Binding of Metal Cations to Chemically Modified Wool and Antimicrobial Properties of the Wool–Metal Complexes

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ABSTRACT: Wool was modified by treatment with tannic acid (TA) or by acylation with ethylenediaminetetraacetic (EDTA) dianhydride. Kinetics of modification with TA and acylation with EDTA–dianhydride was investigated as a function of the reaction time. Wool displayed a higher breaking load and lower elongation at break as the degree of acylation increased. The absorption of metal cations (Ag^+, Cu^{2+}) by untreated and chemically modified wool was studied as a function of the kind of modifying agent, weight gain, and pH of the metal solution. Absorption of Ag^+ and Cu^{2+} at alkaline pH increased with increasing weight gain of both TA and EDTA–dianhydride. The absorption of metal cations by untreated and TA-treated wool below pH 7 was negligible. Acylation with EDTA–dianhydride enabled wool to absorb and bind significant amounts of metal cations at acidic and neutral pHs. The wool–Ag complexes exhibited low levels of metal desorption at acidic pH, irrespective of chemical modification. Higher levels of metal desorption were shown by wool–Cu and wool–EDTA–Cu complexes. Wool–Ag complexes exhibited prominent antimicrobial activity against *Corynebacterium* and *E. coli.* © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 3513–3519, 2001

Key words: wool; chemical modification; tannic acid; ethylenediaminetetraacetic dianhydride; metal cations; antimicrobial fibers

INTRODUCTION

As a protein fiber, wool has polar and ionizable groups on the side chain of constituent amino acid residues able to bind other charged organic or inorganic molecules. Studies on binding of metal ions to wool were previously reviewed by Maclaren and Milligan.[1a] The rate and extent of uptake depend on various factors, such as the kind of metal and its valence state, the solution concentration, pH, time, temperature, and so forth. The most likely binding sites are the free carboxyl groups of acidic amino acids, which may provide negatively charged binding sites over a wide range of pH conditions. Coordination bonds can be formed with nitrogen atoms of amine and amide groups, especially at alkaline pH. It was previously shown that cystine reacts as well, leading to formation of stable metal mercaptides. The absorbed metal can be quantitatively desorbed at acidic pH, apart from that bound to cystine. The reversibility of the wool–metal interaction is attributed to competition between metal and hydrogen ions for the same binding sites.

The treatment of wool with metals may induce useful changes of fiber properties.[1a] Mercury salts

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confer improved wrinkle recovery and abrasion and shrinkage resistance on wool. However, their use has never been industrially exploited because of toxicity, low durability, and so forth. Zirconium and titanium salts can be used to impart flame resistance to wool. Only the reaction of chromium salts with wool has been successfully applied at the industrial level for chrome dyeing. The use of wool as sorbent to remove heavy metal pollutants from industrial effluents and to purify contaminated water supplies has awakened some interest. Although technically feasible, it has never yet resulted in practical applications.[2] However, in recent years there has been an increasing interest in exploiting the metal-absorption capacity of natural protein fibers for the production of new types of sorbents,[3] as well as in incorporating metal ions into fibrous polymers, the aim of which is to confer new textile performances on them, such as antistatic properties, electrical conductivity, and antimicrobial activity.[4–6]

In the context of a research project aimed at developing functional textiles, based on natural protein fibers, with barrier effect against microorganisms and antiodor performance, we recently reported a new method for the chemical modification of the silk fiber substrate that was effective in enhancing the uptake of metal cations over a wide range of pH conditions.[5] The resulting silk–metal complexes displayed enhanced antimicrobial activity, confirming that the increase of the number of binding sites and of the selectivity toward metal cations may represent an interesting approach to address the preparation of new functional materials.[7–9]

The present study deals with the chemical modification of wool by treatment with tannic acid (TA) or by acylation with ethylenediaminetetraacetic (EDTA) dianhydride. Absorption and binding of metal cations (Ag^+, Cu^{2+}) to chemically modified wool was then investigated as a function of fiber weight gain and pH of metal solution. Finally, the antimicrobial activity of the different wool–metal complexes against *Cornebacterium* and *Escherichia coli* was evaluated. The results are discussed in view of the application of the wool–metal complexes as materials with enhanced barrier properties against microorganisms. Other possible applications, such as the preparation of absorbing devices for removing and recycling metal ions, is considered as well.[3]

EXPERIMENTAL

Materials

White wool in the form of yarn was used after preliminary solvent degreasing. Tannic acid and EDTA–dianhydride were purchased from Wako Pure Chemicals (Tokyo, Japan) and used without further purification.

