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Kinetic Approach to the Nonlinear Curve of Bovine Serum Albumin Desorption from Stainless Steel Surfaces

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The kinetics of the desorption of bovine serum albumin (BSA) from stainless steel particles at various surface coverage values (θ) or at various cleaning temperatures were studied in a plug-flow column fed by a 0.1M NaOH solution. The desorption curves obtained by plotting the logarithm of the amount of residual BSA against cleaning time was analyzed by using an integrated model for two first-order processes occurring simultaneously. At each θ or temperature, the curve of BSA desorption could be successfully reduced to the sum of two independent and simultaneous first-order processes occurring at different rates. The desorption rate constant (k') for the faster-desorbing BSA (BSA') was influenced by θ , especially above 0.6. On the other hand, the rate constant (k^s) for the slower-desorbing BSA (BSA^s) was nearly constant when θ was above 0.6. The k^s was approximately 10 to 20 times lower than k' . The k' showed an Arrhenius-type temperature dependence. The apparent activation energy (E_a) of k' was estimated to be 33kJ/mol. The k' increased by approximately 1.4 times for every 10°C rise in temperature. The proportion of BSA^s to the total amount of adsorbed BSA decreased with increasing cleaning temperatures.

Key words : Nonlinear kinetics of protein desorption/Stainless steel/Surface coverage/Cleaning temperature/Caustic alkali cleaning.

INTRODUCTION

In the food industry, the cleaning of the product-processing equipment is an important process in maintaining the safety of the product. Many studies on the cleaning of hard surfaces in contact with the product have been conducted by using a variety of soils, substrates, and detergents under various cleaning conditions (Dunsmore et al., 1981; Jennings, 1959; Koopal, 1985). The cleaning efficiency is determined not only by the amount of detergent, water, and energy used, but also by the cleaning time. The cleaning process can be described by a mathematical equation as a function of time. Therefore, a kinetic study of

cleaning is needed for the optimization of cleaning.

Rhodes and Brainard (1929) have found that semi-log plots of detergency versus time gave a linear relationship and pointed out that the slope was an index of detergent power. Jennings (1959) studied the removal of milk solids from stainless steel by NaOH solutions in a circulation system. His data showed that the removal process in the initial stage of cleaning is kinetically first-order with regard to soil and detergent concentrations. The first-order rate constant was shown to have an Arrhenius-type temperature dependence. Bourne and Jennings (1963a, b) studied the nonlinear curve of tristearin removal from stainless steel surfaces by 0.03M NaOH solution. They suggested that two species of tristearin are present on the stainless steel surface, i.e., species 1 and 2, each of which is removed independently and

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simultaneously according to a first-order process. Species 1 was shown to be removed 10 to 100 times faster than species 2. Jennings (1965) has reviewed that a number of cleaning curves as a function of time could be represented by the sum of two linear semi-log plots, and that the concept of two soil species covered a wide range of soils, solid surfaces, detergents, and cleaning conditions.

Recently, we formulated an integrated model for first-order desorption based on the concept of two soil species (Urano and Fukuzaki, 2002). This kinetic model closely fitted the experimental data of the desorption of bovine serum albumin (BSA) from alumina particles at various surface coverage values (θ) during caustic alkali cleaning. The process of BSA desorption could be reduced to the sum of two independent and simultaneous first-order reactions occurring at different rates, and the rate constants for two first-order reactions were evaluated as a function of θ . This kinetic approach seems to be applicable to the evaluation of the overall cleaning efficiency.

The aim of this study is to investigate the effects of θ and cleaning temperatures on the kinetics of the desorption of irreversibly adsorbed BSA from stainless steel particles during caustic alkali cleaning. The curve characterizing BSA desorption, a semi-log plot of the residual BSA versus time, was analyzed by the integrated model for two first-order reactions occurring simultaneously. This paper shows the methodology for analyzing the nonlinear kinetics of BSA desorption and precisely evaluating the effects of the variables on the overall cleaning process.

