MSE (Malvern Instruments Ltd, Worcestershire, U.K.) was used to determine the volume−surface average diameter (d_{32}) and specific surface area (area per unit mass). The d_{32} and specific surface area values were accurate to within 3%.

Determination of Surface Protein Concentration and Composition. Emulsions (30 g) were centrifuged at 45000*g* for 40 min at 20 °C in a temperature-controlled centrifuge (Sorvall RC5C, DuPont Co., Wilmington, DE) and the subnatants were carefully removed using a syringe. The cream layer was dispersed in deionized water (purified by reverse osmosis followed by treatment with a Milli-Q apparatus, Millipore Corp., Bedford, MA) and recentrifuged at 45000*g* for 40 min. Again, the subnatant and cream layer were collected carefully. Each subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore). The filtrates were analyzed

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separately for total protein using Kjeldahl (1026 Distilling Unit and 1007 Digestor Block, Tecator AB, Höganäs, Sweden). A factor 6.38 was used to convert nitrogen to protein content.

The surface protein concentration (mg protein/m^2) was calculated from the surface area of the oil droplets determined by Mastersizer and the difference in the amount of protein used to prepare emulsion and that measured in the subnatants after centrifugation.

The protein composition of the subnatant was determined using SDS−PAGE as described by Singh et al. (1993). A certain amount of subnatant was mixed with SDS buffer (0.5 M Tris, 2% SDS, 0.05% mercaptoethanol, pH 6.8) and a portion 5 μL applied to the SDS gels previously prepared on Mini-Protean II system (Bio-Rad Laboratories, Richmond, CA). The separating gels contained 15% acrylamide, made up in Tris/HCl buffer, pH 8.3, and stacking gels were composed of 4% acrylamide in Tris/HCl buffer, pH 6.8. After destaining, the gels were scanned on a laser densitometer (LKB Ultroscan XL, LKB Produkter AB, Bromma, Sweden). The percentage composition of each sample was determined by scanning the areas for α_s- ($\alpha_{s1} + \alpha_{s2}$), β-, and κ-caseins and expressing the individual casein peaks as a fraction of the sum total.

The reproducibility of these methods was determined by analyzing 9 separate emulsions made with 2.5% sodium caseinate and 30% soya oil. Variations were ~4% for surface protein concentration, ~5% for α_s-casein, ~4% for β-casein, and ~9% for κ-casein.

RESULTS AND DISCUSSION

Effect of Protein Concentration. Figure 1A shows that increasing caseinate concentration from 1.0 to 7.5% (w/w) had no effect on d_{32} but increased the surface protein concentration. The increase in surface concentration was gradual from 1.0 to 2.0% caseinate concentration; the surface concentration was stable between 2 and 4%, but at higher caseinate concentrations, there was a sharp increase in surface concentration. The plateau region of the curve at surface concentration of 1.36 ± 0.05 mg/m^2 probably corresponds to saturated monolayer coverage of adsorbed casein molecules, while a sharp increase in surface concentration at caseinate concentration above 4% may be attributed to the formation of secondary or multilayers at the interface. Similar behavior has been observed for β-casein, bovine serum albumin, and lysozyme adsorption on to a planar oil−water interface by Graham and Phillips (1979). Alternatively, an increase in caseinate concentration may cause formation of large casein aggregates in solution which may be subsequently adsorbed at the interface, resulting in high values of surface protein concentration. Pepper and Farrell (1982) using gel permeation chromatography showed that with increasing protein concentration in the range 0.1−3.0%, casein components of soluble casein associated to form polymers that approach molecular radii of ~10 nm.

Fang and Dalgleish (1993) and Hunt and Dalgleish (1994) also reported increase in surface protein concentration with increase in casein concentration, although the values of surface concentration obtained at different caseinate concentrations were considerably lower in our study than those reported by them. For example, Fang and Dalgleish (1993) found surface concentration of ~3 mg/m^2 at 2% caseinate concentration as compared to 1.36 mg/m^2 in our study. These differences could be due to the different types of homogenizer and/or caseinates used to prepare emulsions. Fang and Dalgleish (1993) used a microfluidizer for making emulsions and obtaining smaller droplet diameter (~0.3 μm), and they used freeze-dried sodium caseinate prepared in a laboratory under relatively mild conditions.

