Production of Antimicrobially Active Silk Proteins by Use of Metal-Containing Dyestuffs

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ABSTRACT: The work presented here discusses a new technique for preparing silk fibers and films with persistent antimicrobial activity through use of metallic dyestuffs during the fiber dyeing process. The length of the silk fibers investigated contracted when the fibers were immersed in concentrated neutral salt solutions, such as calcium or potassium nitrate, at elevated temperature levels. The birefringence and molecular orientation of the silk fibroin molecules became less ordered by the action of the neutral salt solutions, resulting in increased dyestuff absorption. Subsequently, contracted silk fibers were dyed with metallic dyestuffs containing Cr or Cu for the purpose of obtaining silk fibers with antimicrobial activity. Silk fibers dyed with metallic dyestuffs showed significant antimicrobial activity against the plant pathogen *Cornebacterium* and the human pathogen *Coli bacillus*. Tensile strength of the silk fibers after the salt shrinking and dyeing processes did not show a significant change, whereas the elongation at break was increased slightly. The techniques described here for preparing significantly active antimicrobial silk fibers are effective and economic ways of providing new materials for industrial and biomedical applications. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 1181–1188, 2002

Key words: birefringence; silk protein; fibers; dyes

INTRODUCTION

Silks are among the most prevalent and well characterized of the fibrous proteins and have been used extensively as an excellent textile material.[1–6] In addition, silk fibers exhibit many outstanding properties such as biocompatibility toward human tissue,[7] dyeability, humidity absorption, and handling texture that have distinguished them from other natural and synthetic fibers.[1–6] The comfortable handling texture of silk fabric is mainly ascribed to the silk protein's specific physical properties. These properties are derived from the protein's distinct primary structure, which leads to the highly ordered secondary and tertiary structures of silk fibroin, the major component of silk fibers.

As the popularity of a safe and healthy lifestyle continues to grow, especially among the young, demand for consumer products aimed at this target audience increases. This is most evident by the numerous items available with antimicrobial activity in the textile field as well as the cosmetic and medical industries. For example, silk used in innerwear could encounter problems with minor bacterial growth attributed to the high humidity absorption property of silk. The preparation of silk fibers with antimicrobial activity against various bacterial strains could provide a practical solution.

After considering these points, we have been conducting research on the absorption of metals to silk fibers and wool yarns by chelating agents introduced into the fibers.[8,9] To achieve this end, the fibers were preliminarily modified by acylation with ethylenediaminetetraacetic (EDTA) dianhydride in organic solvent, after which the metals were combined by immersing the modified silk fibers in the metallic solution. The coordination sites of the EDTA organized within the protein fibers become the combining sites for the metals. All these metal-containing silks exhibited significant antibacterial activity. In our previous studies,[7,8] we examined the amount of the metals released from the modified proteins fibers because metal desorption from the samples seems to be an important parameter for the development of useful textile materials with a persistence of metals coordinated within the silk fibers. It was concluded that some amount of metals is released from the samples, which could produce disadvantageous atmospheric contamination ef-

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fects. It is therefore desirable to reduce the amount of released metals to as small a measure as possible.

Katoh[10] has developed on the contracted silk fiber by immersion in calcium nitrate at elevated temperatures and on the resin curing treatment of the contracted silk fibers. He observed that the yarn size and area factor increased with increasing shrinking rate. He concluded that air volume within the yarn area factor increased to levels about 85% higher than those of the untreated silk fiber.[10] It is therefore of interest to determine the dyeing behavior of contracted silk fibers in the dyeing process.

Because the brilliancy of dyed and printed silk fabrics is a decisive factor for evaluating the quality of silk goods, dyeability of silk fibers is one of the most attractive topics for applied and basic research in this area. In the present study we focused on metallic dyes, that is, dyestuffs containing antimicrobially active metal ions, such as Cr or Cu. From this point of view, it is possible to produce antimicrobially active silk fibers after dyeing with these types of metallic dyestuffs.

The most important themes for producing silk fibers containing antimicrobial metals are to prepare the antimicrobially active silk fibers using minimum quantities of metals and preserving the stability of metals within the fibers. The production of silk fibers with antimicrobial activities using metallic dyestuffs is a feasible research goal, given the inherent antimicrobial activity of the stuffs. Additionally, it has been shown that shrinkage treatment for silk fibers can be an effective way for preparing silk fibers with excellent handling by immersing them in a neutral salt solution, such as calcium chloride, or lithium bromide at elevated temperatures. This technique could be applied to the production of silk fibers with high metallic dyestuff absorption in the same process as the dyeing.

