Antifungal Activity of Silver Doped Hydroxyapatite on Leather

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Abstract

In this study, silver doped hydroxyapatite (Ag-HA) prepared by microwave method was applied in the finishing composition on leather and its antifungal effect was investigated. By using X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) structural properties of the prepared Ag-HA have been determined.

The antifungal activities of the control leather and the 0% – 5% Ag doped hydroxyapatite treated leather samples to the test fungi *Aspergillus niger* TEM and *Trichoderma viride* TEM were evaluated according to ASTM D 4576-08:2008 Standard Test Method for Mold Growth Resistance of Wet-Blue (Leather). According to the results of the study, the leather treated with Ag doped hydroxyapatite containing 2% and above amount of silver showed strong antifungal activity and it was decided about possible usage of Ag doped hydroxyapatite as antifungal agent on leathers.

Keywords: Silver doped hydroxyapatite, Leather, Antifungal activity, Mold resistance

1. Introduction

Leather is an ideal nutrient source for moulds because as a natural product it contains grease, dyes and tanning agents. The risk of mould growth is present in nearly all process stages of leather production as well as the subsequent processing and use (Leppchen et al. 2011). Mould not only has a negative effect on the appearance and odor of the goods attacked. It can also cause considerable material damage or even render articles unfit for use. Under some circumstances a health hazard may exist for persons coming into contact with the affected goods (Würtz 2007).

More stringent hygiene demands have led to the development and widespread use of materials and components with antimicrobial finishes in leather industry as well. Antimicrobial finishes are used to avoid microbial colonization and its consequences as well as to prevent the spread of infection in the case of various shoe components or in certain areas such as orthopaedics, work shoes, and the like (Würtz 2011). Currently, adequate protection of leather is ensured solely by using toxic and ecologically critical preservatives such as benzothiazoles or phenol derivatives. However, Gu et al. (2009) mentioned that because of the disparate antimicrobial spectrum and the problem of toxicity, normal leather fungicides, such as 2-(thiocyanomethylthio) benzothiazole are not appropriate for use in shoe lining leather. Antimicrobial treatment can be applied in a number of ways including coating to the finished leather.

One of the most studied biomaterial for its extraordinary biocompatibility, bioactivity, and osteoconductivity is the hydroxyapatite (HA), a bioceramic material from the family of apatites with the general formula $Ca_{10}(PO_4)_6(OH)_2$. Being the main inorganic constituent in human bones and teeth, HA is widely used in medical applications such as implants, coatings, prostheses (Ciobanu et al. 2013). By taking advantage of the ion-exchange capabilities, HA can be effective in controlling microorganisms with the introduction of transient metal ion such as silver (Oh et al. 2003).

Silver has been known as a disinfectant for many years and has a broad spectrum of antimicrobial activity while exhibiting low toxicity. Several *in vitro* studies reported that the silver ions in the HA coatings play an important role in preventing or minimizing initial microbial development (Ciobanu et al. 2013). Recently, the antimicrobial effects of silver-substituted HA have been studied on different materials, including textile (Lv et al. 2006).

The purpose of our work was to evaluate the antifungal property of leathers treated with the silver doped hydroxyapatite prepared by microwave method.

2. Materials and Methods

Preparation and characterization of HA and Ag-HA solutions

Analytical grade calcium hydroxide ($Ca(OH)_2$, Merck), diammonium hydrogen phosphate (DAP, $(NH_4)_2HPO_4$, Merck), and silver nitrate ($Ag(NO)_3$, Fluka) were used for the preparation of the Ag-HA according to the literature (Rameshbabu et al. 2006). The phase analysis of the 0%-5% Ag-HA powders was characterized by X-ray Diffraction (XRD, Phillips X'Pert Pro, Japan) and the functional groups present in the 0% - 5% Ag-HA were ascertained by Fourier Transform Infrared Spectroscopy (FTIR, Perkin Elmer, Spectrum 100, USA).