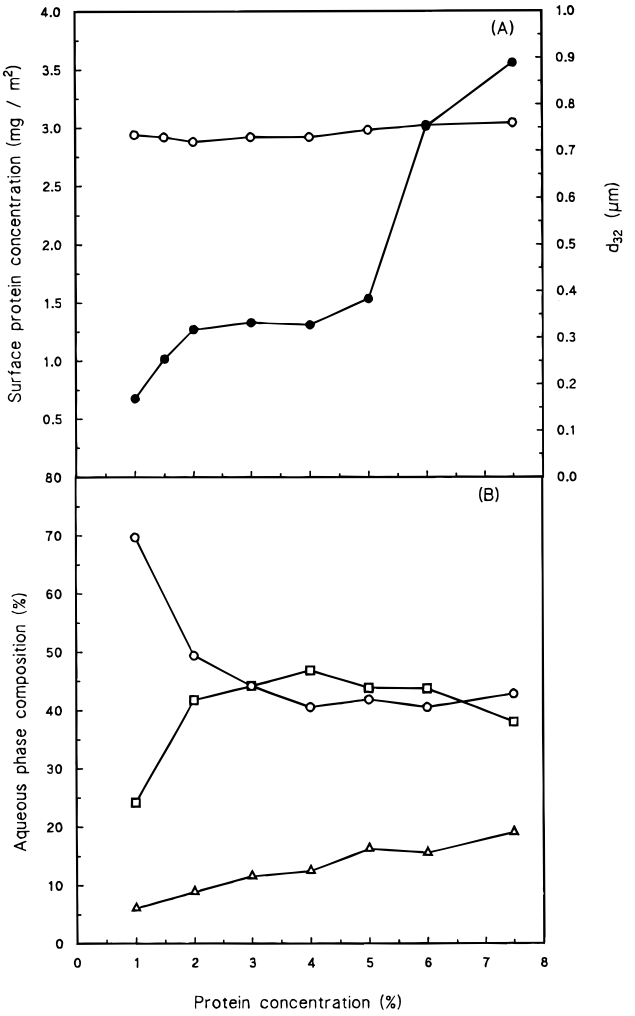

Figure 1. (A) Changes in surface protein concentration (mg/m^2) and droplet diameter (μm) in emulsions containing 30% soya oil and varying amounts of caseinate. (●) Surface protein concentration and (○) average droplet diameter (d_{32}). (B) Aqueous phase composition of caseins in sodium caseinate-stabilized emulsions (30% soya oil) as a function of caseinate concentration. (○) α_s-, (□) β- and (△) κ-casein.

Figure 1B shows the protein composition of the subnatant, i.e., unadsorbed proteins as a function of caseinate concentration. In the emulsion made with 1% protein, the ratio of unadsorbed α_s- to β-caseins was considerably greater than in the original sodium caseinate solution (the composition of original sodium caseinate solution was α_s-casein 45.05%, β-casein 40.05%, and κ-casein 14.9%), suggesting that β-casein was adsorbed in preference to α_s-casein under these conditions. As the concentration of caseinate was increased, the preference for β-casein adsorption diminished. The proportion of unadsorbed κ-casein increased slightly with increase in protein concentration. This change in behavior may again be related to the formation of casein aggregates in sodium caseinate solutions or formation of β-casein micelles at higher protein concentrations which affected its adsorption behavior (Euston et al., 1995).

Dickinson et al. (1988b) showed that in emulsions stabilized by a mixture of pure α_{s1}- and β-caseins [10% (w/w) tetradecane, 0.5% (w/w) protein], β-casein was adsorbed in preference to α_{s1}-casein. This was attributed to greater surface activity of β-casein (Dickinson et al., 1987; Castle, 1987). Robson and Dalgleish (1987) reported no preference for adsorption of β-casein in sodium caseinate-stabilized emulsions immediately

