Elution of Metals with Artificial Sweat/Saliva from Inorganic Antimicrobials/Processed Cloths and Evaluation of Antimicrobial Activity of Cloths

Harunobu Nakashima,*,[a] Naoko Miyano,[a] and Tadashi Takatuka[b]

[a]*Department of Environmental Health, Osaka Prefectural Institute of Public Health, 1–3–69 Nakamichi, Higashinari–ku, Osaka 537–0025, Japan and* [b]*Technology Research Institute of Osaka Prefecture, 2–7–1 Ayumino, Izumi, Osaka 594–1157, Japan*

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Inorganic agents, such as Ag, Cu, and Zn compounds, are known to be relatively safe, and these agents are used in many products. In Europe, the standard levels of heavy metals eluted with artificial sweat and saliva are specified in the self-imposed safety criteria (OEKOTEX Standard) for textile products. We prepared metal zeolites (Ag, Cu, Zn, and Cr) and standard cloths loaded with these zeolites and a silver antimicrobial agent, AG300. The agents, standard processed cloths, and regions of commercially available products in which metals were detected at a high concentration were subjected to metal elution with artificial sweat (JIS L 0848: 2004), saliva (BS 6684: 1987), and purified water according to the JIS shake-flask antimicrobial test method (JIS L 1902–1900). The metal concentrations in the extracts were measured using an inductively coupled plasma-mass spectrometry (ICP-MS). A similar tendency was noted in elution from the agents, standard processed cloths, and commercial products. No metals were eluted with purified water alone, while the metals were eluted with artificial sweat and saliva. Large amounts of Cu and Zn were eluted, the elution of Ag was low, and almost no Cr was eluted. Furthermore, the antimicrobial activity of the standard processed cloths was evaluated in the antimicrobial test (JIS L 1902: 2002) using *Staphylococcus aureus* (*S. aureus*) and *Klebsiella pneumoniae* (*K. pneumoniae*). Cu- and Ag (Ag zeolite and AG300)-processed cloths exhibited strong antimicrobial activities against both bacteria. Zn-processed cloth also showed antimicrobial activity against *S. aureus*.

Key words —— inorganic antimicrobial agents, artificial sweat, artificial saliva, inductively coupled plasma atomic emission spectrometry, inductively coupled plasma-mass spectrometry, evaluation of antimicrobial activity

INTRODUCTION

Antimicrobial agents used in antimicrobial-processed products are classified into inorganic, organic, and natural organic compounds.[1–3] Inorganic agents, such as Ag, Cu, and Zn compounds, are known to be relatively safe, and these agents are used in many types of household and medical products due to their good balance between antimicrobial activity and endurance.[3–6] However, patients with metal allergy due to Cu or Zn have been reported.[7–12] For fiber products that come into contact with the skin, excessive use of processing antimicrobials has been suggested to upset the balance of indigenous bacteria in the skin, inducing disorders such as dermatitis. In Europe, the OEKOTEX Standard[13,14] comprises self-imposed safety criteria for textile products. This standard sets the value limits for the elution of heavy metals (such as Hg, Cr, Cu, and Ni) with artificial sweat and saliva, with particularly strict limits for products for infants. Although there is a movement toward the use of this standard in Japan, no such self-imposed restriction standard has yet been established. In the OEKOTEX Standard, the elution method, amount of solution, elution time, and metal measurement methods are closed. For the establishment of such guidelines in Japan, we have evaluated the safety of inorganic antimicrobials.[15–17] First, analysis of metals in commercially available products was performed by X-ray fluorescence spectrometry and inductively coupled plasma atomic emission spectrometry (ICP-AES).[16,17] This study was performed to establish limits on the amount of metal elution from processed products.[15,16] As

*To whom correspondence should be addressed: Department of Environmental Health, Osaka Prefectural Institute of Public Health, 1–3–69 Nakamichi, Higashinari–ku, Osaka 537–0025, Japan. Tel.: +81-6-6972-1321; Fax: +81-6-6972-2393; E-mail: hrnakaji@iph.pref.osaka.jp

inorganic antimicrobials, metal zeolites containing Ag, Cu, or Zn are widely used.[18–20] Therefore, we prepared metal zeolites and standard cloths processed with these zeolites or Novaron AG300 as an Ag antimicrobial agent, and measured metal elution with artificial sweat/saliva using inductively coupled plasma-mass spectrometry (ICP-MS). Similar elution tests were also performed on commercially available antimicrobial products that showed a high metal concentration.[16,17] In addition, the antimicrobial activity of standard processed cloths was evaluated with the antimicrobial test method (JIS L 1902: 2002)[21] to confirm their antimicrobial effects.