Application of Ag-HA solutions to lining leather

The shoe lining leathers (crust) used in this study were produced by a conventional process using neither bactericide nor fungicide. Leather samples were cut under sterile conditions and, except the control sample, 0% - 5% Ag-HA solutions in finishing composition were applied to the grain side of these samples. After application, the leather specimens were passed through a drying process at 105°C for 15 min and ironed at 100°C. In this way, a thin film containing Ag-HA was formed on the leather samples.

Antifungal activity assessment

The antifungal activities of the control leather and the 0% – 5% Ag doped hydroxyapatite treated leather samples were evaluated according to ASTM D 4576-08:2008 Standard Test Method for Mold Growth Resistance of Wet-Blue (Leather). In the experiments, *Aspergillus niger* TEM ve *Trichoderma viride* TEM as fungi were studied.

Samples of the leather were placed in the center of the petri dishes and then the growing medium (potato dextrose agar - PDA) was filled up to the upper level of the leather samples. The petri dishes were incubated for two weeks at the temperature 26°C. After 3, 7 and 14 days of incubation periods, the degree of growth observed on the specimens were visually assessed using the rating scale according to standard (ASTM 2008).

3. Results and Discussion

Ag doped hydroxyapatite characteristics

The phase analysis of the 0%-5% Ag-HA powder samples were done by XRD (Phillips X'Pert Pro, Japan) using a 40 kV current, with a monochromatic CuKα (target) radiation and a scan range from 2θ = 10 to 70°. The XRD patterns of pure HA and the as-synthesized 0.5%-5% Ag-HA powders formed is depicted in Figure 1. The d-values correspond to that of calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). From the XRD pattern, the characteristic peak of calcium phosphate clearly shows the development of poorly crystallized apatite phase at 2θ~25–34.5°. The HA crystals that were synthesized revealed characteristic peaks in the XRD pattern that were consistent with International Centre for Diffraction Data files for hydroxyapatite (JCPDS 2003). It is clearly seen from Figure 1 that apatite peaks were generally found at 2θ of 25.40°, 29.00° and 31.50°, corresponding to (002), (102) and (211)

orientations for the powder sample. In addition, the low intensity peaks were determined at 2θ of 39.20°, 39.60°, 46.50° and 49.00°.

Figure 2 summarizes FTIR spectra of pure HA and of 0.5%-5% Ag-HA, respectively. The presented significant absorbance agreed with HA component in all powders (Chung et al. 2005). Major characteristic HA bands are assigned as: ν_1 PO_4^{3-} band around 962 cm^{-1}; ν_2 PO_4^{3-} band around 471-472 cm^{-1}; ν_3 PO_4^{3-} band around 1023-1025 and 1090 cm^{-1}; ν_4 PO_4^{3-} band around 560-562 and 600-602 cm^{-1}; adsorbed water and hydroxyl groups of broad absorption around 631-633 cm^{-1}. For pure HA the presence of strong O–H vibration peak could be noticed at wave number of 3570 cm^{-1}. However, for Ag-HA samples, the intensity of O–H vibration peak is reduced. The functional groups in FTIR spectra of the 0-5% Ag-HA in as synthesized condition are given in Table 1.