Loading with tannic acid was performed by immersing wool into a 4.7% (w/v) aqueous solution of the reagent (material-to-liquor ratio 1 : 100) at 70°C for various times (1–7 h). Wool was then washed with running water, rinsed with distilled water, and dried at room temperature before metal absorption experiments.

Acylation of wool was performed with 10% (w/v) EDTA–dianhydride in *N,N*-dimethylformamide (material-to-liquor ratio 1 : 40) at 75°C for various times (1–5 h). Samples with increasing weight gain were thus obtained.

For the preparation of wool–metal complexes, either untreated or modified wool specimens were immersed in aqueous solutions of 33.3 m*M* $AgNO_3$, containing 46.7 m*M* KNO_3, or 33.3 m*M* $Cu(NO_3)_2$, at 25°C for 30 h. The solution pH was adjusted with acetic acid or ammonia.

Metal desorption experiments were performed by immersing the wool–metal complexes in an aqueous solution at pH 3.8 by acetic acid, at room temperature, for 120 h.

The different wool–metal complexes prepared in this study are identified by the following designations:

Wool–Ag (or –Cu): untreated wool containing the metal cation

Wool–EDTA–Ag (or –Cu): acylated wool containing the metal cation

Wool–TA–Ag (or –Cu): wool treated with tannic acid containing the metal cation

Measurements

Moisture regain was determined on dried samples kept at 20°C and 65% RH for 7 days and expressed as grams of moisture/100 g wool fiber.

Tensile properties of wool yarns were measured with a Tensilon UTM-II (Orientec Co., Ltd., Saitama, Japan), in standard conditions at 20°C and 65% RH at a gauge length of 100 mm and strain rate of 40 mm/min (ISO 2026).

The amount of metal cations absorbed by wool was quantitatively determined by using a Per-

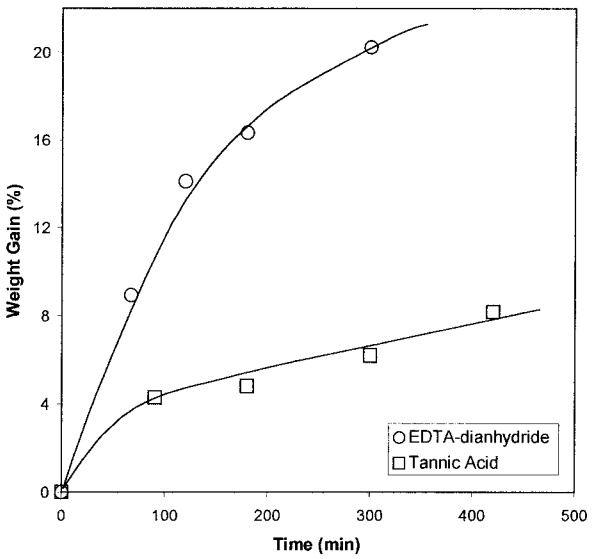

Figure 1 Weight gain of wool modified with tannic acid and acylated with EDTA–dianhydride as a function of reaction time.

kin–Elmer inductive coupled plasma–atomic emission spectrometer (ICP-AES) model Plasma 400 (Perkin Elmer Cetus Instruments, Norwalk, CT). Samples of 5–10 mg were completely digested with HNO_3 65% (2 mL), by using a microwave digestion system model MDS-81D (CEM Co., Matthews, NC), and then diluted to 10 mL with distilled water before ICP-AES analysis. The results are the average of duplicate tests.

The antimicrobial activity of the different wool–metal complexes against *Cornebacterium* and *E. coli* was evaluated according to the following procedure. From a bacteria cell culture with a cell density of about 100 cells/mL, 2 mL were taken and mixed with 25 mL agarose containing King B growth medium (Wako Pure Chemicals) at 55°C, poured into a glass petri dish, and allowed to solidify at 25°C. The wool specimens (5 $\times$ 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 zone of growth inhibition.

RESULTS AND DISCUSSION

Chemical Modification of Wool

Figure 1 shows the kinetics of absorption of TA and of acylation of wool with EDTA–dianhydride as a function of the reaction time. Once absorbed, TA is held into the fiber substrate by means of weak interactions (no covalent bonds are formed). The uptake of TA showed an initial rise with a turning point at 90 min, corresponding to a weight gain of 4%. Afterward, it proceeded at a markedly lower rate, reaching a maximum weight gain of 8% after a 7 h treatment. This behavior is in striking contrast with that recently reported for silk, which exhibited a noticeably higher affinity for TA (about 18% weight gain in 90 min).[5] The complex cellular structure of wool, characterized by the presence of a highly hydrophobic external layer at the level of the cuticular cells and different structural and morphological internal compartments, with highly crosslinked domains of keratin chains, may account for the low uptake level.