MATERIAL AND METHODS

Preparation of Metal Zeolites —— Inorganic antimicrobials include Ag, Cu, and Zn compounds. Therefore zeolites of these metals and a Cr zeolite with antimicrobial activity were prepared. Zeolite was used as a synthetic zeolite A-4 powder (75 μm: Wako Pure Chemical Industries, Ltd., Osaka, Japan). Silver nitrate (anhydrous), copper sulfate (pentahydrate), zinc sulfate (heptahydrate), and chromium nitrate (nonahydrate) were analytical-grade reagents (Wako Pure Chemical Industries, Ltd.). As purified water Milli-Q water after passing through Milli RO 5 plus and Milli Q plus (Millipore Corporation) was used.

Each metal solution was prepared so that the metal content was 5 wt% of the metal zeolite. Each metal salt (0.15748 g silver nitrate, 0.39293 g copper sulfate, 0.43976 g zinc sulfate, and 0.76958 g chromium nitrate) was weighed in a 500-ml polypropylene beaker and dissolved in 200 ml of purified water. Zeolite (2.0 g) was added to this solution and suspended by ultrasonication at room temperature (20–24°C) for 24 hr. The suspension was passed through a polycarbonate membrane filter (pore size, 0.2 μm: Advantech, California, U.S.A.), and the metal zeolite was collected. This metal zeolite was vacuum-dried at 60°C for 72 hr.

Preparation of Standard Processed Cloths —— Standard processed cloths were prepared by fixing each metal zeolite or Novaron AG300 (antimicrobial agent in which silver is carried by zirconium phosphate; Toagosei Co., Ltd., Tokyo, Japan) using the binder Light-Epoch S-60NFE (Kyoeisha Chemical Co., Ltd., Osaka, Japan). This binder contains 25% effective components (drug components effective for antimicrobial fixation to cloth). Each metal antimicrobial and the resin binder were suspended in purified water so that the weight of the agent and that of the effective components of the binder were equal. Each suspension was diluted in purified water so that the percentage of the final metal antimicrobial weight was 1% or 2% of the weight of the fiber (o.w.f.). White cotton fabric [Japanese Standards Association (Tokyo, Japan), JIS L 0803, Kanakin No. 3] was immersed in each solution and wrung using a mangle (Tsujii Machine Manufacturing Co., Ltd., Osaka, Japan) with a cloth : suspension weight ratio of 1 : 1. The cloth was dried at 110°C using a hot air-circulating baking machine (Daiei Kagaku Seiki Mfg. Co., Ltd., Kyoto, Japan) for 1 hr for the fixation of the metal antimicrobial to the cloth.

Quantification of Metals in Antimicrobials and Processed Cloths Using ICP-AES —— The metal antimicrobials (metal zeolites and AG300) and standard processed cloths were analyzed. The analyzed elements were Ag, Cu, Zn, and Cr with bactericidal activity and Al as a component of antimicrobial zeolites.[18] As the standard solution, the custom-made multielement standard XSTC-13 (SPEX Centriprep Inc., New Jersey, U.S.A.) containing the metals for measurement was used. Nitric acid for poisonous metal analysis (Kanto Chemical Co., Inc., Tokyo, Japan) and Milli-Q water as purified water were also used.

ICP-AES was performed using an IRIS 1000 (Thermo Electron Corp., Kanagawa, Japan) under the following conditions: radio frequency power, 1150 W; auxiliary gas flow, 0.5 l/min; nebulizer pressure, 26.06 psi; and pump rate, 130 rpm. The main analysis wavelengths were: Cu, 324.757 nm; Ag, 328.068 nm; Zn, 213.856 nm; Cr, 267.716 nm; and Al, 309.271 nm. Depending on samples, wavelengths with less interference were used. The calibration curve of each metal showed a good linearity in the range of 0–1.0 μg/ml (correlation coefficient, 0.991–1.000).