MATERIALS AND METHODS

Materials

Non-porous 316L stainless steel particles with a mean diameter of 8-10 μm (manufacturer's analysis) were obtained from The Nilaco Corp. (Tokyo). The specific surface area, as determined by the BET method (Fukuzaki et al., 1995), was 0.36 m^2/g . BSA (Fraction V; Lot M7G9556) was purchased from Nacalai Tesque Inc. (Kyoto). All other chemicals were of analytical grade.

Preparation of BSA-fouled stainless steel particles at various θ

BSA solutions were prepared by dissolving 0.001 to 0.5g of BSA in 100ml of 10^{-3} M KNO_3 solution, the pH of which was adjusted to 5.2. BSA-fouled stainless steel particles were prepared at 40°C by introducing 5 ml of the BSA solution and 1.0g of stainless steel particles into a 25ml glass vial, which was reciprocally shaken (140 oscillations per min) for 2h as described

previously (Fukuzaki et al. 2001). The thus obtained BSA-fouled particles were dried at 40°C for 16h and stored in a desiccator before use in the cleaning experiment. θ was expressed as the relative amount adsorbed divided by the saturated amount adsorbed ($3.0\text{mg}/\text{m}^2$).

When BSA-fouled stainless steel particles at θ of 0.11 to 1.0 were immersed in 10^{-3} M KNO_3 solution at 40°C, no desorption of BSA occurred, indicating that the adsorption of BSA on stainless steel particles was irreversible.

Cleaning experiment

The cleaning experiment was conducted at 40, 50, 60, 70, and 80°C in a stainless steel column (4mm $\phi \times 50\text{mm}$) installed vertically, into which 1-g portion of the dried BSA-fouled stainless steel particles at various θ were packed, leaving approximately 80% void space. First, 10^{-3} M KNO_3 solution (pH 5.2) was allowed to flow upward through the column at a space time (τ) of 2 min for 120 min at 40°C to wet the BSA-fouled stainless steel particles. Second, 0.1M NaOH solution was fed from the bottom of the column at τ of 2 min at 40, 50, 60, 70, and 80°C. When the NaOH solution reached the column outlet, alkaline eluent fractions were collected at 4-min (2τ) intervals and then at appropriate intervals depending on the amount of desorbed BSA. The BSA concentration of each fraction was measured by the Lowry-Folin method (Lowry et al., 1951).

Kinetic analysis

The kinetic analysis of BSA desorption from stainless steel particles was performed by using a model based on the concept of two soil species, proposed by Urano and Fukuzaki (2002):

$$\ln \Gamma = \ln \{ \exp(\ln \Gamma_o^f - k^f t) + \exp(\ln \Gamma_o^s - k^s t) \} \quad (1)$$

where Γ , Γ_o^f , and Γ_o^s are the amount of adsorbed BSA at any given time t , and the amounts of a faster-desorbing BSA (BSA^f), and a slower-desorbing BSA (BSA^s) at zero time, respectively; k^f and k^s are the desorption rate constants of BSA^f and BSA^s , respectively. The kinetic parameters were calculated by a data-fitting procedure based on a nonlinear least-squares regression method (Urano and Fukuzaki, 2002).

RESULTS

Nonlinear kinetics of BSA desorption

Figure 1 shows the experimental data for BSA desorption at θ of 0.55, where $\ln \Gamma$ was plotted against the cleaning time. The data-fitting procedure was conducted by using eq. 1 and the nonlinear

regression method. The zero time denotes the time when NaOH solution reached the column outlet. The desorption curve had a short length of lag phase which was probably needed for the exchange of the KNO_3 solution for the NaOH solution in the column and for the wetting and/or swelling of the adsorbed BSA. The rate of BSA desorption was higher in the initial stage of cleaning and gradually decreased as the residual amount of BSA decreased toward the later stage of cleaning.

