Synergistic Action of Temperature and pH Factors in the Cleaning of Fatty Acids Soils; Analyzed by Probability Density Functional Method

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Abstract: In this study, the interaction between the temperature and the pH of soil containing fatty acids with sodium dodecyl sulfate (SDS) aqueous solution was investigated to elucidate their synergistic effect in cleaning. A tergotometer was used for the cleaning test, and the cleaning results were analyzed by the probability density functional method, using the calculated parameter, $\mu_{rl}$, as an index of the cleaning power. The increase in $\mu_{rl}$ by one of the factors was defined as $\Delta X$ or $\Delta Y$ and the increase in $\mu_{rl}$ by the both factors was defined as $\Delta (X + Y)$. It is assumed that there is a synergistic effect when $\Delta (X + Y) > \Delta X + \Delta Y$. The cleaning of fatty acid stains followed the addition rule pertaining to mechanical force and the pH effect. However, synergy was observed between the temperature and the pH effect. This was also supported by the plot of $\mu_{rl}$ vs $1/T$ and observations using a phase-contrast microscope.

Key words: oily soil, kinetics, washing, laundry, synergy, first-order equation

1 Introduction

Cleaning is a complex phenomenon involving various factors, such as surfactant concentration, pH, temperature, and mechanical force. When considering its mechanism, it is useful to understand the interactions, that is, the additive, synergistic and offsetting effects between each condition. However, there are relatively few research studies on interactions in the cleaning process.

Studies aimed at clarifying interactions like synergism have been developed mainly in the field of biochemistry, specifically in pharmacy, to clarify the effects of the concentrations of two different chemical substances. The most typical methods for determining the interactions between two chemicals are the isobologram and the CI method. These methods can be used to determine the interaction of two drug components, using doses with specific efficacy levels, such as half a toxic dosage or half a lethal dosage. These are fairly well-established methodologies; numerous studies using these methods were reviewed by Tallarida and Huang.

Other studies in various fields have witnessed different approaches. Selvakumar compared the antioxidant effects of ginger tea and green tea individually or in combination, and concluded that the antioxidant effects are synergistic. Pasqualino stated that there is a synergistic effect on the biodegradability of diesel and biodiesel after comparing their biodegradability separately and in combination. Mobin stated that sodium dodecyl sulfate and cetyltrimethylammonium bromide have a synergistic effect on the suppression of L-methionine corrosion. Furthermore, Song studied the effect of increasing the reducing sugar content of corn and observed a synergistic effect between cellulase and xylanase. Jadidi and Bera examined the synergistic effects of two-component mixtures of anionic-nonionic and anionic-cationic surfactants on their surface activity. Yamamoto presented a synergistic enhancement in the refolding yields of modified and reduced lysozymes by using surfactants as aggregation inhibitors and water-miscible organic co-solvents as surfactant modulators.

Normally, synergistic, additive, and offsetting effects were determined by mixing two chemical substances. For example, when comparing the effects of substance $A$ and $B$, separately and that of mixing half the amount of substances $A$ and $B$, a synergistic effect is recognized when the results of mixing are highly desirable.

The method proposed in this paper can be used for de-
termining whether any observed effects are synergistic, additive, or offsetting in nature. For example, it can determine not only the effect of substance concentration, but also the effects of temperature, pH, mechanical force, etc. on the interaction. This is a detergency analysis method that employs the probability density functional theory technique developed by the authors’ group.

It is a kinetic analysis assuming that the soil adhesion and soil removal forces follow a normal distribution. It was concluded from our previous work that the cleaning results can be analyzed using the probability density function with oily pigments in soil\(^{10}\), iron(III) oxide particles in soil\(^{11}\), and test fabric treated with mixed soil\(^{12}\). Subsequently, we showed that one of the two parameters \(\sigma_d\) was related to the type of soil\(^{13}\) and soil removal mechanism\(^{14}\). Using the parameter \(\mu_d\) calculated from the cleaning result of a hemoglobin stain, an additive rule was observed between the pH and temperature, and between the surfactant concentration and the temperature\(^{15}\).