Test solutions were prepared *via* the following procedure: 10 mg of metal antimicrobial and 100 mg of standard processed cloth were ashed. Each sample was put into a 50-ml Teflon vessel, mixed with 5 ml of concentrated nitric acid, and ashed in a microwave digestion system (ETHOS model 900: Milestone General Co., Ltd., Kanagawa, Japan) continuously at a power of 300 W for 4 min, 400 W for 6 min, and 800 W for

15 min. After acid degradation, the samples were placed in a beaker. Following the addition of 5 ml of nitric acid 0.1 M, the samples were heated on a hotplate for 10 min, placed in test tubes, and mixed with nitric acid 0.1 M to obtain a volume of 20 ml as the test solution. Recovery experiments showed a good recovery rate (94–101%) for each metal.

Metal Elution Experiments —— Metal antimicrobials (metal zeolites and AG300), standard cloths processed with these agents, and 20 commercially available antimicrobial products (20 regions) in which metals were detected at a high concentration[16, 17] were used for elution experiments.

Acidic artificial sweat (pH 5.5) and alkaline artificial saliva (pH 8.0) were prepared by the method described in the "Test method for color fastness to perspiration" (JIS L 0848: 2004).[22] For the preparation of acidic artificial sweat, L-histidine hydrochloride monohydrate (0.5 g), NaCl (5 g), and $Na_2HPO_4 \cdot 12 H_2O$ (2.2 g) were dissolved in purified water, mixed with NaOH 0.1 M (15 ml) to adjust the pH to 5.5, and 1000 ml of the solution was obtained. For the preparation of alkaline artificial sweat, L-histidine hydrochloride monohydrate (0.5 g), NaCl (5 g), and $Na_2HPO_4 \cdot 12 H_2O$ (5 g) were dissolved in pure water, mixed with NaOH 0.1 M (25 ml) to adjust the pH to 8.0, and 1000 ml of the solution was obtained. Artificial saliva was prepared by the method described in BS 6684 British Standard Specification for Safety Harnesses, 1987.[23] In this study, a solution (pH 2.3) obtained by dissolving NaCl (4.5 g), KCl (0.3 g), Na_2SO_4 (0.4 g), NH_4Cl (0.4 g), DL-lactic acid (3.0 g), and urea (0.2 g) in purified water (1000 ml) was used.

Elution experiments of the metal antimicrobials (metal zeolites, AG300) were performed using the following procedure: Each antimicrobial (0.1 g) plus artificial sweat, saliva, or purified water (10 ml) were placed in a beaker and stirred for 1 min to create an antimicrobial suspension. The suspension was passed through a membrane filter (pore size 0.2 μm, ϕ 47 mm, Japan Millipore JGWP04700).

Metal elution from the standard cloths and commercially available antimicrobial products was performed using the JIS shake-flask antimicrobial test method (JIS L 1902: 1990).[24] Each cloth (0.75 g) was put into a 200-ml Erlenmeyer flask and shaken at 310 rpm using a wrist-action shaker at room temperature (20°C) for 1 hr. The obtained eluate was passed through a membrane filter (pore size 0.2 μm).

Determination of Metals Using ICP-MS —— After elution with artificial sweat/saliva/purified water from the antimicrobials, standard processed cloths, and commercially available antimicrobial products, Ag, Cu, Zn, and Cr in the eluate were determined using ICP-MS. As a metal standard solution, the multielement standard XSTC-13 (SPEX Centriprep Inc.) was used. Nitric acid was employed as an ultrapure reagent (Kanto Chemical Co.). As purified water, Milli-Q water was used. With nitric acid 0.1 M, the standard solution and each eluate was diluted to 1/50 or 1/100 as a test solution.

ICP-MS analysis was performed with an ICPM-8500 (Shimadzu Co., Ltd., Kyoto, Japan) under the following conditions: forward power, 1.2 KW; sampling depth, 5.0 mm; Ar auxiliary gas flow rate, 7.01 l/min, Ar plasma gas flow rate, 1.5 l/min; and Ar carrier gas flow rate, 0.56 l/min. The monitored ion was m/z 65 for Cu, m/z 66 for Zn, m/z 52 for Cr, and m/z 45 for Sc as an internal standard. Ag was detected at m/z 107, and Y as an internal standard was detected at m/z 89. At m/z 63, where the highest sensitivity for Cu is obtained, a large amount of Na in artificial sweat/saliva interferes with measurement. Therefore Cu was detected at m/z 65 with less interference. Each metal showed a calibration curve with a good linearity (correlation coefficient, 0.991–1.000) in the range of 0–1.0 ng/ml.