For data-fitting using eq. 1, it is necessary to put aside the data in the lag phase and to give the suitable initial values of the parameters of eq. 1. Figure 1A shows the graphical method to find the data in the lag phase to be put aside and to estimate the provisional initial values of the parameters. First, the broken line (1), which was an approximated straight line to show the fast desorption phase, was extrapolated back to $\ln \Gamma_0$ ($=0.52$) to give a provisional zero time (t_0^p). Second, the broken line (2), which was an approximated straight line to show the slow desorption

phase, was extrapolated back to t_0^p to give a provisional Γ_0^s ($\Gamma_0^{s,p}$). We then subtracted $\Gamma_0^{s,p}$ from Γ_0 to give a provisional Γ_0^f ($\Gamma_0^{f,p}$). From the slopes of the lines (1) and (2), provisional k^f and k^s values ($k^{f,p}$ and $k^{s,p}$) were obtained. After putting aside the data from zero time to t_0^p and substituting the obtained provisional values into eq. 1, desorption data were analyzed by a data-fitting procedure based on a nonlinear regression method and eq. 1. The solid line in Fig. 1B was derived from the computer analysis. The broken lines (f) and (s) in Fig. 1B represent the estimated first-order desorption for BSA^f and BSA^s, respectively. The slopes of the broken lines (f) and (s) give the k^f and k^s , respectively. Optimum t_0^p (t_0) was identified as the time to give $\ln \Gamma_0$ on the estimated solid line. In this way, a set of the best parameters (Γ_0^f , Γ_0^s , k^f , and k^s) of eq. 1 and t_0 were estimated as follows: $k^f = 0.051 \text{ min}^{-1}$, $k^s = 0.0058 \text{ min}^{-1}$, $\Gamma_0^f = 0.94 \text{ mg/m}^2$, $\Gamma_0^s = 0.75 \text{ mg/m}^2$, and $t_0 = 2.24$ (multiple coefficient, $R = 0.9999$). At this point, BSA^s includes the molecules that could not be removed under the cleaning conditions in this study.

FIG. 1. Graphical method to put aside the lag phase and estimate Γ_0^f and Γ_0^s from the experimental desorption data at θ of 0.54. (A) Estimation of $\Gamma_0^{f,p}$, $\Gamma_0^{s,p}$ and t_0^p . (B) Nonlinear regression fit of eq. 1 (see text). The solid line in panel B was computed by using eq. 1, and broken lines (f) and (s) represent the estimated first-order desorption for BSA^f and BSA^s, respectively. The cleaning experiment was conducted in a plug-flow column by feeding 0.1M NaOH solution at τ of 2 min (40°C).

Effect of θ

Figure 2 shows the nonlinear regression fit of eq. 1 to the desorption data at θ of 1.0, 0.69, 0.38, 0.21, and 0.10. Each of the solid lines was derived from the computer analysis by using eq. 1 based on a nonlinear regression method. At all θ , except for the lag time, the estimated solid lines were in good agree-

FIG. 2. Curves characterizing the desorption of BSA from stainless steel particles at various θ by feeding 0.1M NaOH solution at τ of 2.0 min (40°C). Symbols (θ): $\circ$, 1.0; $\square$, 0.69; $\diamond$, 0.38; $\triangle$, 0.21; ∇ , 0.10.

ment with the experimental data ($R > 0.999$). This indicates the validity of kinetic analysis by using eq. 1 in this experimental system.

Figure 3 shows the estimated Γ_o^f and Γ_o^s as a function of θ . With increasing θ from 0.11 to 1.0, the Γ_o^f increased proportionally from 0.06 to 2.2 mg/m². The Γ_o^s also increased with the increase of θ , but it became close to a saturation value of 0.86 ± 0.10 mg/m² (mean $\pm$ standard deviation) at θ above 0.6. At θ of 0.11 to 0.38, BSA^s was a dominant species on stainless steel surfaces.

FIG. 3. The Γ_o^f and Γ_o^s derived by computer analysis as a function of θ . Symbols: $\circ$, Γ_o^f ; $\triangle$, Γ_o^s .

FIG. 4. The k^f and k^s derived by computer analysis as a function of θ . Symbols: $\circ$, k^f ; $\triangle$, k^s .

The relationship between the estimated k^f or k^s and θ is shown in Fig. 4. The k^f value tended to increase gradually as θ increased from 0.11 to 0.56. At θ above 0.66, an abrupt increase in k^f was observed. The k^s increased slightly with the increase of θ from 0.11 to 0.50, whereas it approached a constant value of 0.0071 ± 0.0012 min⁻¹ at θ above 0.6. The k^s ranged between 0.0003 and 0.0083 min⁻¹, and it was approximately 10 to 20 times lower than k^f .

