Derivation of Quantitative Removal Efficiency of Protein Stain from K/S Value of Washing Test Fabric Soiled with Hemoglobin

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1 INTRODUCTION

Cleaning of protein stains is important in many industrial and household fields such as the food processing industry, pharmaceutical processing industry, maintenance of dental and surgical equipment, contact lenses cleaning, dish washing and laundry. In recent years, many laundry detergents containing protease have been developed in order to improve their stain removal efficiency. Nevertheless, a robust method for testing the cleaning power of novel detergents has not been developed.

The efficiency with which protein stains can be removed from solid surfaces has been studied using various methods. Sakiyama et al. conducted an experiment in which cleaning liquid was passed through a glass column in which stainless steel particles soiled with protein were packed. The test particles were prepared by immersing them in protein solution and discarding the supernatant, and the Lowry method was used for quantifying the protein. Turner et al. conducted a cleaning test using glass slides soiled with casein and hemoglobin. The test samples were heated at 100°C, and the protein was quantified by the visible light absorption, UV absorption, and ninhydrin reaction method. Gordon et al. prepared protein films by baking egg yolk and calculated the cleaning efficiency on the basis of the thickness of the films. Like these studies, the method of quantifying protein and the protein heating processes are major subjects in this field, but no standard test for measuring removal of protein soils adhered on solid surfaces has been developed.

More complex factors can affect the efficiency with which protein soils adhered on fibrous substrates are removed. EMPA 111 is a commercially available soiled fabric that is soiled with blood as a model protein soil that is used in laundry tests. However, removal efficiency should ideally be evaluated on the basis of reflectance without considering the quantity of the protein soil. Banerjee et al. evaluated the cleaning efficiency of blood-soiled fabrics by visual examination without showing numerical values.

To quantify the adherence of protein soil on fibers, the protein soil is generally extracted using liquids such as alkanis, and the extraction liquid is measured by chemical reaction analysis such as the Kjeldahl method, Biuret method, bicinchoninic acid assay, and Lowry method.

Abstract: We have improved a previous method for the preparation of hemoglobin-soiled fabrics in order to facilitate quantitative calculation of the efficiency with which protein stains can be removed from such materials. We then evaluated the sensitivity of surface reflectance as a method for stain quantification. Test fabrics were made by spotting a white fabric with a certain amount of hemoglobin solution and drying it. We observed a large difference between the percentage stain removal as measured by surface reflectance when compared with chemical analysis. Deformities in the surface of the soiled fabric caused by capillary action in the drying process likely contributed to this difference. Quantitative removal percentage could be predicted easily from the K/S values of test fabrics that were dry-heated without steam, although soil adhesion was too weak to evaluate the washing power of commercial detergent. Overall, we found that practical test fabrics with adequate soil adhesion properties can be prepared by adopting a steam heating process after dry heating.

Key words: washing test, soiled fabric, protein soil, detergent, K/S value, surface reflectance
However, these methods are time-consuming and laborious. Minagawa et al. used the Lowry method to quantify the proteins soil on test fabrics prepared by immersion into wheat gluten solution, squeezing with a mangle and drying at room temperature\(^\text{10}\). Tokoro et al.\(^\text{11-13}\) conducted washing tests using cow’s blood as a model protein soil according to the Minagawa’s method. However, no attempt was made to evaluate the amount of the protein soil on the basis of appearance. Additionally, there have been no studies on the effect of the drying conditions on the state of soiled fabrics.

These problems can be resolved by developing a method of calculating the quantitative removal efficiency via the surface reflectance. In our previous study\(^\text{14}\), a method for the preparation of hemoglobin-soiled fabric was proposed, and the relationship between the 2 removal percentages, 1 that is calculated using K/S values derived from the reflectance and the other that is obtained by chemical analysis, was discussed. The results showed that although there was a correlation between the 2 methods, there was a relatively large difference in the absolute value of the removal percentages. In this paper, the method of preparing washing test fabric soiled with hemoglobin was improved by changing the drying method. This yielded a better relationship between the K/S values derived from the reflectance and the protein quantity.