In this study we conducted shrinking treatment of silk fibers in calcium nitrate solutions at elevated temperatures, examined the antimicrobial activity of several dyestuffs containing metals such as Cr and Cu ions, and prepared antimicrobial active silk fiber and films by using these metallic dyestuffs against plant and human pathogens. These goals were examined to encompass the combination of conventional dyeing techniques with new materials properties.

EXPERIMENTAL

Materials

Contracted silk fibers

Silk fibers with shrinking rates of 0, 11, 21, 43, 61, and 72% were prepared by immersing silk fibers in calcium nitrate or potassium nitrate at a concentration of 1.43 g/cm^3 at 80°C for 0 s, 30 s, 2 min, 3 min, and 3 min 30 s, respectively. Calcium nitrate tetrahydrate and potassium nitrate were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and used without further purification.

Shrinkage of silk fibers

Silk fibers were immersed at 80°C in potassium nitrate solution, where the specific gravity was adjusted to 1.41 g/cm^3. The specific gravity of the salt solution was measured by use of Bohme specific gravimetry, which corresponds to the concentration of neutral salt solution. Treatment times were varied up to 40 min to obtain silk fibers with different shrinkage ratios.

Antimicrobial silk fibroin film

For production of silk films, *Bombyx mori* silk fibers were dissolved in aqueous 8*M* LiBr solution at 55°C for 20 h, then dialyzed with cellulose dialysis tubing against water at 5°C for 3 days. Silk fibroin films were obtained by casting the regenerated silk solution onto polyethylene film at room temperature for 12 h. Thus regenerated silk fibroin films, which are soluble in water, were obtained. The nonsolved silk fibroin films were obtained by immersing the soluble silk fibroin film in 50% methanol for 30 min. Before the dyeing, nonsolved silk fibroin films were treated with aqueous 0.2% NaOH solution at 20°C for 5 min, then dried at room temperature. After this alkali treatment, a large number of dyestuffs immerse into the silk fibroin films.

The films thus obtained were dyed by use of conventional dyeing techniques in a dye bath containing 20% Kayakalan Yellow GL, Kayalax Yellow G, and Kayakalan Black, all of which contain Cr ions. Dyeing tests were carried out in an acidic bath, using Suminol Fast Red B conc. (C.I. Acid Red 6) purchased from Sumitomo Chemicals (Japan). The dye concentration was 1% owf (on the basis of fiber weight). Acetic acid (1% owf) and ammonium acetate (2% owf) were added to the dyeing bath in a material-to-liquor ratio of 1 : 50. The temperature was increased from 40 to 80°C over 40 min, and maintained at 80°C for 30 min. Dyed samples were taken out and thoroughly rinsed with water.

Measurements

Moisture regain

Equilibrium moisture regain was determined under standard conditions at 20°C and 65% relative humidity (RH).

Optical properties

According to the procedure demonstrated in a previous study, the refractive indices, either parallel or perpendicular to the fiber axis, were measured with Beche's line method, using polarizing light microscopy under monochromatic light (Na light) at 20°C and 65% RH. The measurement procedure is described in detail elsewhere.[11]

DSC

Differential scanning calorimetry (DSC) measurements were performed on a Rigaku Denki (Japan) instrument (model DSC 10-A) at a heating rate of 10°C/min. The DSC range and sample weight were 2.5 mcal/s and 2 mg, respectively. The open aluminum cell was swept with N_2 gas during the analysis.

Mechanical properties

Tensile properties were measured with a Tensilon UTM-II (Orientec, Saitama Prefecture, Japan), under standard conditions at 20°C and 65% RH at a gauge length of 100 mm and strain rate of 40 mm/min.

Antimicrobial activity

The antimicrobial activity of the silk fibers against *Cornebacterium* and *Escherichia coli* was evaluated according to the following procedure. From a bacterial cell culture with a cell density of 100 cells/mL, a 2 mL, alignot was taken and mixed with 25 mL agarose containing King B growth medium (Wako Pure Chemical Industries, Ltd.) at 55°C, poured into a glass petri dish, and allowed to solidify at 25°C. The specimens (5 × 5 mm) were placed onto the surface of the solid gel and incubated at 25°C for 2 days. The antimicrobial activity was evaluated by measuring the size of the growth inhibition zone.