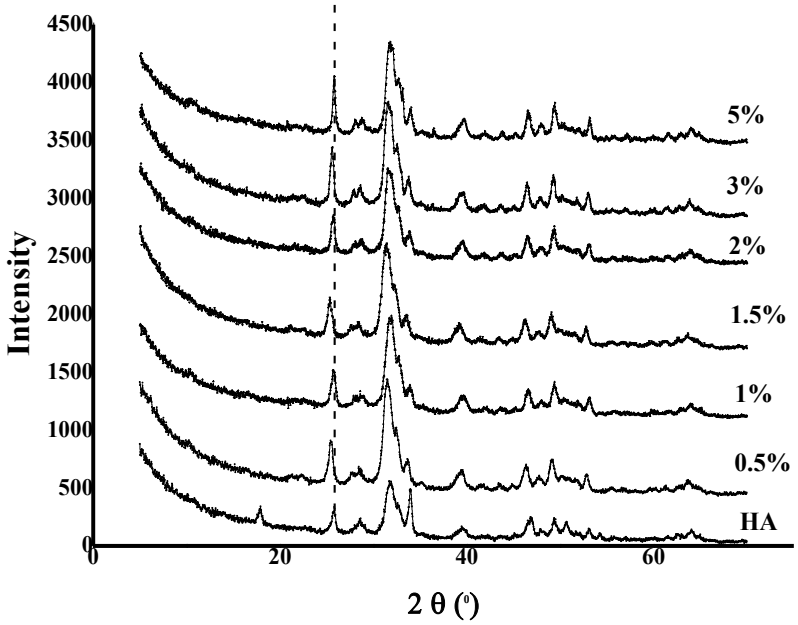

Figure 1. XRD patterns of the 0%-5% Ag-HA

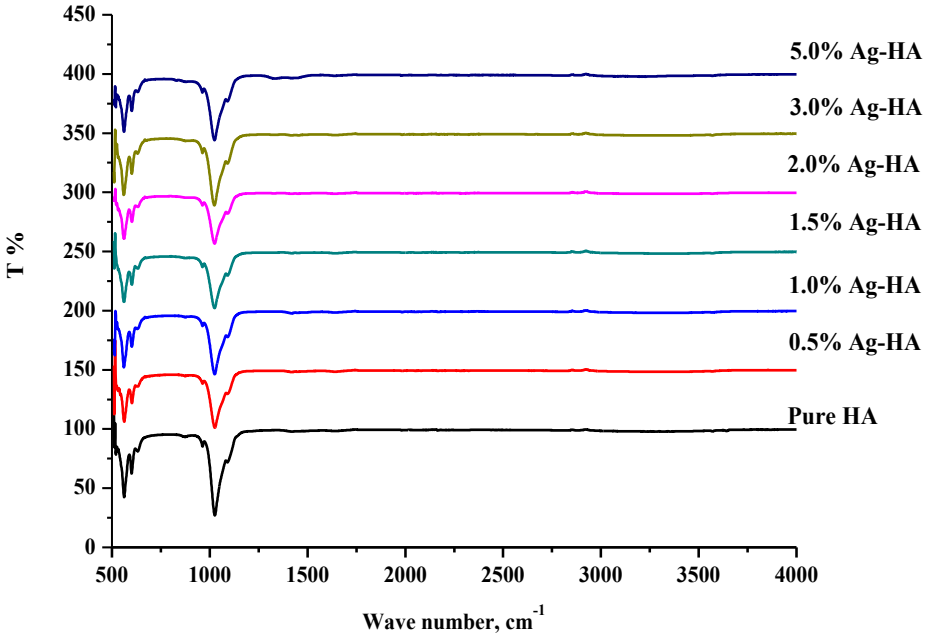

Figure 2. FTIR spectra of the 0%-5% Ag-HA in as synthesized condition

Table 1. The functional groups in FTIR spectra of the 0% – 5% Ag-HA in as synthesized condition

Functional groups	Pure HA	0.5% Ag-HA	1% Ag-HA	1.5% Ag-HA	2% Ag-HA	3% Ag-HA	5% Ag-HA
	Wavenumber (cm^{-1})						
ν_2 PO_4 vibration band*	471	471	471	471	471	471	472
ν_4 PO_4 vibration band	562	563	560	562	562	559	562
ν_4 PO_4 vibration band	600	602	601	601	602	601	601
ν_1 PO_4 vibration band	962	962	962	962	962	962	962
ν_3 PO_4 vibration band	1025	1025	1025	1024	1024	1024	1023
-OH band	632	632	632	633	632	631	632
ν_3 PO_4 vibration band	1090	1090	1090	1090	1090	1090	1090
-OH band	3570						