2 EXPERIMENTAL

2.1 Preparation of soiled fabrics

2.1.1 Fabrics

Cotton broad (Shikisensha, #40) with a thread density of 130/inch (warp) × 70/inch (weft) was used as a test fabric substrate. Desizing of the fabric was conducted using an aqueous solution of 0.5% sodium carbonate (120 g/L) at 70-80°C for 60 min. The fabric was cut into 5 cm × 5 cm pieces after being dried at an ambient room temperature.

2.1.2 Soiling liquid

Soiling liquid was prepared by dissolving hemoglobin (Wako, from Bovine) in 0.1 N ammonia aqueous solution. The concentration of hemoglobin was adjusted to 2%, except for the experiments examining the effect of hemoglobin concentration. A fabric was spread on a pinholder and 400 μL of soiling liquid was uniformly dripped onto the fabric using a micro pipet.

2.1.3 Drying conditions

We used the steaming method proposed in our previous study\(^\text{14}\). Briefly, soiled fabrics were heated using a steam heater for 30 min after drying at ambient room temperature for 30 min.

In this paper, soiled fabrics were dried under 2 temperature conditions; normal room temperature and 150°C in a box type dryer (Toyo Engineering, DRD 320DA). Heat drying was conducted using a drying rack on which test fabrics were placed. Comparisons were conducted among 3 types of drying rack, the top faces of which were, metal net, silicone coating sheet (cooking sheet, Asahi Kasei Home Products Co.) and parallel stretched yarns (cotton spun yarn, #90, 20 mm interval) (Fig. 1).

Test fabrics soiled with and without the steam heating process were used in this study. The steam heating was conducted for 10 min using a microwave oven with a steam heating function (Sharp, Helsio AX-2000) in which the fabrics were placed on a metal net rack. The temperature of steam heating process was adjusted to 100 ± 2°C and was checked with a thermo-couple thermometer. The steaming was needed to strengthen the soil’s adhesion to fibrous substrates, because hemoglobin without steam heating tends to be easily removed by washing in a general washing machine and a general-purpose detergent solution.

2.2 Washing test

2.2.1 Washing test using a previously proposed test fabric

Washing test was conducted using this washing test fabric to compare the removal efficiency of different kinds of laundry detergents (Table 1). A01-A07 are weakly alkaline detergents and N01 - N03 are neutral detergents.

Washing liquid was prepared by dissolving commercial detergents into 1 L of distilled water, and the concentration was adjusted to each standard usage. Five pieces of soiled fabric were washed in each experiment. Soiled fabrics dried on a metal net rack were washed using Terg-O-Tometer at 30°C under the condition of agitation at 120 rpm, temperature of 30°C, and a washing time of 5 min. Rinsing was performed for 1 min using tap water. Two types of removal percentages were derived by chemical analysis (Lowry method) and by surface reflectance.

2.2.2 Washing test using test fabrics without steam heating

Three kinds of racks were used to dry the test fabrics soiled with hemoglobin solution. Washing liquid was prepared by dissolving sodium dodecyl sulfate (SDS) or sodium hydrogen carbonate into 1 L of distilled water. Washing test was conducted using Terg-O-Tometer at 30°C. Rinsing was...
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performed for 1 min using tap water. In addition to adjusting the agitation and washing times, the washing conditions were controlled to yield relatively low detergency levels as shown below.

A: 0.3 \( \text{SDS}, 120 \text{ rpm}, 5 \text{ min} \)
B: 0.3 \( \text{SDS}, 80 \text{ rpm}, 5 \text{ min} \)
C: 0.3 \( \text{SDS}, 80 \text{ rpm}, 3 \text{ min} \)
D: 0.3 \( \text{SDS}, 80 \text{ rpm}, 1 \text{ min} \)
E: 0.1 \( \text{NaHCO}_3, 120 \text{ rpm}, 5 \text{ min} \)