Degree of exhaustion

Degree of exhaustion was measured photometrically using a spectrophotometer (Spectronic 20; Shimadzu, Kyoto, Japan) for the extracted solution at 25°C with 25–75% pyrizine from the dyed silk fibers. The material-to-liquor ratio was maintained at 1 : 20. The degree of exhaustion was estimated from the correlation curves of the absorption and the differing dyestuff concentrations.

Absorption amount of dyestuffs

Absorption amounts of dyestuffs were measured photometrically by using a UV–vis spectrophotometer (MPC-3100/UV-3100S) at a wavelength of 505 nm after a 1 : 5 dilution of the residual dyeing solution.

Dyeing

Dyeing tests were carried out with several different dyestuffs. The dyeing processes for the various dyestuffs are differentiated below. For Kayanol Black 2RL, dyeing tests were carried out in an acidic bath, using Kayanol Black 2RL (C.I. Acid Red 6) purchased from Sumitomo Chemicals. The dye concentration was 1% owf and without ammonium acetate in the dyeing bath. The material-to-liquor ratio was 1 : 50. The temperature was increased from 40 to 80°C over 40 min, and maintained at 80°C for 30 min.

Dyeing of fibers with Suminol Fast Red B conc. was also carried out in an acidic bath, using Suminol Fast Red B conc. (C.I. Acid Red 6) purchased from Sumitomo Chemicals. The dye concentration was 1% owf. Acetic acid (1% owf) and ammonium acetate (2% owf) were added to the dyeing bath. The material-to-liquor ratio was 1 : 50. The temperature was increased from 40 to 80°C over 40 min, and maintained at 80°C for 30 min.

The remaining dye tests were carried out in an acidic bath, using Kayarus Supra Rubine BL, or Kayarus Tug. Blue GL, both containing copper ions, and Kayakalan Brown GL, which contains Co ions, purchased from Sumitomo Chemicals. Silk fibers were first treated at 40°C in a 5% owf ammonium acetate, 2% acetic acid mixture system for 15 min; 20% dyestuffs solution was then added into this mixture system; and the fibers were treated for an additional 15 min. The temperature of the system was increased from 40 to 90°C over 40 min, and maintained at 80°C for 30 min. After adding 1% acetic acid into the dyeing bath, the temperature of the dyeing bath remained at 90°C for another 30 min. The samples were taken out of the dyeing bath, after the temperature was allowed to slowly decrease to 50°C. After all dye tests, the fibers were thoroughly rinsed with water.

The dyeing behavior was determined by measuring the absorbance of the residual dyeing bath with a Shimadzu Seishakusho spectrophotometer (UV3100S), at a wavelength of 505 nm.

RESULTS AND DISCUSSION

Shrinking rate of silk fiber

Before fiber dyeing of the silk, we first conducted preliminary tests for preparation of the shrunk silk fibers by immersion in aqueous calcium nitrate solutions at elevated temperatures. Silk fibers were immersed in various concentrations of aqueous calcium nitrate, having specific gravities of 1.41, 1.43, and 1.49 g/cm^3, at 80°C for different immersing times (Fig. 1). In all cases, the length of the silk fibers shrunk in the early stages of the immersion process within an immersion time of 30–60 s. The silk fibers exhibited higher shrinking rates with increasing concentrations