In this paper, we focused on fatty acid in soil to observe a synergistic effect using a probability density functional method. Solid fatty acid stains can be removed by solubilization with an aqueous surfactant solution\(^{16-18}\); however, under alkaline conditions, the removal proceeds by the production of a fatty acid soap by neutralization\(^{19-21}\). Moreover, other studies have shown that the detergency increases significantly as the temperature rises\(^{22-24}\).

From these results, it can be expected that a synergistic effect can be obtained by manipulating two factors, namely pH and temperature. Therefore, in this study, the effect of pH and temperature on the cleaning of fatty acid soils were analyzed using the probability density functional method. Furthermore, the cause of the synergistic effect was verified by phase-contrast microscopy.

2 Experimental Procedures

2.1 Materials
The materials used to prepare the artificially soiled fabric were cotton (Cotton Science Association in Japan), oleic acid (Fujifilm Wako Chemicals), palmitic acid (Fujifilm Wako Chemicals), and Sudan IV (Fujifilm Wako Chemicals). Sodium hydroxide (Fujifilm Wako Chemicals, guaranteed grade) was used to adjust the pH of the cleaning solution, and sodium dodecyl sulfate (SDS, Fujifilm Wako Chemicals, for Biochemistry) was used as a surfactant.

2.2 Preparation of soiled fabric
Approximately 250 g of cotton fabric was soaked in 2 L of a 0.5% aqueous sodium carbonate solution at 60–70°C, then rinsed with distilled water, dehydrated, air-dried, and cut into \(5 \times 5 \text{ cm}^2\) samples. The soiling liquid was prepared by dissolving 9 g of oleic acid, 1 g of palmitic acid and 0.02 g of Sudan IV in 100 g of toluene. Subsequently, a total of 500 µL of the mixture was applied to multiple places on the piece of cotton fabric to render the soiling state uniform. After that, it was stored in an incubator (20°C) for 1 d before commencing with the cleaning test.

2.3 Cleaning test and calculation of cleaning efficiency
For each cleaning solution having a different pH, an appropriate amount of aqueous sodium hydroxide solution was added to a 0.6% SDS aqueous solution and diluted with distilled water to make the SDS concentration 0.3%. The pH was measured with a pocket-sized pH/temperature meter (Milwaukee pH 56, 0.05 pH accuracy). The final pH adjustment was carried out by adding a weakly stirred aqueous solution of sodium hydroxide in a washing device cup while adjusting the temperature.

A tergotometer (Daiei Kagaku, TM-4) was used for the cleaning test, and five test fabrics were washed with 1 L of cleaning solution. The washing time was 5 min per unit in four repeated washing experiments. In the experiment of varying the pH and mechanical force, the SDS concentration was 0.3% and the temperature was 30°C; while observing the influence of pH and temperature, the agitation was adjusted to 80 rpm.

The surface reflectance was measured by stacking five contaminated fabrics of the same color using a digital color difference meter (ZE-2000, Nippon Denshoku Industries Co., Ltd.). The \(K/S\) value was calculated using the Kubelka-Munk equation (Eq. 1), where \(R\) is the average value of the surface reflectance (Y value) of the front and back surfaces of the fabric. The detergency (\(D\%\)) was calculated using the \(K/S\) value derived from the surface reflectance (Eq. 2),

\[
K/S = \frac{(1 - R)^2}{2R} \quad (1)
\]

\[
D(\%) = \frac{K/S_0 - K/S_w}{K/S_0 - K/S_b} \times 100 \quad (2)
\]

where, \(K\) is the light absorption coefficient, \(S\) is the light diffusion coefficient, \(D\) is the cleaning efficiency, \(K/S_0\) is the \(K/S\) value of the soiled fabric, \(K/S_w\) is the \(K/S\) value of the washed fabric, and \(K/S_b\) is the \(K/S\) value of the raw white fabric.

It was confirmed in advance that the \(K/S\) detergency (%) of the soiled fabric prepared by this method and the detergency (%) obtained by GC analysis exhibited a linear relationship. Therefore, the \(K/S\) detergency was converted to the detergency (%) in units of mass of fatty acids using a calibration curve. In the GC analysis, the sample was extracted with toluene, methyl-esterified by the boron trifluoride methanol method, and detected by a flame ionization detector (FID).
2.4 Calculation of two parameters by provability density functional method

This method assumes that both the soil adhesion force and the cleaning force follow a normal distribution, and calculates the two parameters, $\sigma_{rl}$ and $\mu_{rl}$, from the time-dependent changes in the detergency. The two parameters were calculated in the same way as previously reported\(^{13-15}\).