Effect of cleaning temperatures

Figure 5 shows the desorption curves of BSA ($\theta = 0.86$) at various cleaning temperatures. The rate of BSA desorption increased remarkably with increasing temperatures from 40 to 80°C, resulting in small amounts of BSA remaining on surfaces after 60 min of cleaning. Each of the solid lines was derived from the computer analysis using eq. 1 as described above. At each temperature, a fairly good fit of eq. 1 to the experimental data was obtained ($R = 0.9985$ to 0.9996). The estimated parameters of eq. 1 at each temperature are summarized in Table 1. The Γ_o^f increased gradually with increasing temperatures from 40 to 80°C. On the other hand, the percentage of Γ_o^s decreased and reached about 8% at 80°C. This indicates that the Γ_o^f/Γ_o^s ratio depends on the temperature. The k^f value apparently depended on temperature. The k^s also increased by 1.6 to 1.8 times at temperatures of 50°C to 70°C compared with the k^s at 40°C, whereas it showed no significant temperature-

FIG. 5. Curves characterizing BSA desorption from stainless steel particles at various cleaning temperatures by feeding 0.1M NaOH solution at τ of 2.0 min. Symbols: $\diamond$, 40°C; ∇ , 50°C; $\triangle$, 60°C; $\square$, 70°C; $\circ$, 80°C.

TABLE 1. Kinetic parameters derived from cleaning curves by computer analysis.

Cleaning temperature (°C)	BSA ^f		BSA ^s	
	Γ_o^f (mg/m ²)	k^f (min ⁻¹)	Γ_o^s (mg/m ²)	k^s (min ⁻¹)
40	1.97	0.069	0.66	0.006
50	2.01	0.122	0.62	0.011
60	2.21	0.176	0.42	0.011
70	2.38	0.240	0.25	0.010
80	2.42	0.280	0.21	0.006

All of data were average values of two individual experiments.

dependence, probably due to a gradual decrease in Γ_o^s values with the increase in temperature.

The relationship between k^f and temperature was represented by Arrhenius plot (Fig. 6):

$$\ln k^f = -E_a/RT + \ln A \quad (2)$$

where E_a is the apparent activation energy, kJ/mol; R is the gas constant, 8.314 J/K · mol, T is the temperature, K; and A is the constant. A linear relationship between $\ln k^f$ and $10^3/T$ existed, from which the E_a and A were estimated to be 33 kJ/mol and 2.8×10^4 min⁻¹, respectively. By substituting these values into eq. 2, the k^f values were calculated to be 0.074 min⁻¹ at 40°C, 0.110 min⁻¹ at 50°C, 0.161 min⁻¹ at 60°C, 0.229 min⁻¹ at 70°C, and 0.319 min⁻¹ at 80°C, respectively. It was noted that the k^f increased by a factor of approximately 1.4 for every 10°C rise in temperature.

FIG. 6. Arrhenius plot of the k^f values. For cleaning conditions, see legend for Fig. 5.

DISCUSSION

Nonlinear regression analysis using eq. 1 showed that the curves characterizing the desorption of irreversibly adsorbed BSA from stainless steel particles at various θ or at various temperatures could be successfully reduced to the sum of two independent and simultaneous first-order reactions occurring at a different rate. It was suggested to be convenient for a kinetic approach to the nonlinear cleaning curve to assume the presence of two major groups, i.e., a faster-desorbing BSA^f and a slower-desorbing BSA^s, on stainless steel particles. The relative abundance and first-order rate constants of BSA^f and BSA^s depended on given cleaning conditions.

In this study, BSA was adsorbed in an irreversible fashion on stainless steel particles at all θ . The saturation amount adsorbed was 3.0 mg/m². Norde and Giacomelli (2000) calculated the maximum amount of the BSA adsorbed of around 4 mg/m² for a closely packed monolayer based on the assumption that the BSA molecule can be approximated by an equilateral triangle with a side of ~ 8 nm and a depth of ~ 3 nm. The saturation amount obtained in this study was less than this calculated value. Judging from the irreversibility of BSA adsorption and the saturation amount, both BSA^f and BSA^s are thought to constitute less than the coverage of one monolayer.