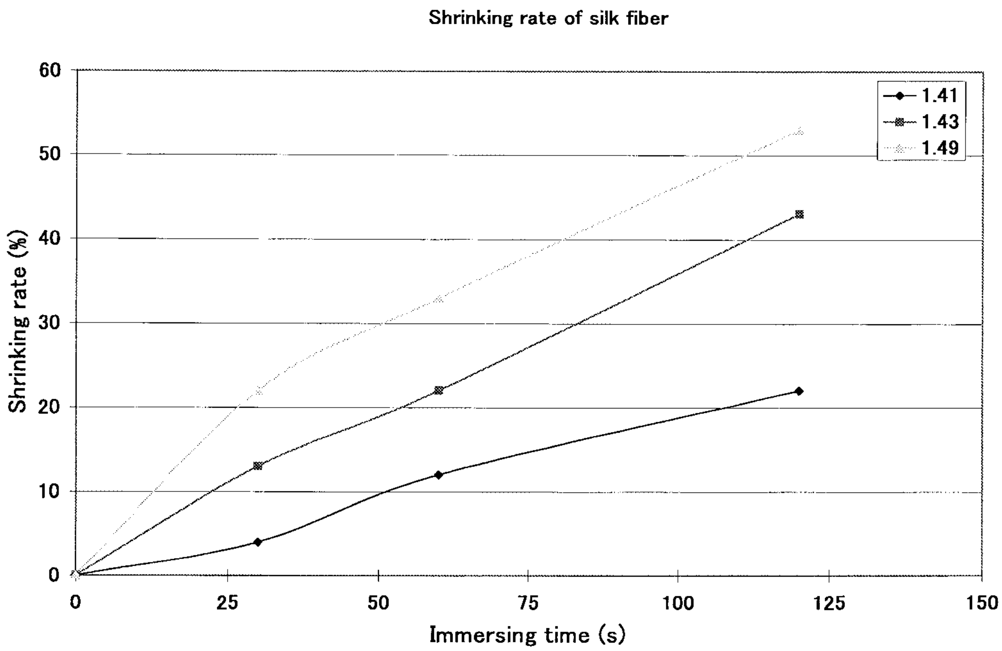

Figure 1 Shrinking rate of silk fibers, which are produced by immersing in various concentrations of aqueous calcium nitrate at 80°C for different periods of immersing time.

of calcium nitrate solution and immersion times. The value of the shrinking rate of silk fibers immersed in 1.43 g/cm^3 calcium nitrate solution was about two times larger than that of silk fibers immersed in 1.41 g/cm^3 calcium nitrate solution. This suggests that the shrinking rate of the silk fibers is directly and chiefly related to the concentration of the neutral salt solution.

The shrinking rate of silk fibers immersed in the 1.49 g/cm^3 calcium nitrate solution showed the highest value among the three neutral salt concentrations examined. The shrinking rate of the fiber increased in the very early stages of immersion up to 20% at an immersion time of 30 s and then the shrinking rate increased linearly thereafter. It is important to note that the adoption of moderate shrinking conditions using 1.43 g/cm^3 is preferable for preparing shrunk silk fibers because of the low risk of obtaining an over-shrunk specimen. The shrinking rate of silk fibers can be controlled easily under these conditions by simply adjusting total immersion time and neutral salt concentration.

Birefringence and molecular orientation

To evaluate the changes in the optical properties, molecular orientation and dyeing behavior of silk fibroin, the birefringence, molecular orientation, and degree of dyestuff exhaustion of the silk fibers were assessed. These parameters were measured after treatment in neutral salt solutions as a function of shrinking rate. Table I represents the decrease in birefringence attributed to the crystalline and amorphous regions of silk fibroin molecules with increasing shrinking rate. These birefringence data suggest that the silk fibroin molecules become more disordered after shrinking treatment. It is easily observed that the molecular orientation of silk fibroin molecules in a more crystalline or ordered array decreased with increasing shrinkage ratio.[12]

The degree of dyestuff exhaustion is an important factor to determine to assess the efficiency of dyeing. First, the dyeability of the shrunk silk fibers as a function of the shrinking rate was examined for silk fibers shrunk with potassium nitrate. The most interesting finding from the view of dyeing behavior is that the degree of exhaustion of dyestuff increased with increasing shrinking rate. These results suggest that

TABLE I
Physical Properties of Silk Fibers Treated in Neutral Salt Solutions

Shrinking rate (%)	Birefringence ($\times 10^{-3}$)	Molecular orientation[a] (%)	Degree of exhaustion ($\times 10^{-5}$ equiv/g fiber)
0	5.5	82	18.5
11	4.9	72	19.3
21	4.6	68	20.6
43	4.2	62	21.9
61	3.5	51	22.3
72	2.5	37	22.6

[a] Molecular orientation (MO) is evaluated by the following equation: MO = Δn/(maximum refractive index) $\times 10^3 \times 100$ (%), where maximum refractive index = 6.8×10^{-3}. Maximum refractive index is the refractive index corresponding to the crystalline region of silk fiber.

dyestuff diffuses more easily within the silk fibers when the silk fibroin molecules become slightly loosened and disordered.

Because the degree of exhaustion increased slightly with an increased rate of shrinkage, the results indicate that shrinkage treatment is an effective and economic technique for increasing dyestuff absorption. It seems that shrinkage finishing processes can be an effective method for antimicrobial silk fabric production by utilizing metallic dyestuffs during the dyeing procedure, thus reducing the need for increased amounts of dyestuffs.