The $\sigma_{rl}$ parameter is related to how the cleaning rate decreases during the cleaning process. If $\sigma_{rl}$ is small, the cleaning speed is high at the initial stage, but decreases sharply thereafter. On the other hand, when $\sigma_{rl}$ becomes large, the cleaning speed does not exhibit a significant decrease even after the cleaning time elapses.

In addition, $\sigma_{rl}$ is related to the dirt removal mechanism, and as $\sigma_{rl}$ increases, the cleaning mechanism changes in the following order: (1) solid particle dirt removal behavior; (2) dissolution of water-soluble dirt having affinity with the substrate; (3) solubilization of oily stains in the surfactant solution; (4) emulsification of oily soil. In contrast, the $\mu_{rl}$ value represents the level of detergency, and as the detergency rate generally increases, $\mu_{rl}$ also increases; if the $\sigma_{rl}$ value is constant, the $\mu_{rl}$ value also increases as the detergency increases.

Because the $\mu_{rl}$ value varies depending on the magnitude of the $\sigma_{rl}$ value, it is desirable to use a $\mu_{rl}$ value calculated under constant $\sigma_{rl}$ values when comparing different $\mu_{rl}$ values. Therefore, each $\mu_{rl}$ value was calculated based on the average value of $\sigma_{rl}$ obtained in the cleaning test.

2.5 Macroscopic observation of emulsification and solubilization

A pH-adjusted solution of SDS (20 mL, 3.0 g/L) was placed in a test tube. Four SDS solution conditions were set: [20°C / pH 7], [20°C / pH 11], [50°C / pH 7], and [50°C / pH 11]. To obtain a temperature of 50°C, the SDS solution was heated using a water tank incubator (FTB-01, YAMATO). Following this, 100 µL of oleic acid was added dropwise to the test tube with shaking. The test tube was allowed to stand and the state of the sample was observed.

2.6 Microscopic observation of dissolution or solubilization

In this experiment, 80 µL of oleic acid was placed on a glass slide, and 80 µL of a 0.3% SDS solution prepared under the conditions mentioned above was added dropwise onto the oleic acid. Subsequently, the mixed state was observed with a phase-contrast microscope (BX-51, YAMATO), and an image was captured with a microscope camera (microscope digital-DP-12, YAMATO). The SDS solution was prepared under four conditions: [20°C / pH 7], [20°C / pH 11], [50°C / pH 7], and [50°C / pH 11]. For 50°C, the SDS solution was warmed using a water bath, and the glass slide was warmed in a drying oven (DG400, YAMATO). With the glass slide still in the drying oven, oleic acid was added onto it and it was brought into contact with the SDS solution.

3 Results and Discussion

3.1 Detergency under various conditions of pH and mechanical power

Time-dependent curves of the detergency obtained by varying the pH in four increments to obtain values of 9.2, 10.4, 10.9, and 11.1 respectively, and with mechanical stirring values of 30 rpm, 80 rpm, and 130 rpm respectively, are shown in Fig. 1. In this experiment, no change in pH was observed before and after washing. In any mechanical power condition, the washing efficiency continuously increases with the washing time; this was observed here, i.e., detergency increased with increasing pH and mechanical power.

When the results of the cleaning test were analyzed by the probability density functional method, the fluctuation of $\sigma_{rl}$ due to the change in pH was larger than the fluctuation of $\sigma_{rl}$ due to the change in the mechanical force (Table 1). Furthermore, as the pH increased, $\sigma_{rl}$ increased sharply.

The $\sigma_{rl}$ parameter is related to the cleaning mechanism; $\sigma_{rl}$ varies between 0.01–0.6 when removed by the detachment of solid particle soils, 0.3–1.4 when removed by the dissolution of water-soluble soil into water, 1.0–2.0 when removed by the solubilization of oily soil into micelles, and 3.0 or higher when removed by the emulsification or dispersion of oily soil\(^{14,15}\).