Evaluation of the Antimicrobial Activity of Standard Processed Cloths Using the JIS Standard Test (JIS L 1902: 2002)[21] —— *Staphylococcus aureus* (*S. aureus*) IFO 12732 as a gram-positive coccus and *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 4352 (IFO 13277) as a gram-negative bacillus were used as test microorganisms. The test bacteria were cultured with shaking in nutrient broth (NTB) overnight. After adjustment of the concentration, shaking culture was repeated for 2 hr. The bacterial suspension was diluted again in NTB at a 1/20 concentration to obtain $3–5 \times 10^6$ cells/ml of test bacterial suspension. Three standard cloths (0.4 g each) processed with each metal were used for the antimicrobial activity test. Each cloth was placed in a vial and autoclaved. A test bacterial suspension (0.2 ml) was used to inoculate the test cloth, and, after culturing at 37°C for 18 hr, cells were washed out with 20 ml of sterilized buffered saline containing 0.2% Tween 80. After serial dilution of the solution obtained by washing with buffered saline, each diluted solution (0.1 ml) was applied to a standard agar plate. After culture for 24–48 hr, the number of viable cells was counted.

As controls, 6 white cotton fabric samples for the JIS color fastness test (JIS 0803 Kanakin No. 3) were used. From the bacterial count in 3 white cotton fabric samples immediately after inoculation (A), that in 3 white fabric samples after 18-hr culture (B), and that in 3 test cloths after 18-hr culture (C), static bacterial activity (b–c): log B–log C, and bactericidal activity (a–c): log A–log C, were calculated.

RESULTS AND DISCUSSION

Preparation of Metal Zeolites

The goal metal content of each metal zeolite was determined as 5 wt%. In Na substitution by the metal, the metal salt was added after the adjustment of its amount so that the metal weight was 5% of the metal zeolite weight when all sodium and metal ions had been exchanged.

The Al content in zeolites is high (≥ 10%). Therefore, in nitric acid solution, Al may be dissolved, resulting in the elution of all of the metal that has replaced the Na. Therefore each metal zeolite was ashed with nitric acid, and each metal in the solution was determined using ICP-AES. Good substitution was observed, showing a weight ratio of 3–5% (30–50 mg/g) for Ag, 3–4% for Cu, 3–4% for Zn, and 2–3% for Cr. When each metal was measured in the solution after metal substitution, no Ag, Cu, or Zn was detected (below detection limits). Novaron AG 300 was similarly analyzed, but Ag was detected at only 0.5–1 mg/g (0.05–0.1%). Since the principle component of the substance carrying Ag is Zr in Novaron AG300, Ag may not have been eluted into the nitric acid solution, resulting in the detection of only a low concentration.

Preparation of Standard Processed Cloths

To select the optimal binder for the fixation of metal antimicrobials to the cloth, the fixation effects of the following binders were evaluated: Kesmon binder KB4900 (Toagosei Co., Ltd.; acrylic type); Light-Epoch S-60NFE (Kyoeisha Chemical Co., Ltd.; silicon acrylic type); Light-Epoch T-23 M (Kyoeisha Chemical Co., Ltd.; acrylic type); and no binder. Zeolites are crystalline minerals composed of Al, Si, and Na.[18] Therefore cloths to which zeolite A-4 was fixed using each binder were prepared, and Al before and after washing with water was measured using ICP-AES. The attachment rate of each metal antimicrobial was compared. For washing, cloths were shaken using a wrist-action shaker for 1 hr in the JIS shake-flask antimicrobial test (JIS L 1902–1990).[24] As a result, the amount of Al in processed cloths after washing was 98% of that before washing using Light-Epock S-60NFE, which showed the lowest reduction rate. In addition, this binder did not contain metals such as Ag, Cu, Zn, or Cr. Therefore, using this binder, each metal zeolite or Novaron AG300 was fixed to cloths, and antimicrobial-processed cloths (standard processed cloths) were prepared. Each metal concentration was about 500 μg/g in 1% and about 1000 μg/g in 2% processed cloths. As a blank, cloths processed with only this binder (Light-Epoch S-60NFE, 1% and 2%) were prepared and similarly analyzed. The results of metal analysis in cloths processed with each metal antimicrobial are shown in Table 1 (1). The low Ag concentration in cloths processed with Novaron AG300 may be due to the incomplete ashing of Zr as the principle component of the carrier, as was observed in the ashing of this antimicrobial.