Dyeing absorption of silk fiber treated with neutral salt solution

As demonstrated in Figure 1, it is important to note that intermediate concentrations of calcium nitrate, such as 1.43 g/cm^3, are preferable for the purpose of effective production of shrunk silk fibers. We therefore examined the dyeing absorption of silk fibers with a shrinking rate of 25%, which had been prepared by immersion in potassium nitrate tetrahydrate with a specific gravity of 1.41 g/cm^3 at 80°C for 10 min. Potassium nitrate was chosen over calcium nitrate in this dyeing absorption section, given the economical factors that might be considered for implementation of these processes in industry. The commercial product, calcium nitrate tetrahydrate, costs approximately 50 times that of potassium nitrate, making potassium nitrate a worthy candidate for these experiments. For the specific dyeing procedure, Kayakalan Black 2RL dyestuff was used, and the material-to-liquor ratio was maintained at 1 : 100.

Table II shows the dyeing absorption of this dyestuff for silk fibers with shrinking rates of 0 and 25% at various dyeing temperatures. From these experiments it was shown that the dyeing absorption of the silk fibers increased with increasing dyeing temperature. It is important to note that dyeing absorption for the shrunk silk fiber with a shrinking rate of 25% always exhibited higher values than those for silk fibers with a shrinking rate of 0%. These results imply that larger amounts of dyestuff absorb within the silk fiber when the sample is shrunk by immersion in a neutral salt solution.

TABLE II
Dyeing Absorption of Silk Fibers with Various Shrinking Rates at Different Dyeing Temperatures

Dyeing temperature (°C)	Dyeing absorption for silk fibers with 25% shrinking rate ($\times 10^{-5}$ equiv/g fiber)	Dyeing absorption for silk fibers with 0% shrinking rate ($\times 10^{-5}$ equiv/g fiber)
40	21.8	18.2
60	22.4	19.3
80	24.6	22.1

TABLE III
Antimicrobial Activity of Dyestuffs Containing Metal Ions Against *Cornebacterium*

Name of dyestuff	Contained metal	Size of inhibition zone (mm)
Kayakalan Brown GL	Co	3
Kayakalan Yellow GL	Cr	12–25
Kayalax Yellow	Cr	19
Kayakalan Black 2RL	Cr	11
Kayakalan Black BGL	Cr	7
Kayakalan Orange RL	Cr	9

Antimicrobial activity of dyestuffs

We examined first whether the dyestuffs containing metal ions showed antimicrobial activity toward bacteria. Table III lists the antimicrobial activity of various dyestuffs containing metals against *Cornebacterium michiganese* pv. *Michiganese*. Among the dyestuffs examined, Kayakalan Yellow GL and Kayalax Yellow, both containing Cr ions, displayed comparatively higher antimicrobial activities against *Cornebacterium*. Therefore, these dyestuffs may be highly useful for preparing silk fibers with antimicrobial activity by the combination of conventional dyeing techniques and use of metallic dyestuffs.

Antimicrobial silk fibers

According to the traditional dyeing procedures, silk fibers are usually dyed in a dyeing bath containing 1–3% owf dye and the necessary auxiliary dyeing assistants. Because of the low dyestuff concentrations commonly employed by these methods, there may be a risk that silk fibers dyed in this manner do not show any antimicrobial activity. To be certain that silk fibers with comparatively higher antimicrobial activities were prepared, we dyed the silk fibers with an excess amount of dyestuff. We therefore measured the antimicrobial activity for silk fibers, which had been dyed using different metallic dyes at concentrations of 20% owf and not 1–3% owf dyestuffs.

Table IV represents the antimicrobial activity of silk fibers dyed with dyestuffs containing metal ions against *Cornebacterium michiganese* pv. *Michiganese* and *Coli bacillus*. From these data it is evident that there are several dyestuffs producing antimicrobial activity toward both bacterial strains. Table V lists the color and metal contained in the dyestuff, as well as the antimicrobial activity of the silk fibers and silk films dyed with these metallic dyes at a dye concentration of 20% owf. It was demonstrated that silk fibers and films displayed significant antimicrobial activity against *Cornebacterium* and *Coli bacillus*. Figure 2 shows the antimicrobial activity of silk fibers dyed with 20% Kayarus Supra Rubine BL, which is a copper azo dye, and 20% Kayarus Supra Tug. Blue GL, a copper phtha-

TABLE IV
Antimicrobial Activity of Silk Fibers Dyed with Various Dyestuffs Containing Metal Ions Against *Cornebacterium* and *Coli bacillus*