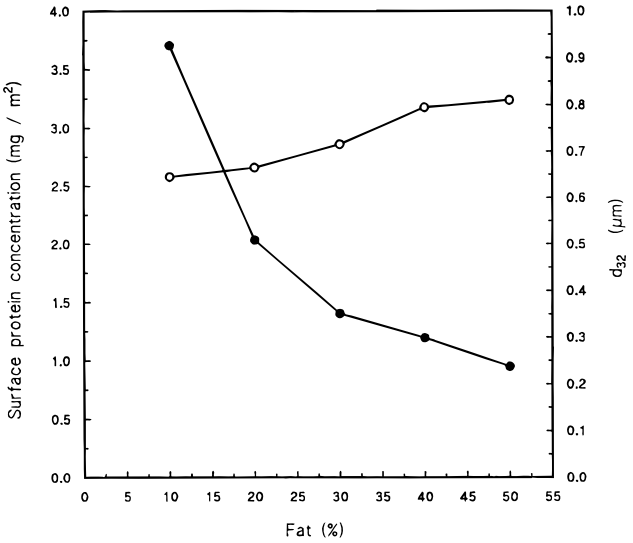

Figure 2. Changes in surface protein concentration (mg/m^2) and droplet diameter (μm) in emulsions containing 2.5% caseinate and varying oil concentration. (●) Surface protein concentration and (○) average droplet diameter (d_{32}).

after homogenization. However, on aging, β-casein displaced some of the adsorbed α_{s1}-casein. Recently, Hunt and Dalgleish (1994) reported no preference for any of the caseins either immediately after homogenization or after aging the emulsions (20% soya oil, 1.0−3.0% proteins).

Effect of Oil Concentration. Oil concentration was varied by adding different quantities of soya oil to the caseinate solution (2.5%, w/w) prior to homogenization. As the concentration of oil in the emulsion was increased from 10 to 50%, the d_{32} increased (from 0.60 to 0.81 μm). These results are consistent with earlier studies by Tornberg et al. (1990) who attributed this increase to the greater incidence of coalescence and bridging at higher oil concentrations, both of which lead to reduction in total fat surface area.

Surface protein concentration decreased from 3.7 ± 0.3 to 1.4 ± 0.04 mg/m^2 as the oil concentration increased from 10 to 30%, but further increase in oil concentration caused only a slight decrease (Figure 2). The greater surface concentration at low oil concentrations may be mainly due to the formation of multilayers because of high protein to oil surface area ratio, a situation similar to that observed in Figure 1A. Increasing oil concentration (i.e., increase in total oil surface area) probably causes spreading of the adsorbed protein into thinner layers, resulting in a decrease in surface concentration.

The relative proportions of α_s-, β-, and κ-caseins were largely unaffected by varying oil concentrations in the range 10−50%.

Effect of Homogenization Pressure. Emulsions made with soya oil (30%, w/w) and sodium caseinate (2.5% w/w, pH 7.0) were homogenized at different first stage pressures, ranging from 34 to 272 bar at 55 °C. The d_{32} decreased with increase in homogenization pressure (Figure 3A), whereas the surface protein concentration decreased sharply (from 2.2 ± 0.1 to 1.5 ± 0.05 mg/m^2) as the first-stage pressure rose from 34 to 136 bar (Figure 3A). Higher pressure had no further effect. These results are consistent with the studies reported by other workers (Tornberg, 1978; Murphy and Fox, 1991; Mulvihill and Murphy, 1991), who used a valve homogenizer incorporated into a recirculating