Metal Elution with Artificial Sweat/Saliva and Purified Water

Metal Elution from Metal Antimicrobials (*Metal Zeolites, AG300*): Metals are readily eluted with sweat/saliva from antimicrobials (metal zeolites, AG300). Since extraction for a long time results in the elution of all Cu or Zn, differences in the elution tendency among extracting solutions or among antimicrobials cannot be clarified. Therefore the elution tendency of each metal was observed by extraction for 1 min. The results are shown in Table 2. Each metal was eluted with artificial sweat/saliva but not with purified water. Since metals in zeolites and AG300 are eluted after ion exchange with Na ions,[18–20] their elution may occur after contact with artificial sweat/saliva with a large amount of Na ions. Elution did not occur with neutral, pure water without Na ions. These findings suggest the function of antimicrobials: “Metals are not eluted with water during washing but eluted during sweating with clothes on, exerting antimicrobial effects.”

Compared with Cu or Zn elution, the level of Ag elution with artificial sweat/saliva was low and similar between the Ag zeolite and AG300. This may be because large amounts of Cl ions are contained in artificial sweat/saliva, and Ag ions bind to Cl ions, forming $[AgCl_2]^-$, which is slightly soluble in water.

The amount of Cu elution from the Cu zeolite was high with both sweat/saliva, but no Cu was

Table 1. Metal Elution with Artificial Sweat/Saliva from Standard Processed Cloths

Type of processed cloth	(1) Concentration of metal in processed cloth (ICP-AES)	(2) Amount of eluted metal from standard processed cloth (ICP-MS)			
		Acidic sweat	Alkaline sweat	Saliva	Purified water
	(μg/g)[a]	(μg/g)[b]			
Ag zeolite (1% processed cloth)	477	2.12	8.95	6.21	N.D.
Ag zeolite (2% processed cloth)	1087	5.38	18.28	8.39	N.D.
Cu zeolite (1% processed cloth)	547	514.95	353.09	530.70	N.D.
Cu zeolite (2% processed cloth)	1398	1295.80	793.09	1229.10	N.D.
Zn zeolite (1% processed cloth)	563	77.82	404.14	741.97	N.D.
Zn zeolite (2% processed cloth)	1278	174.83	752.49	1364.80	N.D.
Cr zeolite (1% processed cloth)	656	N.D.	N.D.	N.D.	N.D.
Cr zeolite (2% processed cloth)	1051	N.D.	N.D.	N.D.	N.D.
Novaron AG300 (1% processed cloth)	195	2.30	10.08	4.61	N.D.
Novaron AG300 (2% processed cloth)	333	4.57	19.87	7.72	N.D.

The metal concentrations in the table show those of the metal type as a processing agent. *a*) Detection limit; less than 0.1 μg/g. Three samples of each cloth type were analyzed (n = 3, CV = 0.6–25.1%). *b*) N.D.; Not detected, less than 0.001 μg/g. Three extract samples of each cloth type were analyzed (n = 3, CV = 1.3–20.1%). 1% processed cloth; White cotton fabric processed with a metal agent at 1% of the weight of the fiber (o.w.f). 2% processed cloth; White cotton fabric processed with a metal agent at 2% o.w.f. Eluant volume, 15 ml; sample amount, 0.75 g; elution time, 1 hr; temperature, room temperature.

Table 2. Metal Elution with Artificial Sweat/Saliva from Inorganic Antimicrobials (μg/g)

Inorganic antimicrobials	Element	Acidic sweat	Alkaline sweat	Saliva	Purified water
Ag zeolite	Ag	7.29	26.00	14.50	N.D.
Cu zeolite	Cu	10100.00	8890.00	8110.00	N.D.
Zn zeolite	Zn	379.00	6210.00	3190.00	N.D.
Cr zeolite	Cr	N.D.	N.D.	N.D.	N.D.
AG300	Ag	4.12	13.20	7.00	N.D.

N.D.; Not detected, less than 0.01 μg/g. Three samples of each antimicrobial type were analyzed (n = 3, CV = 3.6–20.3%). The metal concentrations in the table show those of the metal type as a processing agent. None of the 4 types of metal was eluted from zeolite A-4.

eluted with water. The level of Cu elution was the highest with acidic sweat, followed in order by alkaline sweat and saliva. Cu forms chelates with amino acids (such as histidine).[25–27] Such chelates are readily eluted with water. Histidine was added to artificial sweat, which may be associated with the higher amount of elution with sweat than with saliva. In addition, Cu is very soluble in acidic solution, which may be associated with the higher rate of elution with acidic than with alkaline sweat. Organic acids such as lactic acid also facilitate metal elution from zeolites.[25] Therefore lactic acid in saliva also promotes metal elution. However, histidine may have had a more marked influence than lactic acid on Cu elution.