The dissolution mechanism in water is complicated because the soil samples used in this study are not water-soluble. It is presumed that as the pH increases, the cleaning mechanism shifts from the detachment of solid particle soil by mechanical force to the solubilization of oily stains by surfactants and alkaline agents.

3.2 Verification of addition rule of pH effect and mechanical power effect

Increased pH and mechanical strength have the effect of increasing the cleaning efficiency under these experimental conditions. Here, the effects of increased pH, increased mechanical force, and that of simultaneously increased pH and mechanical force are expressed by $\Delta D_{(pH+Mec)}$, $\Delta D_{(pH)}$, and $\Delta D_{(Mec)}$, respectively. The interaction between pH and the mechanical force, that is, the additive, synergistic, or offsetting effect, can be expressed by the following relational expressions\(^{16}\):  

Additive effect: $\Delta D_{(pH+Mec)} = \Delta D_{(pH)} + \Delta D_{(Mec)}$

Synergistic effect: $\Delta D_{(pH+Mec)} > \Delta D_{(pH)} + \Delta D_{(Mec)}$

Offsetting effect: $\Delta D_{(pH+Mec)} < \Delta D_{(pH)} + \Delta D_{(Mec)}$
Consequently, cleaning experiments were conducted under 12 conditions in which the pH and the mechanical force were varied, and each cleaning efficiency was processed by the probability density functional method to obtain the $\sigma_{rl}$ parameter. The $\mu_{rl}$ value of each condition was calculated using the mean value of $\sigma_{rl}$ to investigate if an additive effect was established in this case (Fig. 2).

For example, if the cleaning conditions are changed from $\text{pH 10.4} / 30 \text{ rpm} \rightarrow \text{pH 10.9} / 80 \text{ rpm}$, the effect of increasing only the pH is $\Delta D_{(\text{pH})} = 0.83$, and the effect of increasing the mechanical force only is $\Delta D_{(\text{Mec})} = 0.26$. The sum of both effects is calculated as $\Delta D_{(\text{pH})} + \Delta D_{(\text{Mec})} = 1.09$. Meanwhile, the effect calculated from the experimental values obtained when both the pH and mechanical force are increased is $\Delta D_{(\text{pH},\text{Mec})} = 1.05$. It is evident that the calculated and the measured values are nearly equal.

Upon examining the addition law in six cases, we found a clear addition law in five of them. However, under one condition of $\text{pH 9.2} / 30 \text{ rpm} \rightarrow \text{pH 10.4} / 80 \text{ rpm}$, the calculated value was significantly lower than the measured value. The effect of increasing the mechanical force from 30 rpm to 80 rpm at pH 9.2 is $\Delta D_{(\text{Mec})} = 0.02$, which is unnaturally small. It can be estimated that the cleaning effect at $\text{pH 9.2} / 30 \text{ rpm}$ should be slightly higher. This might be a result of the low cleaning rate and the tendency for errors to occur under conditions of low pH and mechanical force. In the other cases, a fairly clear agreement is obtained, so it can be judged that there is an additive effect between the pH and the mechanical force in cleaning fatty acid soil.

### Table 1

Calculated $\sigma_{rl}$ and $\mu_{rl}$ (assuming $\sigma_{rl} = 0.97$) obtained from cleaning test of fatty acid soiled cloth varying pH and mechanical power.

| pH   | 30 rpm |   | 80 rpm |   | 130 rpm |   |
|------|--------|---|--------|---|---------|---|
|      | $\sigma_{rl}$ | $\mu_{rl}$ | $\sigma_{rl}$ | $\mu_{rl}$ | $\sigma_{rl}$ | $\mu_{rl}$ |
| 9.2  | 0.11   | -0.9 | 0.14   | -0.88 | 0.15     | -0.72 |
| 10.4 | 0.42   | -0.6 | 0.55   | -0.34 | 0.65     | -0.17 |
| 10.9 | 1.26   | 0.23 | 1.42   | 0.45  | 1.56     | 0.65  |
| 11.1 | 1.88   | 0.68 | 1.97   | 0.97  | 1.49     | 1.19  |

Fig. 1  Time dependent detergency curves of fatty acid soil obtained with respect to various pH and agitation levels (soiled fabrics were washed with a tergotometer at 30°C, and pH was controlled with NaOH).