Name of dyestuff	Contained metal[a]	Size of inhibition zone (mm)	
		Cornebacterium	*Coli bacillus*
Kayakalan Yellow GL	Cr	1.5	2.8
Kayalax Yellow G	Cr	1.0	2.2
Kayakalan Black 2RL	Cr	1.3	2.3
Kayarus Supra Rubin BL	Cu	0.1	0.1
Kayarus Tug. Blue GL	Cu	0.5	0.2
Kayakalan Brown GL	Co	3.0	0.1
Kayarus Supra Rubine BL	CuZo	0.1	0.1
Kayarus Tug. Blue GL	CuPht	0.5	0.2

[a] CuZo, copper azo dye; CuPht, copper phthalocyanine dye.

locyanine dye, against *Cornebacterium* and *Coli bacillus*. Figure 3 represents the result for silk fibers dyed with 20% Kayakalan Yellow GL and 20% Kayakalan Black 2RL for both bacteria strains. From these data it is easily observed that the silk fibers, which were dyed in baths containing 20% metallic dyestuffs, show significant antimicrobial activity against bacteria and specifically inhibit the growth of *Cornebacterium michiganese* pv. *Michiganese* and *Coli bacillus* (Figs. 2 and 3).

Therefore, it was shown that use of metallic dyestuffs in conventional dyeing processes can be used to prepare antimicrobially active silk fibroin fibers. Furthermore, dyestuffs containing Co ions seem to show higher antimicrobial activities compared to those of dyestuffs containing Cr ions.

Tensile properties after dyeing process

Because the mechanical properties of silk fibers can be significantly lowered as a result of shrinkage and dyeing processes, it is important to evaluate the effect of these treatments on the mechanical behavior of the silk fibers. If warranted, the treatments can be modified as is practical. Table VI lists the tensil strength and elongation at break of silk fibers after salt-shrinking treatment and dyeing. The tensile strength of the silk fibers does not show a significant decrease after dyeing, whereas the elongation at break was increased slightly. Silk fibers that had been shrunk with potassium nitrate and subsequently dyed with Kayakalan Black 2RL do not show a significant decrease in tensile properties.

The position of the major endothermic peak, which appeared above 300°C on the DSC curves, was shifted to a higher temperature after the dyeing process and neutral salt treatment for the silk fibers. These results suggest that the molecular coagulation of silk fibroin molecules increases in these processes. As is observed from the DSC curves, an increase in tensile strength coincides with the shifting to higher temperatures as well.

CONCLUSIONS

This study proposes a new method for obtaining antimicrobially active silk fabrics, silk fibroin fibers, and

TABLE V
Antimicrobial Activities of Silk Fibers and Silk Fibroin Films, Which Had Been Dyed with Various Dyestuffs Containing Metals

Dye stuff	Metal	Color	Size of Inhibition Zone (mm)	
			Cornebacterium	*Coli bacillus*
S-Kayarus Supra Rubin BL[a]	Cu	Black	0.1	0.1
S-Kayarus Tug. Blue GL	Cu	Blue	0.5	0.2
S-Kayakalan Brown GL	Co	Brown	3.0	0.1
S-Kayarus Supra Rubine BL[b]	Cu	Orange	0.1	0.1
S-Kayarus Tug. Blue GL[c]	Cu	Blue	0.5	0.2
Film-Kayakalan Yellow GL[d]	Cr	Orange	1.5	—
Film-Kayalax Yellow G	Cr	Orange	1.0	—
Film-Kayakalan Black 2RL	Cr	Black	1.3	—

[a] S-Kayarus Supra Rubin BL: Silk fiber dyed with Kayarus Supra Rubin BL.
[b] Copper azo dyestuff.
[c] Copper phthalocyanine dyestuff.
[d] Film-Kayakalan Yellow GL: Silk fibroin film, which had been preliminary immersed in 50% methanol for 30 min at room temperature, then dyed with Kayakalan Yellow GL.