Acylation of wool with EDTA–dianhydride proceeded with higher efficiency. After a 5-h reaction time the weight gain reached 20.2%, corresponding to an acyl content of 0.86 mmol/g. A twofold degree of acylation was reached by using succinic and glutaric anhydrides.[10] Amine groups of basic amino acid residues (lysine, histidine, and arginine) and hydroxy groups of serine, threonine, and tyrosine are the most important reactive sites for anhydrides.[1b] The free –SH groups of cysteine may react as well. On the basis of the amino acid composition, the total amount of potentially reactive sites can be estimated to be about 4 mmol/g. Of course, not all of them are accessible to reagents because they are buried within the fiber structure. Moreover, the reactivity of acid anhydrides is influenced by both steric and chemical factors of the anhydride substituent (size of the side chain, presence of electronegative groups, degree of hydrophobicity, etc.).[9] On the basis of weight gain we can estimate that about 21% of the theoretical reactive sites of wool reacted with EDTA–dianhydride, although this value probably underestimates the true value because crosslinks were formed by the bifunctional anhydride molecules, as indicated by tensile measurements.

The kinetic data shown in Figure 1 allowed wool samples with increasing amounts of TA or EDTA–dianhydride to be prepared for the subsequent metal absorption tests.

Physical Properties of Acylated Wool

The physical properties of wool acylated with EDTA–dianhydride are listed in Table I.

Compared to the control sample, the moisture regain of wool decreased slightly with increasing acyl content. However, the observed changes are

Table I Moisture Regain and Tensile Properties of Wool Acylated with EDTA–Dianhydride

Weight Gain (%)	Moisture Regain (%)	Breaking Load (gf)	Strength (gf/d)	Elongation at Break (%)	Energy (gf × mm)
Control	11.0	288 ± 33	0.72 ± 0.08	28.9 ± 2.8	4110 ± 770
8.9	10.1	284 ± 32	0.65 ± 0.13	18.7 ± 4.6	3222 ± 769
14.1	10.0	326 ± 23	0.73 ± 0.05	14.6 ± 3.0	3285 ± 609
16.3	9.5	310 ± 47	0.70 ± 0.11	12.0 ± 2.0	3155 ± 505
20.2	9.5	311 ± 29	0.63 ± 0.06	12.8 ± 2.1	2615 ± 516

negligible, falling within the range of the natural variability.

The tensile properties showed an interesting trend. Following reaction with EDTA–dianhydride, the breaking load increased, whereas elongation at break decreased, indicating that wool fibers tended to become stiffer with increasing weight gain. This behavior suggests that some bifunctional anhydride molecules could form crosslinks by binding to reactive sites located onto adjacent keratin chains. The changes of breaking load and elongation at break brought about by acylation resulted in a gradual loss of toughness, as indicated by the decreasing values of energy.

Absorption of Metal Cations: Effect of Chemical Modification of Wool

The absorption of Ag^+ and Cu^{2+} by wool treated with TA or acylated with EDTA–dianhydride was preliminarily studied at alkaline pH, with the metal in the form of an amine complex. These conditions are considered more favorable for metal uptake.[1a,13] The results obtained are shown in Figure 2.

Untreated wool absorbed 0.75 and 0.55 mmol/g Ag^+ and Cu^{2+}, respectively. These values fall in the range of those reported previously by various investigators.[2,11–13] It was shown that free carboxyl groups of aspartic and glutamic acid are the main binding sites for both Ag^+ and Cu^{2+} at acidic to neutral pH.[11,13] At alkaline pH, the nitrogen of amine and peptide groups should participate as well in metal coordination.[1a] If only the free carboxyl groups are considered, the theoretical saturation value for metal absorption would be about 1 mmol/g, supposing the formation of 1 : 1 complexes between metal cations and active ligands.[13] We can therefore estimate that the metal uptake observed in our experimental conditions was lower than that theoretically expected, and this may be attributed to various factors, such as the formation of complexes with higher coordination number,[11] the reduced diffusion rate of metals toward the available binding sites ascribed to the low treatment temperature (25°C), or the low concentration of metal in the solution. In fact, treatment conditions (soaking time, temperature, pH, metal concentration, etc.) are known to greatly influence the rate and extent of metal uptake.[1a]

At alkaline pH the –S—S– cystine bridges are susceptible to attack by metal cations, causing cystine degradation and formation of stable mercaptides, which are always accompanied by a color change of fibers from white to deep brown.[11,13] In the present study, the treatment of wool with the alkaline metal solution at ambient temperature prevented extensive cystine degradation, as evidenced by the only slightly yellowish color displayed by wool after a 30-h treatment.