The amount of Zn elution from the Zn zeolite was also high, and the highest with alkaline sweat, followed in order by saliva and acidic sweat. Zn is a typical amphoteric metal that is highly soluble either in acidic or alkaline solution. However, Zn ions bound to SiO_2 as a component of the zeolite are more readily exchanged with Na ions in an alkaline environment,[18] which may be associated with the most marked elution with alkaline sweat. When acidic saliva is compared with acidic sweat, the lower pH and the presence of lactic acid in saliva may have resulted in a higher level of elution with saliva. Zn is not affected by histidine under the conditions.[27]

Cr elution from the Cr zeolite was negligible. This may be because Cr is firmly carried by zeolite.

None of the 4 types of metal was detected from artificial sweat, artificial saliva, purified water, or zeolite A-4.

Metal Elution from Standard Processed Cloths: This study was aimed at not only the observation of the amount of metal elution but also at the evaluation of the influences of the elution concentration on indigenous bacteria in the skin. Therefore elution conditions were evaluated using those for antimicrobial tests as a reference. The JIS shake-flask antimicrobial test (JIS L 1902–1900) allows the application of elution with artificial sweat. In this test method, each cloth (0.75 g) and test solution (75 ml) are placed in a 200-ml Erlenmeyer flask and shaken using a wrist-action shaker at room temperature (20–30°C) for 1 hr. The ratio of the sample weight to the solution volume (bath ratio) is 1 : 100. Considering the actual state of elution from cloth with human sweat/saliva, this volume of solution is excessive. To determine the minimum bath ratio, the amount of solution, amount of the sample that allows adequate stirring and collection as an eluate, and volume of solution were changed in the range from 3 to 15 ml. When the volume of solution was small, the sample attached to the container wall, and stirring was inadequate. Adequate stirring was possible with 15 ml of solution, and a sufficient amount of eluate for analysis could be collected. Therefore, the amounts of solution and sample were determined to be 15 ml and 0.75 g, respectively (bath ratio, 1 : 20). Elution experiments using each standard processed cloth were performed. The results are shown in Table 1 (2). From the processed cloths, metals were not eluted with purified water but were eluted with sweat/saliva. The metal elution rate was lower for the processed cloths in which the antimicrobial is covered with the binder than for the antimicrobials alone. In the processed cloths, the influences of an organic acid (lactic acid) and amino acid (histidine) with large molecular size, which have difficulty passing through the binder film, decrease, while those of Na ions and H ions (pH) with small molecular size may increase.

The level of elution from the cloths processed with the Ag zeolite or AG300 showed a tendency similar to that from the Ag antimicrobials, being low with sweat/saliva and similar between the cloths processed with the two antimicrobials.

The amount of Cu elution from the Cu zeolite-processed cloths showed a tendency similar to that from the Cu zeolite, being high with sweat/saliva and zero with pure water. The order of eluting ability was saliva = acidic sweat > alkaline sweat. Most Cu was eluted with saliva and acidic sweat.

The amount of Zn elution from the Zn zeolite-processed cloths was the highest with saliva, followed in order by alkaline and then acidic sweat. The ratio of Zn elution with saliva increased for the Zn zeolite-processed cloths compared with the Zn zeolite; most Zn was eluted from the processed cloths. No Cr was eluted from the Cr zeolite-processed cloths. Similar elution experiments were performed for cloths processed with the binder alone (1% and 2%), but none of the 4 types of metals was eluted.

Metal Elution from Commercially Available Antimicrobial-processed Products: We previously performed metal analysis of 86 regions of 40 antimicrobial-processed products.[16, 17] Similar elution experiments were performed for 20 regions of the 40 products that showed Ag, Cu, Zn, or Cr at a high concentration. The results are shown in Table 3. Table 3 also shows the metal contents (results of analysis using ICP-AES) of the products. For ICP-AES analysis and elution experiments with sweat/saliva/purified water, 5 measurement areas considered to be similarly processed were used. The elution patterns were not always consistent with those for the standard processed cloths. The amount of metal elution with water from these products was low, but that with sweat/saliva was high.