3.3 Detergency under various conditions of pH and temperature

The interaction between the temperature and the pH
was examined under a constant agitation condition of 80 rpm. Figure 3 shows the time-dependent curves of detergency obtained by changing the temperature in four increments: 20°C, 30°C, 40°C, and 50°C, and changing the pH in

Fig. 2 Determination of additive effect between the pH increase and agitation increase in fatty acid cleaning: The increase in $\mu_r$ due to an increase in pH is expressed as a vertical value, and the increase in $\mu_r$ due to an increase in agitation is expressed as a horizontal value. In addition, the increase in $\mu_r$ due to an increase in the pH and agitation is expressed as a diagonal arrow. Moreover, additivity is confirmed if the calculated value (vertical value + horizontal value) is significantly close to the corresponding experimental value (value of diagonal arrow).

Fig. 3 Time dependent detergency curves of fatty acid soil obtained with respect to various pH and temperature levels (soiled fabrics were washed with a tergotometer under 80 rpm agitation, and pH was controlled with NaOH).
The cleaning efficiency increased continuously with cleaning time and the cleaning power increased with increasing pH and temperature. Under the conditions of 50 \( ^\circ \)C and pH 11.0, the cleaning rate reached 100% after 5 min of washing.

Upon analyzing the cleaning test by the probability density functional method, the change in the value of \( \sigma_{rl} \) with pH was found to be larger than that of \( \sigma_{rl} \) with temperature (Table 2). It is presumed that this is because the cleaning mechanism changed from the detachment of solid particle soil by mechanical force to the solubilization of oily soil by surfactants and alkaline agents as the pH increased, similar to the previous case.

### Table 2

Calculated \( \sigma_{rl} \) and \( \mu_{rl} \) (assuming \( \sigma_{rl} = 0.78 \)) obtained from cleaning test of fatty acid soiled cloth varying pH and temperature.

| pH | 20°C | 30°C | 40°C | 50°C |
|----|------|------|------|------|
| 6  | 0.35 | -0.77| 0.40 | -0.48|
| 10 | 0.42 | -0.71| 0.62 | -0.35|
| 10.5| 0.75 | -0.41| 0.61 | 0.05 |
| 11 | 0.98 | -0.09| 1.31 | 0.6  |

Fig. 4 Determination of synergistic effect between the pH increase and temperature increase in fatty acid cleaning: The increase in \( \mu_{rl} \) due to an increase in temperature is expressed as a vertical value, and the increase in \( \mu_{rl} \) due to an increase in pH is expressed as a horizontal value. In addition, the increase in \( \mu_{rl} \) due to an increase in the pH and temperature is expressed as a diagonal arrow. Synergy will be confirmed if the experimental value (value of diagonal arrow) is greater than the corresponding calculated value (vertical value + horizontal value).

3.4 Verification of addition rule of pH effect and temperature effect

Increased pH and temperatures are expected to enhance the cleaning effect of fatty acids by increasing the reaction rate. Therefore, the interaction between the pH and the temperature was investigated based on \( \mu_{rl} \) calculated by the probability density functional method with \( \sigma_{rl} = 0.78 \). The \( \mu_{rl} \) value was calculated to be greater than 5.0, as the cleaning efficiency reached 100%, after 5 minutes of cleaning at 50\( ^\circ \)C and pH 11. As a result, the measured values were larger than the calculated values in all nine cases (Fig. 4). This is the state where \( \Delta D_{pH + Temp} \geq \Delta D_{pH} + \Delta D_{Temp} \), indicating that there is a synergistic effect between the pH and temperature. In particular, the synergistic effect tended to increase as the temperature approached 50\( ^\circ \)C and pH 11.
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3.5 Discussion from the $\mu_{rl}$ plot for 1/T

When the effects of temperature and pH on protein washing were plotted against 1/T, they exhibited a linear relationship. Similar results were obtained when the effect of SDS concentration vs pH was examined. These results show that $\mu_{rl}$ is an index that can be treated in the same way as the rate constant.