2.2.3 Washing test using improved steam heated test fabrics

Test fabrics were prepared by heating soiled fabrics with a steam heater for 30 min after drying at 150\( ^\circ \)C for 2 min without steaming. Soiled fabrics were dried on a yarns rack. Two kinds of alkaline detergents (A03 and A06 in Table 1) represented as Alkaline A and Alkaline B, respectively and 2 kinds of neutral detergents (N01 and N02 in Table 1) represented as Neutral A and Neutral B, respectively were used as test detergents. The detergents were dissolved to obtain standard use concentration by using distilled water. We used 0.1\% NaHCO\(_3\) solution as the washing liquid. Washing tests were conducted using Terg-O-Tometer with an agitation of 120 rpm, temperature of 30\( ^\circ \)C, and washing time of 5 min. Rinsing was performed for 1 min using tap water.

2.3 Derivation of removal percentage from chemical analysis

Chemical quantitative analysis of hemoglobin was conducted according to the Lowry method. Hemoglobin adhered to a soiled fabric before and after washing were extracted using alkaline solution. Erlenmeyer flasks with a common stopper that contained 100 mL of 0.1 N NaOH aqueous solutions were fixed in a shaking water bath, and extraction was performed at 95 \( \pm 2 \)\( ^\circ \)C for 120 min. Then, the hemoglobin was quantified by the Lowry method using the following reagents.

- Reagent A: 2\% Na\(_2\)CO\(_3\) solution (in 0.1 N NaOH aqueous solution)
- Reagent B: 0.5\% CuSO\(_4\)\(_5\)H\(_2\)O solution (in 0.1\% aqueous solution of Potassium Sodium (+)-Tartrate Tetrahydrate)
- Reagent C: alkaline copper solution (50 mL of Reagent A + 1 mL of Reagent B)
- Reagent D: Folin and Ciocalteu’s phenol reagent (diluted 1:1 with water)

We measured 2 mL of the extract into a test tube, and added 10 mL of Reagent C. We added 1 mL of Reagent D after 10 min, and mixed it by stirring. After incubation for 30 min, the absorbance of the test solution was measured at 750 nm using spectrophotometer (V-530 Nihon Denko). A linear relationship between the concentration of hemoglobin and the absorbance was checked preliminarily.

Removal percentages were calculated using the following equation[1].

\[
D(\%) = \frac{(D_i - D_w)}{(D_s - D_w)} \times 100
\]  
[1]

Here, \(D_i\) is the absorption of extracts from non-soiled white fabric, \(D_s\) is the absorption of extraction of test fabric before washing, and \(D_w\) is the absorption of extracts of test fabric after washing.

For validating the uniformity of hemoglobin adhesion, a sample fabric was cut into 2 pieces, 1 of 3.53 cm \( \times \) 3.53 cm \( \approx 12.5 \text{ cm}^2 \), which was cut out from the center portion of the soiled fabric, and other piece is the remaining portion which has an area of about 12.5 cm\(^2\). The amounts of protein soil on both pieces were chemically analyzed as described above.

Table 1 Ingredients of each detergent used in washing test.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surfactant (K-soap 27%, DEA 11%), Carbonate, Solubilizing agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>Surfactant (Na-soap) 99%</td>
</tr>
<tr>
<td>A03</td>
<td>Surfactant (Na-soap) 60%, Carbonate</td>
</tr>
<tr>
<td>A04</td>
<td>Surfactant (Na-soap) 60%, Carbonate, Sequestering agent</td>
</tr>
<tr>
<td>A05</td>
<td>Surfactant (LAS) 14%, Carbonate, Water softening agent, Anti-soil redeposition agent, Softening agent, Enzyme Surfactant ((\alpha)-SF, Na-soap, AE) 21%, Alumino Silicate</td>
</tr>
<tr>
<td>A06</td>
<td>Carbonate, Sulfate, Anti-soil redeposition agent, Enzyme Fluorescent brightening agent, Bleaching agent</td>
</tr>
<tr>
<td>A07</td>
<td>Surfactant ((\alpha)-SF, Na-soap, AE) 21%, Alumino Silicate, Carbonate, Sulfate, Bleaching agent, Enzyme Fluorescent brightening agent, Bleaching agent</td>
</tr>
<tr>
<td>N01</td>
<td>Surfactant (AE) 18%, Dispersant agent</td>
</tr>
<tr>
<td>N02</td>
<td>Surfactant (AE) 22%, Softening agent</td>
</tr>
<tr>
<td>N03</td>
<td>Surfactant (AE) 31%, Softening agent</td>
</tr>
</tbody>
</table>
By conducting experiments with and without heat steaming under several conditions, it has previously been confirmed that differences in the methods of preparing test fabrics have no effect on the values of absorbance.