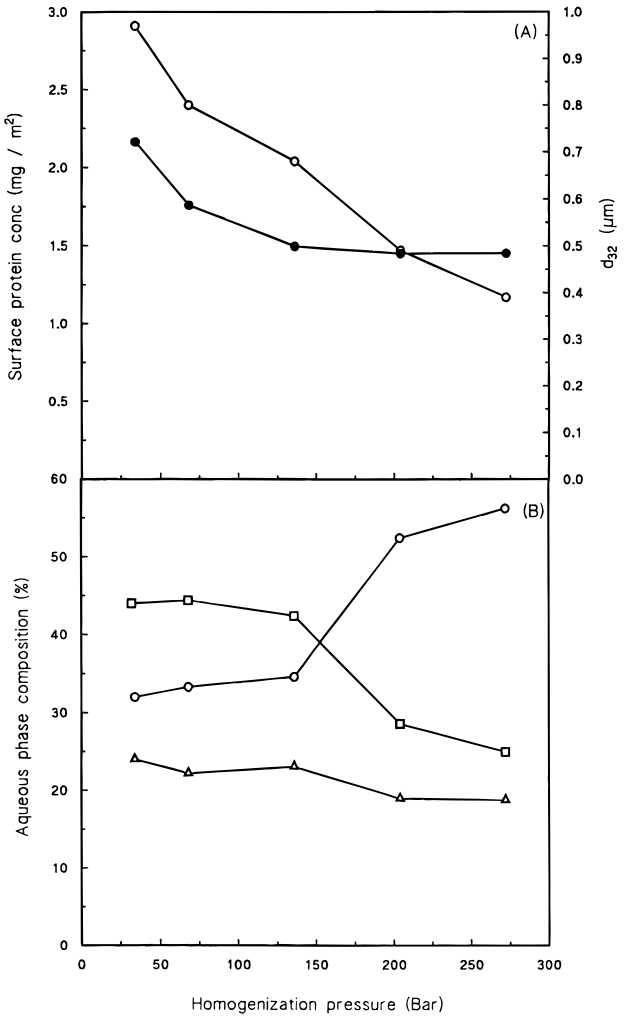

Figure 3. (A) Changes in surface protein concentration (mg/m^2) and droplet diameter (μm) in emulsions containing 30% soya oil and 2.5% caseinate at different homogenization pressures. (●) Surface protein concentration and (○) average droplet diameter (d_{32}). (B) Aqueous phase composition of caseins in sodium caseinate-stabilized emulsions (30 wt% soya oil and 2.5% caseinate concentration) as a function of homogenization pressure. (○) α_s-, (□) β- and (△) κ-casein.

emulsifying system in which the power input could be varied. The decrease in surface concentration on increasing homogenization pressures (i.e., increasing oil surface area) may be attributed to increased spreading and rearrangement of adsorbed protein molecules at the interface. Relatively high surface concentration at low homogenization pressure, i.e., at smaller surface areas, might indicate that multilayers of proteins were formed at the interface, whereas at high homogenization pressure the layers of protein might be thinner and probably approaching a monolayer.

The relative proportions of α_s-, β-, and κ-caseins in the subnatant (unadsorbed protein) remained unaffected with increase in first-stage pressure from 34 to 136 bar but at higher pressures, i.e., 204 and 272 bar, the proportion of β-casein decreased by 40% with a corresponding increase in the proportion of α_s-casein (Figure 3B). The proportions of κ-casein decreased slightly with increase in pressure in the range 34−272 bar. Clearly, β-casein was preferentially adsorbed in emulsions homogenized at 204 and 272 bar. It is possible that during homogenization at high pressures the casein aggregates are broken, allowing β-casein to preferentially adsorb.