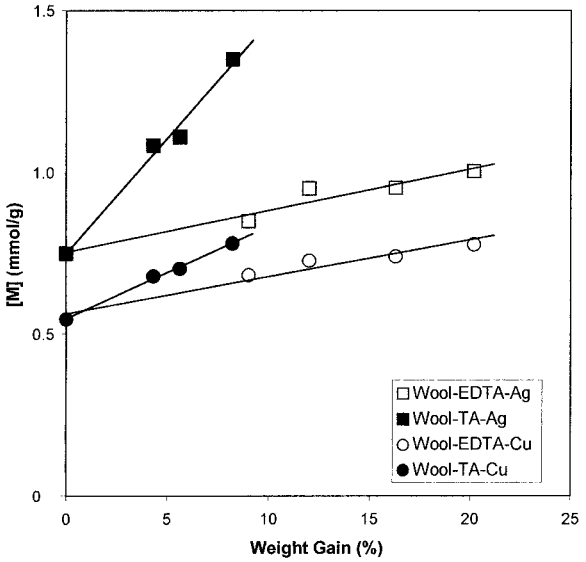

Figure 2 Absorption of metal cations at alkaline pH (11.4) by wool modified with tannic acid and acylated with EDTA–dianhydride as a function of weight gain.

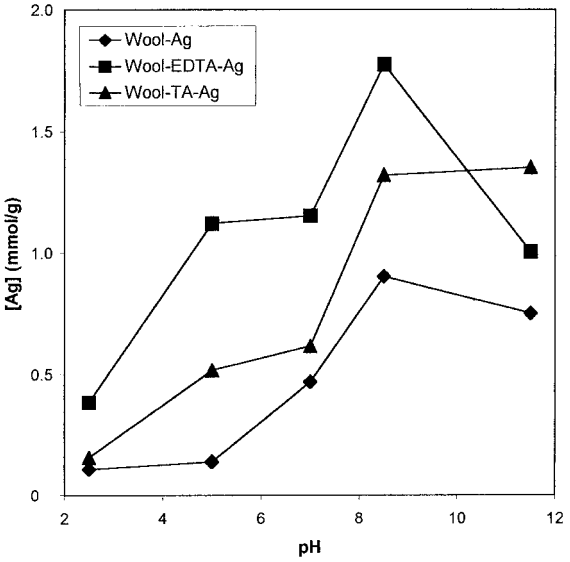

Figure 3 Absorption of Ag^+ by wool untreated, modified with tannic acid (8.2% weight gain), and acylated with EDTA–dianhydride (20.2% weight gain) as a function of solution pH.

Modification of wool with TA or EDTA–dianhydride enhanced the absorption of both Ag^+ and Cu^{2+}, as expected from the increased number of available binding sites introduced into the fiber matrix with the modifying agents. The metal uptake was linearly correlated with fiber weight gain, the increase of which was steeper for wool–TA. These results are consistent with those recently reported for silk fibers.[5] TA appeared more effective than EDTA–dianhydride in enhancing the absorption and binding of metal cations to protein fibers at alkaline pH. This may depend on the properties of the ligand itself, as well as on kinetic and thermodynamic parameters of the metal complex formed within the fiber substrate.

Absorption of Metal Cations: Effect of Solution pH

The pH of the metal solution is known to affect the uptake of metal cations because of its influence on the number of active ligands available.[5,13] To study the effect of pH on the absorption of metal cations, the solution pH was changed over a wide range, from acidic (2.5) to akaline (11.4) conditions.

The absorption of Ag^+ by untreated wool showed a sharp increase at above pH 5, attaining a maximum at pH 8.5, and then decreasing slightly (Fig. 3).

The behavior of wool–TA was closely similar, the curve being shifted to higher values of metal uptake because of the positive influence of the modifying agent in enhancing the capacity of wool to bind Ag^+. As expected, alkaline conditions are more favorable for binding Ag^+ than either neutral or acidic conditions for both untreated and TA-treated wool.