The BSA^s was found to have desorption rate constants (k^s) 10 to 20 times lower than those (k^f) of BSA^f under the cleaning conditions in this study. The difference between k^f and k^s reflects the difference between the resistance of BSA^f and BSA^s to the cleaning with 0.1 M NaOH at 40°C. Sodium hydroxide serves as a source of hydroxide ion (OH⁻), which exerts cleaning power. OH⁻ ions are believed to adsorb on most solid surfaces and proteins (= desorption of H⁺), thereby giving their surfaces more negative charges and creating an electrostatic repulsion between the proteins as well as between the protein and solid surfaces (Koopal, 1985). The swollen and hydrated protein was solubilized and dispersed into the NaOH solution at a mild temperature. When desorption of protein proceeds according to the solubilization mechanism, the rate of protein desorption would be determined by the magnitude of the protein–solid surface interactions. BSA may adsorb in two or more distinct orientations, e.g., in end-on and side-on configurations (Fair and Jamieson, 1980; Norde and Lyklema, 1978) and further undergo conformational changes on solid surfaces (Kondo et al. 1991; Norde and Giacomelli, 2000; Wahlgren and Arnebrant, 1991). This implies the distribution of the binding strength of adsorbed BSA molecules. The

binding strength depends on the conformation of BSA and hence the number of interacting points, mode of interaction, and heterogeneity of stainless steel surfaces. It is reasonably assumed that BSA^s may be adsorbed on stainless steel surfaces by a larger binding strength than BSAⁱ or adsorbed on high-affinity sites on the surface.

Γ_o^s value increased proportionally to θ , and the k^i value increased abruptly at θ above 0.66 (Figs. 3 and 4). Similar results were observed in previous studies concerning the adsorption or desorption behavior of BSA on alumina surface (Urano and Fukuzaki, 2000 and 2002). We previously reported that the isotherms for BSA adsorption onto the stainless steel surfaces showed a small step at θ of 0.8, above which the relative number of ion pairs between BSA and stainless steel decreased (Fukuzaki et al., 1998). These findings were interpreted as a conformational change of BSA in a closely packed monolayer. Proteins are reported to undergo conformational changes on solid surfaces from a flattened conformation to an extended conformation as θ increases (Morrissey, 1977; Urano and Fukuzaki, 2000). It seems that a more extended conformation with fewer interaction points is readily desorbed by cleaning with the NaOH solution. On the other hand, the fact that the k^s was nearly constant and Γ_o^s value reached a saturated value corresponding to θ of 0.2 suggests that BSA^s maintaining a large binding strength coexist in an adsorbed layer on stainless steel surfaces at all θ .

The temperature could affect the rate of BSA desorption and the overall cleanability (Fig. 5). It was shown that the k^i increased by approximately 1.4 times for every 10°C rise in temperature and the estimated E_a was 33 kJ/mol. In addition, the percentage of Γ_o^s decreased gradually from 25% to 8% with increasing temperatures from 40 to 80°C (Table 1). This indicates that some of the slower-desorbing BSA^s were converted into a faster-desorbing BSAⁱ at high temperatures. As a result, the effect of temperature on the k^s was not clearly reflected although the k^s tended to increase at high temperatures. In general, the removal of proteinaceous soils by NaOH solution is enhanced with the increase in temperature (Graßhoff, 1988; Jennings, 1959; 1963; Takahashi and Fukuzaki, 2003). The combination of sodium hydroxide and temperature brings about the hydrolysis of proteins, thereby accelerating removal of the protein. Jennings (1959) studied the effect of temperature on the rate of removal of milk films from stainless steel in the initial stage of cleaning with 0.05M NaOH solution. His study demonstrated that the first-order rate constant had an Arrhenius-type temperature

dependence and increased by 1.6 times for every 10°C rise in temperature. Furthermore, Jennings (1963) obtained the E_a of approximately 27 kJ/mol for the same cleaning system as the earlier report (Jennings, 1959). These data are in good agreement with the results obtained in this study.

The kinetic approach presented here provides an analytical way to estimate the kinetic parameters from a nonlinear cleaning curve, and will be widely applicable to a wide range of soils, solid surfaces, and cleaning conditions.

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