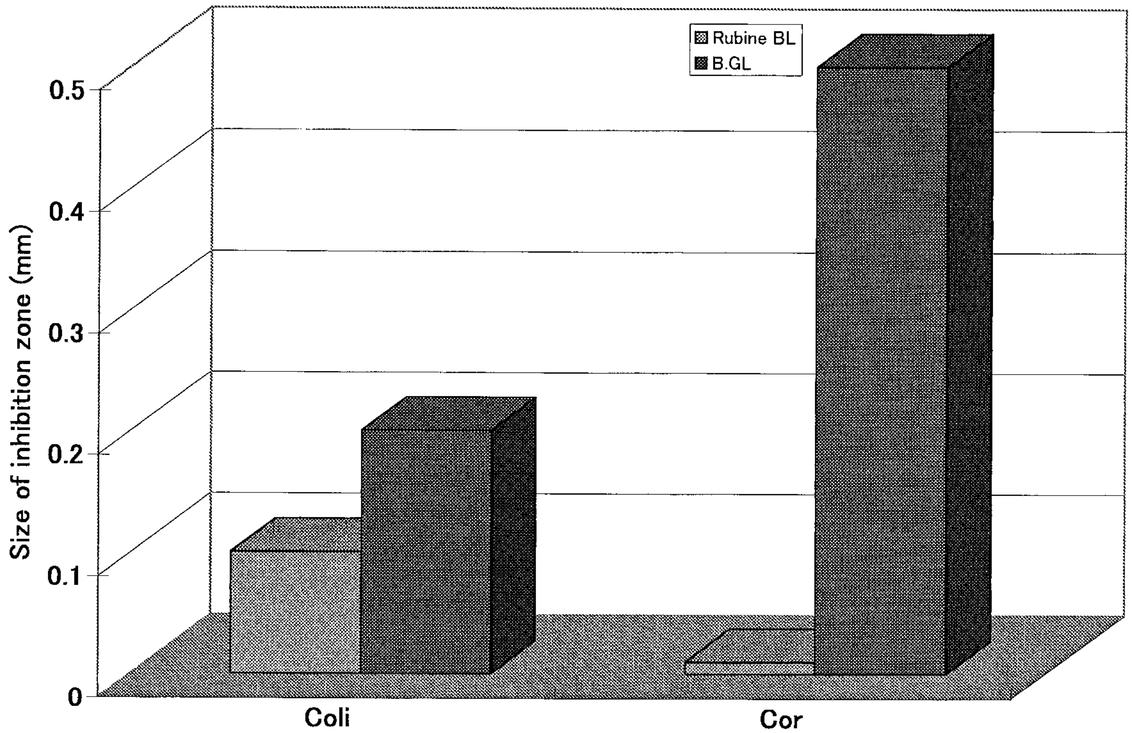

Figure 2 Antimicrobial activity of silk fibers dyed with Kayarus Supra Rubin BL, and with Kayarus Supra Tuq Blue GL against *Cornebacterium* and *Coli bacillus*.

silk films after dyeing the materials in a conventional dye bath containing metallic dyestuffs. After dyeing silk fibers with metallic dyes, the original mechanical properties are retained and the tensile properties of the sample do not decrease significantly.

It is shown that high amounts of dyestuffs can be absorbed by the samples, after shrinkage treatment in neutral salt solutions at elevated temperatures. The tensile properties of the fibers are not lowered when the shrinkage ratio is within 25%. This is an advan-