*not shown in Figure 2

Antifungal activities

Assessment of antifungal activity of the 0%-5% Ag-HA treated leather and the control leather specimens against *T. viridae* is given in Table 2. According to the obtained results, there were intensive growth of fungi on the control leather specimens (85%) and on the leathers treated with pure HA (55%) after 3 days of incubation and it reached to 100% of growth after 7 days on control leathers

and to 100% of growth after 14 days on the pure HA treated leather samples. There were observed a slight growth of *T. viridae* on the 0.5% – 1.5% Ag-HA treated samples after 3 days while there was no growth on the leather samples treated with 2% – 5% of Ag-HA. After 7 days of incubation, the slight growth of *T. viridae* was observed on the 2% Ag-HA treated leather sample whereas no growth was determined on the 3% and 5% Ag-HA treated leather samples. After two weeks of incubation period, the leather specimens treated with 3% and 5% of Ag-HA have shown the highest antifungal activity against *T. viridae* (Table 2).

Antifungal activity assessment of the leather specimens treated with 0%-5% Ag-HA and the control leather against *A. niger* is given in Table 3. Visual examination showed that after 3 days of incubation the growth of *A. niger* on the control leather specimens were about 75% and 65% on the leathers treated with pure HA; after 7 days of incubation it reached to 100% on the control leathers and 90% on the pure HA treated leather samples; after 14 days of incubation the control sample, the pure HA, 0.5% and 1% Ag-HA treated leather samples were covered up by *A. niger* to 100%. There were observed a slight growth of *A. niger* on the 0.5% – 1.5% Ag-HA treated samples after 3 days of incubation while there was no growth on the leather samples treated with 2% – 5% of Ag-HA. The slight growth of *A. niger* was observed on the 2% and 3%Ag-HA treated leather samples after 7 days of incubation whereas such growth was determined on the 5% Ag-HA treated leather samples only after 14 days of incubation (Table 3). Thus, the leather specimens treated with 2% - 5% of Ag-HA have shown better antifungal activity against both fungi species for the entire testing period.

4. Conclusion

In this research, it was found that the Ag-HA provides effective antifungal property to leather material. Its performance was increased by increasing the concentration of Ag. According to the results of the Mould resistance test, the concentrations of Ag-HA which were effective against both tested fungi were 2% and higher of Ag-HA. The results are promising for Ag-HA use in applications as antifungal coating on leather.

Table 2. Antifungal activity of 0% – 5% Ag-HA treated leather specimens against *T. viridae*

Treated leather specimens	Surface overgrown by mold in 3 days (%)	Rate	Surface overgrown by mold in 7 days (%)	Rate	Surface overgrown by mold in 14 days (%)	Rate
Control	85	3	100	4	100	4
Pure HA	55	2	90	3	100	4
0.5% Ag-HA	12	0.5	50	2	100	4
1.0% Ag-HA	5	0	50	2	100	4
1.5% Ag-HA	5	0	25	1	75	3
2.0% Ag-HA	0	0	12	0.5	50	2
3.0% Ag-HA	0	0	0	0	25	1
5.0% Ag-HA	0	0	0	0	25	1

Table 3. Antifungal activity of 0% – 5% Ag-HA treated leather specimens against *A. niger*

Treated leather specimens	Surface overgrown by mold in 3 days (%)	Rate	Surface overgrown by mold in 7 days (%)	Rate	Surface overgrown by mold in 14 days (%)	Rate
Control	75	3	100	4	100	4
Pure HA	65	2	90	3	100	4
0.5% Ag-HA	50	2	85	3	100	4
1.0% Ag-HA	25	1	65	2	100	4
1.5% Ag-HA	12	0.5	50	2	100	4
2.0% Ag-HA	12	0.5	25	1	75	3
3.0% Ag-HA	12	0.5	25	1	50	2
5.0% Ag-HA	5	0	12	0.5	25	1

5. Acknowledgement

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6. References

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