2.4 Calculation of removal percentage using reflectance

Surface reflectance was measured using a color difference meter ZE-2000 (Nippon Denshoku Ind. Co.). The values of reflectance of front and back sides of a soiled fabric were measured at the center area (φ30 mm). Reflectance was measured by keeping the 5 washed fabrics under the same conditions in a pile. K/S values were calculated from Kubelka-Munk equation[2], and the removal percentage was calculated from equation[3].

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

\[
D(\%) = \frac{(K/S_0 - K/S_w)/(K/S_0 - K/S_s)} \times 100 \tag{3}
\]

Here, R is surface reflectance, K/S₀ is the K/S value of the test fabric before washing, K/Sₚ is the K/S value of the test fabric after washing, and K/Sₚ is the K/S value of the non-soiled white fabric.

3 RESULTS AND DISCUSSION

3.1 Washing test result using previously proposed soiled fabrics

The relationship between removal percentage derived from chemical analysis and that calculated by reflectance was checked for previously proposed test fabrics (Fig. 2).

The washing efficiencies of alkaline detergents were generally greater than those of neutral detergents. In particular, A03 and A04, which contain large amounts of alkaline builder (Na₂CO₃), show high removal efficiency.

The above result is in line with other results from tests of the removal of protein soil. However, the removal percentages calculated from the K/S values of reflectance tended to be considerably greater than those derived from chemical analysis. In order to more accurately devise a method for evaluating the quantitative removal efficiency of protein soil by reflectance, the correlation between the 2 removal percentages should be improved.

3.2 Drying method to improve unevenness of soiled fabric

Deformities in a soiled fabric are likely a major source of the differences between the 2 removal percentage values. Indeed, the color of the outer area of a soiled fabric, especially the edge of the fabric, tends to be darker than that of the inner area (Fig. 3). Many conditions, such as the volume of spotting liquid and spotting methods were investigated, but no improvement was observed. The deformities in the test fabric were observed even if a method of immersion into soiling liquid was adopted during the soiling procedure. By process of elimination we then determined that the uneven surface was caused by the drying procedure. The differences of color between the outer and inner area of a test fabric tended to be greater when the drying time became longer. Therefore, the effect of drying method on the uneven surface of soiled fabrics was investigated.

A soiled fabric prepared by the previously proposed method was cut into 2 pieces- an inner area (35 mm × 35 mm in the center part) and the remaining outer area and the protein soil was quantified by chemical analysis. A relatively wide difference in the 2 quantities was observed (Fig. 4). It could be speculated that the unevenness of soil quantity in a test fabric was caused by variations in drying.

![Fig. 2](image)  
**Fig. 2**  
Removal percentages of 10 kinds of commercial detergents tested with previously proposed soiled fabrics (Drying condition: room temperature for 30 min→steam heating at 100 °C for 30 min).

![Dryed at 150 °C for 2 min without steaming](image) and  
**Dryed at room temperature without dry heating nor steam heating**

**Fig. 3**  
Effect of drying temperature on apparent uniformity in soiled fabric.
speed. Generally, the outer area of a wet fabric can be
dried more rapidly than the inner area. When the outer
area is dried but the inner area is still moist, the liquid
in the inner area will be transferred to outer area by capillary
force. Therefore, the uneven surface may be avoided by re-
ducing the drying time. For this reason, a heat drying pro-
cedure without steaming was investigated.