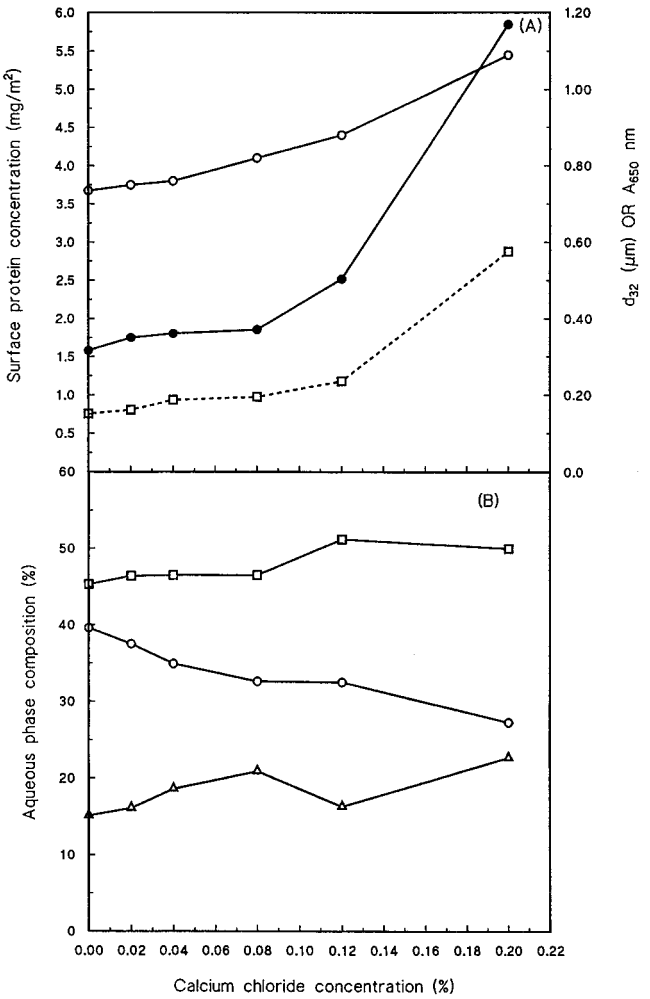

Figure 4. (A) Changes in surface protein concentration (mg/m^2), droplet diameter (μm) and absorbance at 650 nm as a function of calcium chloride concentration in emulsions containing 30% soya oil and 2.5% caseinate. (●) Surface protein concentration, (○) average droplet diameter (d_{32}) and (□) A_{650} nm. (B) Aqeuous phase composition of caseins in sodium caseinate-stabilised emulsions (30% wt % soya oil and 2.5% caseinate concentration) as a function of calcium chloride concentration. (○) α_s-, (□) β- and (△) κ-casein.

Effect of Calcium Chloride Addition. Increasing the concentration of $CaCl_2$ from 0.02 to 0.2% (w/w) gradually increased the d_{32} (Figure 4A). At $CaCl_2$ concentrations up to 0.08%, (w/w) there was no significant change in surface protein concentration, but at higher concentrations of $CaCl_2$ the surface protein concentration increased markedly (Figure 4A).

Addition of $CaCl_2$ to sodium caseinate solutions above a certain concentration may cause the formation of large casein particles/aggregates which subsequently may be adsorbed on to the oil surface, resulting in higher surface concentrations. Turbidity measurements on the caseinate solutions at 650 nm showed marked increase above 0.12% $CaCl_2$, indicating the formation of large casein particles/aggregates (Figure 4A). In fact, the turbidity *vs* $CaCl_2$ curve had a very similar shape to the surface concentration *vs* $CaCl_2$ curve (Figure 4A). These results are consistent with those of Mulvihill and Murphy (1991) who found that the surface protein concentration in emulsions stabilized by calcium caseinate was considerably higher than in sodium caseinate-stabilized emulsions.

Figure 4B shows the proportions of unadsorbed protein in emulsions containing different amounts of $CaCl_2$. The proportions of α_s-casein in the subnatant decreased gradually with increase in $CaCl_2$ concentration, while the proportions of β-casein showed an increase (above 0.08% $CaCl_2$). The proportions of κ-casein varied with no consistent trend.

These results suggest that calcium-induced aggregation of caseins influences their adsorption behavior; possibly large aggregates, rich in α_s-casein, are formed which adsorb in preference to non-aggregated casein material. The preferential adsorption of casein micelles over whey proteins has been previously reported by Oortwijn and Walstra (1979).

It is known that caseins that constitute sodium caseinate are not monomeric, but are aggregated to some extent; the nature and size of aggregates are probably dependent on protein concentration, temperature, presence of ions and processing history. During the dynamic conditions of homogenization much of the protein material is transported to the oil−water interface by convection rather than diffusion (Walstra and Oortwijn, 1982; Walstra, 1983). The rate of adsorption of protein is determined by the size of adsorbing proteins or aggregates and the immediately available binding sites on the molecule, i.e., surface hydrophobicity of the adsorbing proteins (Dickinson, 1992).

Surface coverage and composition of sodium caseinate-stabilized emulsions could therefore depend on the aggregation state of protein at the time of emulsification. Conditions that favor protein aggregation, e.g., addition of $CaCl_2$, high protein concentrations, would increase the surface coverage. Relationships between surface coverage and aggregation state have been demonstrated by Oortwijn and Walstra (1979), Mulvihill and Murphy (1991), and Singh et al. (1993). In addition to the size of aggregates, the structure of aggregates and the type of bonding may also be important. For example, aggregates linked through covalent bonds are less likely to undergo spreading and rearrangement when adsorbed at the interface than those linked through non-covalent interactions, such as hydrophobic interactions. Clearly further work is needed on the aggregation state of proteins in sodium caseinate solutions, how it is affected by various factors, and its relationships to the adsorption behavior.

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