Next, the results of this study were processed similarly, and it was found that all the data were expressed in a clear linear relationship in the range of pH 6 to pH 10.5. Furthermore, the slope of the straight line increased as the pH increased (Fig. 5). This implies that as the pH increases, the temperature dependence increases. Moreover, when the pH reached 11.0, a straight line was observed in the temperature range between 20°C and 40°C, and the slope was greater than when the pH was low. In addition, the slope between 40°C and 50°C increased further. This indicates that when the pH reaches 11, the temperature dependence increases, and at around 50°C, the removal proceeds at a significantly different rate than before. As described above, it was confirmed that the temperature dependence increased with changing pH, and that the reaction proceeded more rapidly when the temperature reached 50°C. Therefore, it can be concluded that there is a synergistic effect between the temperature and pH in fatty acid removal.

3.6 Observation of emulsification, solubilization and dissolution of fatty acid soil from a macroscopic perspective

Figure 6 shows the state after mixing oleic acid with 0.3% SDS aqueous solution under various conditions and stirring. From the left, the conditions are [20°C / pH 7], [20°C / pH 11], [50°C / pH 7], and [50°C / pH 11]. At [20°C / pH 7], strong cloudiness was observed in these solutions, indicating complete emulsification. In contrast, at [20°C / pH 11], it is slightly transparent. Presumably, this is because under alkaline conditions, both emulsification and oleic acid neutralization for soap formation and dissolution occur. Furthermore, at [50°C / pH 11], the entire solution becomes transparent, indicating that oleic acid is in a dissolved state rather than an emulsified one. Therefore, from a macroscopic point of view, the oleic acid removal mechanism changes from emulsification to dissolution or solubilization as the temperature and pH increase.

3.7 Observation of emulsification, solubilization and dissolution of fatty acid soil from a microscopic perspective

Figure 7 shows a comparison between bright-field and phase-contrast observations using a microscope. In the bright field observation, only the boundary between the aqueous phase and the oil droplets could be observed. However, in the phase-contrast observation, the state where the oil begins to dissolve can be observed from the interface. This confirms that the state of dissolution and solubilization can be observed by phase-contrast observations.

Figure 8 shows images captured by a phase-contrast microscope in two different locations and at each condition under which the sample was observed. At [20°C / pH 7], the oil droplets formed clean spheres and did not dissolve in the aqueous phase. At [20°C / pH 11] and [50°C / pH 7], the oil dissolved in the aqueous phase, as observed from the surfaces of some oil droplets. At [20°C / pH 11], it is
Fig. 7  Comparison between bright-field observation and phase-contrast observation.

Fig. 8  Images captured by a phase-contrast microscope of how oleic acid oil droplets change in an SDS aqueous solution under various conditions.
presumed that the oleic acid became sodium oleate and dissolved under alkaline conditions. Furthermore, at[50°C / pH 7], the oleic acid appears to be completely solubilized by SDS. It contrasts, at[50°C / pH 11], none of the oil droplets were forming clean spheres, and most oils dissolved or solubilized rapidly. Thus, by using a phase-contrast microscope, it was possible to observe that the oil was completely dissolved in the aqueous phase as the temperature and pH increased. Both macro and micro observations show that fatty acid stains were synergistically removed with an increase in the temperature and pH.

4 Conclusion

In this study, the interaction between the pH and temperature in the cleaning of fatty acid stains was analyzed using the probability density functional method. A synergistic effect was confirmed because the cleaning effects of the simultaneous increase in the pH and temperature are greater than the sum of the effects when each is used separately. It was speculated that solubilizing the soil and the dissolving action of the soap formation worked together.

Conventional studies of interactions have often been conducted on the effects of two chemical substances at varying concentrations. However, by using the \( \mu_{d} \) parameter calculated using the probability density functional method, the effects of the mechanical force and the temperature can also be calculated separately. From this, it is expected that this method can be widely used for analyzing interactions of the additive, synergistic, and offsetting nature. It is a powerful method to evaluate the interaction of various elements that exist in complex phenomena, such as cleaning.

Acknowledgment

The authors thank Mr. Yosuke Taniguchi for his experimental assistance. This work was supported by Grant-in-Aid for Scientific Research (B) (17H01953) from Japan Society for the Promotion of Science (JSPS).

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