A metal net rack, silicon sheet rack and parallel yarns
rack were used as drying racks on which soiled fabrics
were placed during the drying process. Immediately, the
metal net rack was found not to be suitable for this usage,
because dark brown lines remaining on the fabrics were
observed after the heat drying procedure. We assume that
the brown lines were raised by the ability of the metal sub-
strate to retain both moisture and heat.

A silicon sheet rack was used in the next step because of
its low propensity to absorb water and its low heat conduc-
tivity, but this attempt also proved to be a failure. Consid-
erably wide differences in surface reflectance values were
measured between the front side (upper surface) and the
back side (lower surface) of the test fabrics (Fig. 5, Fig. 6).
This was probably caused by the difference in drying speed
between the front and back sides. The soiling liquid can be
transferred from the back to the front side by capillary
force, because the front side can be dried more rapidly
than the back side attaching to the silicon sheet.

This problem was resolved using a parallel yarns rack in
the heat drying process. The difference in surface reflect-
ance between the front and back sides was greatly de-
creased compared with the case where a silicon sheet rack
was used.

The improvement in the unevenness in a soiled fabric
was checked by comparing the reflectance values of test
fabrics prepared with and without the heat drying process.

The K/S values of test fabrics with the heat drying process
were greater than those of the fabrics dried at ambient
room temperature (Fig. 7). This trend may be due to dif-
fferences in the amount of soil between the outer area and
inner area of the soiled fabric. However, a linear relation-
ship between K/S values and the soil quantity by chemical
analysis can be observed for test fabrics prepared without
heat drying.

Additionally, little differences in the amount of protein
soil between the inner area and outer area were observed
by chemical analysis (Fig. 8).

3.3 Washing test results using heat dried test fabrics

Soiled fabrics prepared with the heat drying process
(without steam heating) were used for this test. Five pieces
of test fabric were washed in 1 L of washing liquid using
Terg-O-Tometer.

A wide range of removal percentages, from about 30%
to 85%, was obtained from this washing test (Fig. 9). Rela-
tively small differences (less than 5%) were observed
between the 2 removal percentages. Therefore, it can be
concluded that the heat drying process is capable of pre-

**Fig. 4** Comparison between the soil amount of the
inner area and that of the outer area (Drying
condition: room temperature for 30 min→steam
heating at 100°C for 30 min).

**Fig. 5** Appearance of test fabric dried on a silicon sheet
(Drying condition: dry heating at 150°C for 2
min without steam heating).

**Fig. 6** Comparison between the surface reflectance of
the test fabrics prepared using silicon sheet and
paralleled stretched yarns as drying rack (Drying
condition: dry heating at 150°C for 2 min without
steam heating).

**Fig. 7** Comparison between the soil amount of the
inner area and that of the outer area (Drying
condition: room temperature for 30 min→steam
heating at 100°C for 30 min).

**Fig. 8** Comparison between the soil amount of the
inner area and that of the outer area (Drying
condition: room temperature for 30 min→steam
heating at 100°C for 30 min).
paring uniformly soiled test fabrics, of which reflectance can be used to predict the quantitative removal percentage.

3.4 Washing test results using improved test fabrics with steaming process

A washing test was conducted using test fabrics heated with steam after a dry heating process. The uniformity of soiled fabrics could be obtained by performing a dry heating process, and the soil adhesion was strengthened (through the steam heating process) to a level suitable for the evaluation of commercial detergents.

There were no significant differences between the removal efficiency derived from chemical analysis and that calculated from K/S values (Fig. 10). Hence, it can be concluded that quantitative removal efficiency can be derived from the surface reflectance of this soiled fabric. Additively,
4 CONCLUSION

Here, we present a new method of preparing washing test fabric soiled with protein. In the preparation method, hemoglobin is used as the model protein soil, and soil liquid is dripped onto the fabrics using a micro pipet; the soiled fabric is then steamed after dry heating process. We found that the test fabric enables the calculation of quantitative removal efficiency using K/S values derived from the reflectance. Finally, the level of removal efficiency can be controlled by adjusting the time of steam heating.

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