Ag elution from 4 products (nos. 7, 16, 19, and 20) in which Ag was detected using ICP-AES analysis was as low as that from the Ag-processed standard cloths. Ag elution from no. 7 was not detected, possibly due to a low Ag content. However, the products applied in this experiment contained a large amount of Al and were considered to use the Ag zeolite based on the indications of the product.[17]

The amount of Cu elution from 7 products (nos. 2, 4, 13–16, and 18) in which Cu was detected using ICP-AES analysis was lower than that from the Cu zeolite-processed cloths (standard processed cloths). The products evaluated in elution experiments may have been processed with other methods. The OEKOTEX Standard provides a limit value for Cu elution with artificial sweat/saliva of 25 (infants)–50 ppm/fiber. In the OEKOTEX Standard, the elution method, volume of solution, elution time, and metal measurement methods are closed. Therefore simple comparison with the results of this study is impossible, but 20 ppm was eluted with artificial saliva from socks (no. 4).

Based on values of ICP-AES analysis, there were 15 products processed with 30 ppm or more of Zn[17] (nos. 1, 3, 5–9, 12–15, and 17–20) among those used in elution experiments. The amount of Zn elution tended to be high for the commercially available products as well as the standard cloths: most Zn was eluted with saliva from some products (nos. 3, 7, 19, and 20). However, the amount of Zn elution was low from 2 products (nos. 17 and 18),

Table 3. Metal Elution with Artificial Sweat/Saliva from Commercially Available Products

No.	Product	Element	Metal content of product ICP-AES (μg/g)[a]	Metal elution from product (ICP-MS) Acidic sweat (μg/g)[b]	Alkaline sweat	Saliva	Purified water	Antimicrobial agents indicated on the product label
1	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Inorganics: antibiotic zeolite
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	2495.0	678.530	798.200	1258.300	20.054	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
2	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Natural organics
		Cu	75.5	4.856	3.545	5.695	N.D.	
		Zn	14.2	7.558	10.845	16.699	0.309	
		Cr	30.4	N.D.	N.D.	N.D.	N.D.	
3	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Natural organics: flavonoid series
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	Inorganics: metal oxides
		Zn	1115.0	655.540	828.800	1076.310	45.386	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
4	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	342.6	10.674	12.098	19.922	N.D.	
		Zn	2.1	6.845	8.817	14.224	1.193	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
5	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	1018.0	51.730	22.179	57.408	N.D.	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
6	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Chitosan
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	1080.0	90.364	102.640	151.300	12.118	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
7	Socks	Ag	4.6	N.D.	N.D.	N.D.	N.D.	Inorganics: silver compound
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	168.7	145.740	130.500	240.620	0.455	
		Cr	316.0	N.D.	N.D.	N.D.	N.D.	
8	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Inorganics
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	3354.0	987.300	1139.800	2572.000	27.654	
		Cr	380.4	N.D.	N.D.	N.D.	N.D.	
9	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Inorganics
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	3200.0	980.430	1178.130	2498.300	40.369	
		Cr	369.6	N.D.	N.D.	N.D.	N.D.	
10	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	5.0	3.447	3.448	3.862	0.556	
		Cr	378.3	N.D.	N.D.	N.D.	N.D.	
11	Socks	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	3.6	2.752	1.645	4.318	N.D.	
		Cr	375.5	N.D.	N.D.	N.D.	N.D.	
12	Lavatory seat cover	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Inorganics: antibiotic zeolite
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	2221.4	68.574	73.005	544.610	6.928	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
13	Lavatory seat cover	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Quarternary ammonium salt
		Cu	26.5	2.223	1.461	3.228	N.D.	
		Zn	148.7	11.418	11.270	24.858	3.515	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
14	Insole	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Zeolite
		Cu	21.4	1.027	2.073	0.882	N.D.	Aliphatic amine
		Zn	5076.0	912.270	1345.200	2903.400	85.751	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
15	Insole	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	Zeolite
		Cu	246.2	N.D.	0.371	N.D.	N.D.	
		Zn	20180.0	1032.900	1449.200	7798.500	106.220	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
16	Bedpad	Ag	149.1	0.563	2.052	2.055	N.D.	Inorganics: silver
		Cu	233.4	1.703	2.624	10.881	N.D.	
		Zn	20.6	2.826	7.425	13.671	N.D.	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	