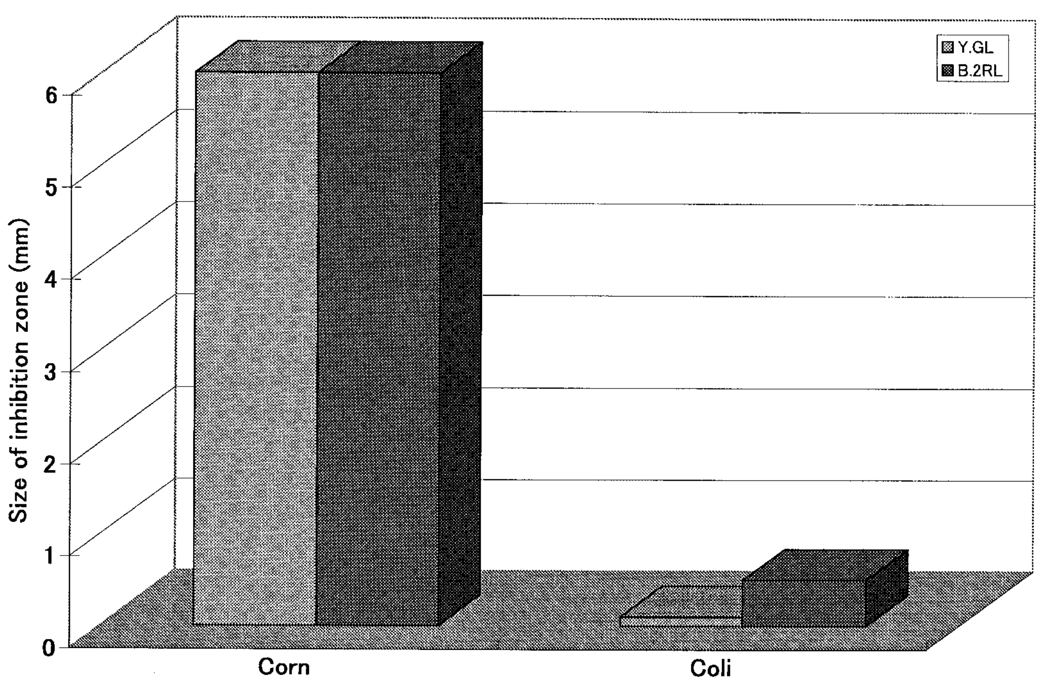

Figure 3 Antimicrobial activity of silk fibers dyed with Kayakalan Yellow GL, and Kayakalan Black 2RL against *Cornebacterium* and *Coli bacillus*.

TABLE VI
Physical Properties of Silk Fibers Dyed at Different Conditions

Sample	SR (%)	PP (°C)	Strength (g/denir)	Elongation (%)	Energy (gf mm)
B (control)	—	317	3.7 ± 15	8.2 ± 1.1	2512 ± 194
B + dye	—	318	3.9 ± 14	10.3 ± 0.7	2757 ± 222
B + salt	25.9	324	2.3 ± 26	14.7 ± 4.9	3032 ± 345
B + salt + dye	25.9	322	2.2 ± 41	17.3 ± 3.1	2997 ± 175

SR (%), shrinking rate; PP (°C); position of decomposition temperature, evaluated from DSC measurement; Strength, tensile strength of silk fibers. Strength is expressed in terms of g/denir; Elongation, elongation at the breaking point of fiber; B + dye, B + salt, B + salt + dye, represent untreated control silk fiber, silk fiber dyed with Kayakalan Black 2RL, silk fiber shrunk with potassium nitrate, and silk fiber shrunk and dyed with Kayakalan Black 2RL, respectively.

tage, in that the shrinkage ratio is closely connected to or is a measure of molecular orientation and birefringence of the silk fibroin molecules. However, it should be taken into consideration that the tensile properties, including tensile strength and elongation of the silk fibers, deteriorate significantly for the overshrunk specimen in the neutral salt solution, when the shrinking rate is beyond 50%.

Therefore, the value of the shrinkage ratio should be controlled at a lower value of 20%, to retain the original tensile properties.

The dyeing concentration in the dye bath for silk fibers adopted in this study was 20% dyestuffs, which is significantly higher compared with that of conventional dyestuff concentrations, usually 2–3%. We have concluded that the techniques described here for preparing antimicrobially active silk fibers are an effective and economic way of providing new materials, which could be useful for industrial and biomedical applications. However, use of these high concentrations can be avoided with future research aimed at finding more effective antimicrobial dyestuffs among the wide variety of dyestuffs available as commercial products.

When compared to the antimicrobially active silk fibers prepared through acylation with EDTA dianhydride, the dyeing technique developed in this work is more energy saving, economical, and interesting. The EDTA procedure requires that the sample first be acylated in organic solvent containing EDTA dianhydride at elevated temperatures for more than 1 h, and then be immersed in metallic solution at room temperature for more than 100 h. The last immersion of the silk sample allows the coordinating metals to form complexes with the EDTA reactive sites introduced within the silk fiber, although this is a time- and labor-intensive step compared to the use of metallic dyes in the dyebath. However, it should be noted that the antimicrobial potential for silk fibers prepared with the metallic dyestuff technique is significantly lower when the silk fibers are dyed with the conventional dyeing procedure containing the usual concentrations of the metallic dyestuffs.

According to the results presented here, dyed silk fibroin fibers with antimicrobial activity can be prepared simultaneously in the dyeing procedure without significantly altering the conventional dyeing process already in place industrially. Furthermore, this method has proved to be both an energy-saving and low cost expenditure technique.

The experimental results of the antimicrobial activities of silk fiber dyes with metallic dyes are probably attributable to the release of dissolved metal ions from the dyed sample. Future developments of these techniques will strive to minimize the amount of metals released from the sample. Because excess amounts of released metal ions are harmful to the human body and the environment, such experiments will meet the technical goal of safe textile material production.

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