Acylation with EDTA–dianhydride allowed wool to attain constantly higher levels of Ag^+ uptake in the pH range 2.5–8.5. The maximum Ag^+ concentration of 1.78 mmol/g was reached at pH 8.5, after which it decreased. The same feature was observed for acylated silk fibers.[5] It is worth noting that Ag^+ was effectively absorbed by acylated wool, even in the acidic and neutral pH range, where untreated and TA-treated wool showed a significantly lower binding capacity.

The absorption of Cu^{2+} by untreated and TA-treated wool (Fig. 4) was almost negligible below pH 8.5, although it increased significantly at strongly alkaline pH. On the other hand, acylation with EDTA–dianhydride resulted in an effective absorption of Cu^{2+}, even in the acidic and neutral pH range.

The most interesting aspect of these results is that the reaction with EDTA–dianhydride significantly widened the pH range at which absorption and binding of metal cations proceeded effectively. Following opening of the anhydride rings, the molecules of the acylating agent provided additional binding sites, that is, carboxyl groups with pK_a values lower that those of the aspartyl

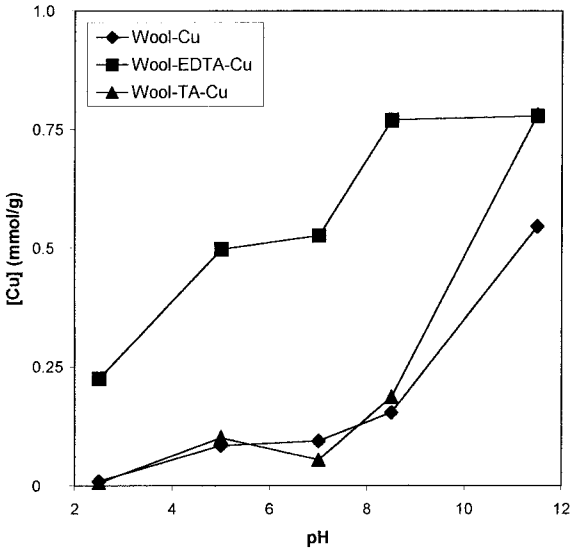

Figure 4 Absorption of Cu^{2+} by wool untreated, modified with tannic acid (8.2% weight gain), and acylated with EDTA–dianhydride (20.2% weight gain) as a function of solution pH.

Table II Release of Metal Cations by Immersion of Wool–Metal Complexes in Aqueous Solution at pH 3.8 for 120 h

Sample	[*M*] (mmol/g)		Metal Released (%)
	Before	After	
Wool–Ag	0.705	0.683	9.0
Wool–EDTA(20.2%)–Ag	1.005	0.931	7.3
Wool–TA(8.2%)–Ag	1.351	1.301	3.7
Wool–Cu	0.546	0.325	40.5
Wool–EDTA(20.2%)–Cu	0.779	0.578	25.7
Wool–TA(8.2%)–Cu	0.702	0.657	6.4

and glutamyl residues of wool.[5] Thus, additional negatively charged carboxyl groups were available for binding metal cations, even at acidic and neutral pHs.

Reversibility of Metal Absorption

The absorption of metal cations by protein fibers is a reversible process.[1a,13] Once immersed in an aqueous solution, the fibers may release variable amounts of metal ions as a function of solution pH and stability of the metal complex formed. The process is enhanced at acidic pH as a result of the competition of hydrogen ions for the same binding sites. In the case of the wool–Ag complex it was shown that silver can be completely released by treatment with strong mineral acids, the small amount retained being that bound to cystine residues, which formed insoluble mercaptides.[13]

Table II lists the results of the desorption tests carried out on the different wool–metal complexes prepared in this study. Wool–Ag, wool–EDTA–Ag, and wool–TA–Ag systems revealed the highest stability of the metal complex, as shown by the relatively low amount of Ag^+ released in the experimental conditions adopted (<10 mol %, irrespective of the chemical modification). The release of Cu^{2+} was always higher, except for the wool–TA–Cu complex. By comparing the two sets of samples, the extent of metal release of untreated and chemically modified wool followed the same order: untreated wool > wool–EDTA > wool–TA, in which the difference among samples of the same series was much larger for the wool–copper system. The variable tendencies of the wool–metal complexes to release metal cations must be regarded as a useful functional property that may address the application of metal-containing fibers.