Table 3. Continued

No.	Product	Element	Metal content of product	Metal elution from product (ICP-MS)				Antimicrobial agents indicated on the product label
			ICP-AES	Acidic sweat	Alkaline sweat	Saliva	Purified water	
			(μg/g)[a]	(μg/g)[b]				
17	Sheet/cover	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	2328.0	2.521	2.988	111.180	N.D.	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
18	Sheet/cover	Ag	N.D.	N.D.	N.D.	N.D.	N.D.	
		Cu	36.5	2.952	0.920	5.850	1.498	
		Zn	294.2	4.787	3.197	16.539	3.490	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
19	Undershirt	Ag	49.3	1.220	2.749	1.653	N.D.	Silver zeolite
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	205.0	152.660	212.480	348.000	6.403	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	
20	Undershirt	Ag	52.8	1.646	3.172	1.635	N.D.	Silver zeolite
		Cu	N.D.	N.D.	N.D.	N.D.	N.D.	
		Zn	200.0	142.500	245.480	332.410	9.044	
		Cr	N.D.	N.D.	N.D.	N.D.	N.D.	

a) N.D.; Not detected, less than 0.1 μg/g. *b*) N.D.; Not detected, less than 0.001 μg/g.

Table 4. Antimicrobial Activity of Standard Processed Cloth

Type of processed cloth	*S. aureus*		*K. pneumoniae*	
	a–c	b–c	a–c	b–c
Ag zeolite (1% processed cloth)	2.4	4.1	2.1	4.3
Ag zeolite (2% processed cloth)	3	4.5	2.8	4.3
Cu zeolite (1% processed cloth)	1.3	3.2	2.8	4.3
Cu zeolite (2% processed cloth)	2.7	4.5	2.8	4.3
Zn zeolite (1% processed cloth)	0.8	2.7	−0.7	1
Zn zeolite (2% processed cloth)	1.4	3.3	−0.5	1.1
Cr zeolite (1% processed cloth)	−1.7	0.1	−0.3	1.4
Cr zeolite (2% processed cloth)	−1.6	0.2	−0.5	1.1
Novaron AG300 (1% processed cloth)	0.5	2	2.1	4.3
Novaron AG300 (2% processed cloth)	2.4	3.9	2.1	4.3
LIGHT-EPOCH (1% processed cloth)	−1.3	0.3	−1.4	0.2
LIGHT-EPOCH (2% processed cloth)	−0.4	1.2	−1.7	0.1

A; Number of inoculated bacteria. B; Number of bacteria on the standard white cloth contacted for 18 hr. C; Number of bacteria on the test cloth after incubation for 18 hr. a = log A, b = log B, c = log C. Bactericidal activity level, a–c; bacteriostatic activity level, b–c.

probably because they were waterproof. Based on the Al content (ICP-AES analysis) and product indications, 10 products (nos. 1, 3, 7–9, 12, 14, 15, 19, and 20) were considered to use a Zn zeolite.[17]

Patients with metal allergy due to Cr have frequently been reported.[12] The OEKOTEX Standard provides a limit of Cr elution with artificial sweat/saliva of 1 (infants)-2 ppm.[13, 14] Elution experiments showed no Cr elution from 6 products (nos. 2 and 7–11) in which Cr was detected with ICP-AES analysis.

Evaluation of the Antimicrobial Activity of Inorganic Antimicrobial-Processed Cloths

The antimicrobial activity of standard cloths processed with metal zeolites (Ag, Cu, Zn, and Cr) or the silver antimicrobial AG300 was evaluated using the antimicrobial test (JIS L 1902: 2002).[21] The results are shown in Table 4. The antimicrobial effects of cloths processed with Ag (Ag zeolite, AG300) or Cu were high against both *S. aureus* and *K. pneumoniae*. The Zn-processed cloths exhibited antimicrobial effects against *S. aureus*. The Cr-processed cloths and cloths processed with the binder alone (blank) had no antimicrobial effects.

Although the rate of Ag ion elution from the Ag-processed cloths was low, the highest antimicrobial effects of Ag-processed cloths may be due to the high antimicrobial activity of Ag ions. The strong antimicrobial effects of Cu-processed cloths may be due to the large amount of Cu ion elution, as shown in Table 1, as well as the marked antimicrobial activity of Cu ions. Although the amount of Zn elution from the Zn-processed cloths was high, its low antimicrobial effects may be due to a lower antimicrobial activity of Zn than that of Ag or Cu.[19,20] No Cr was eluted from the Cr-processed cloths, suggesting no antimicrobial effects.

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