Antimicrobial Activity

As reported in a previous study,[5] metal-containing protein fibers may be suitably applied for manufacturing antimicrobial materials (textiles or other kinds of devices). The antimicrobial activity of either untreated or modified wool fibers containing various amounts of metal cations was evaluated by means of the agar-based test.[14] Figure 5 shows the results obtained by using a wide range of wool–Ag complexes, which were tested for their ability to inhibit the growth of two kinds of bacteria characteristic of plants (*Cornebacterium*) and humans (*E. coli*).

The antimicrobial activity is expressed in terms of size of the zone of growth inhibition induced by the wool samples (wool without metal was ineffective against bacterial growth). The different wool–Ag complexes showed a high activity against *Cornebacterium.* A positive correlation was observed between the amount of metal absorbed and antimicrobial activity, irrespective of chemical modification of the wool substrate. It is interesting to note that a relatively high level of activity was also expressed by samples with low metal content. The activity against *E. coli* was significantly lower, probably because of the lower sensitivity of this bacterium toward the antimicrobial agent (Ag^+), and no concentration dependence was observed (the line runs almost parallel to the *x*-axis).

These results do not allow highlighting of any specific influence of the modifying agent (TA or

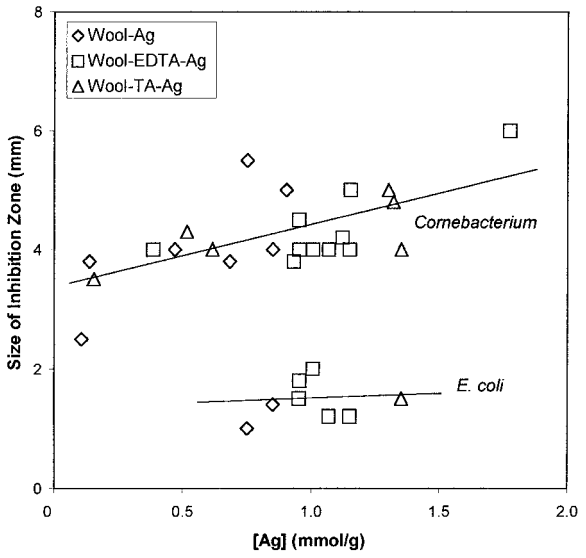

Figure 5 Antimicrobial activity of various wool–Ag complexes against *Cornebacterium* and *E. coli.*

EDTA–dianhydride) on the ability of the different wool–Ag complexes to kill bacteria. Because the size of the zone of growth inhibition on the agar matrix is dependent on the extent of Ag^+ diffusion from fibers to agar,[14] and the amount of Ag^+ released by untreated and chemically modified wool fibers was closely similar (see Table II), data points in Figure 5 overlapped within a narrow range, without showing any correlation with chemical modification of wool.

The different wool–Cu complexes exhibited a significantly lower activity than the corresponding wool–Ag complexes against *Cornebacterium,* as previously reported for silk fibers.[5] Wool–Cu and wool–TA–Cu samples were almost ineffective, irrespective of the metal content. No zone of growth inhibition was observed after incubation with bacteria or, when it was present (i.e., at the highest Cu^{2+} concentration), it was very difficult to evaluate its size because of the strong opacity induced by extensive bacterial contamination. Only wool–EDTA–Cu samples displayed detectable antimicrobial activity. The size of the inhibition zone ranged from 1 to 2 mm for Cu^{2+} concentrations of 0.5–0.7 mmol/g. No antimicrobial activity was detected against *E. coli.*

CONCLUSIONS

The chemical modification of wool fibers with TA or EDTA–dianhydride was effective in enhancing their intrinsic ability to absorb and bind Ag^+ and Cu^{2+}, where the extent of metal uptake was dependent on weight gain. The acylation technique appeared particularly attractive because metal cations were effectively absorbed over a wider pH range, especially in the acidic and neutral regions where untreated or TA-treated wool absorbed only negligible amounts of metal ions.

Wool–Ag, wool–EDTA–Ag, and wool–TA–Ag complexes were characterized by low levels of metal release at acidic pH, and displayed prominent antimicrobial activity against bacteria, whereas the different wool–Cu complexes showed correspondingly higher values of metal release and much lower barrier effect against microbial growth.

Finally, the observation that the chemical modification with EDTA–dianhydride did not significantly improve the antimicrobial properties of the wool–Ag complexes suggests that this technique could be better exploited for applications other than the preparation of wool-based antimicrobial textiles. In particular, the noticeably higher capacity of wool–EDTA fibers to absorb metal cations could be the basis for developing new absorbing devices to be used for removing and recycling metals from aqueous solutions.[3] Studies on this subject are in progress.

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