Polyamide/Silver Antimicrobials: Effect of Filler Types on the Silver Ion Release

Radhesh Kumar,[1] Steve Howdle,[2] Helmut Münstedt,[1]

[1] Institute of Polymer Materials, Friedrich Alexander University, Erlangen-Nurenberg, Martensstrasse 7, 91058 Erlangen, Germany

[2] School of Chemistry, University of Nottingham, University Park NG7 2RD United Kingdom

Received 15 October 2004; revised 30 December 2004; accepted 25 January 2005
Published online 6 July 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30306

Abstract: The efficiency of various silver-based antimicrobial fillers (elementary silver and silver substituted materials) in polyamide (PA) toward their silver ion (Ag^+) release characteristics in an aqueous medium was investigated and discussed. Anode stripping voltammetry (ASV) was used for the quantitative estimation of Ag^+ release from these composites. The biocidal (Ag^+) release from the composites was found to be dependent on the time of soaking in water and the nature of the filler. The long-term Ag^+ release capability of the elementary silver-based PA/Ag composite is promising compared with the commercial counterparts. The silver ion release potential of polyamide composites where the silver filling was performed by using supercritical carbon dioxide ($scCO_2$) is also discussed. The composites release Ag^+ at a concentration level capable of rendering antimicrobial efficacy and proved to be active against the microbes. A good agreement exists between the Ag^+ release experiments and antimicrobial test results. The observed results on the influence of the nature of the filler and crystallinity on the biocidal release and the varying long-term release properties could be helpful in the design of industrially relevant biomaterials. © 2005 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 75B: 311–319, 2005

Keywords: polyamide; silver ion release; antimicrobial; supercritical carbon dioxide

INTRODUCTION

There is a tremendous thrust on the research on silver-based antimicrobial materials.[1–7] Antimicrobials are integrated into various matrix materials wherever the microbial growth is detrimental to the products. Metallic silver is relatively nonreactive, but in aqueous environments, silver ions (Ag^+) are released, and the antimicrobial activity depends on the intracellular accumulation of low concentrations of silver ions.[8–10] Colloidal silver is a completely nontoxic, tasteless, internally and externally applicable, broad-spectrum germ fighter and disinfectant, which can significantly reduce the length and severity of many bacterial infections.[11–13] Silver is used as coating[14,15] and also as particulate filler[16,17] in various polymers to generate antimicrobial property. Many commercial fillers (containing silver) available in the markets rely on certain carrier materials, such as titanium dioxide, sodium hydrogen zirconium phosphate, or some zeolite, as carriers of silver whose presence will improve the water permeability of the composite specimen. Of all such antimicrobials, silver-substituted zeolites are the most widely used antimicrobial additives (e.g., food-packaging materials). Sodium ions present in zeolites are substituted by silver ions. These substituted zeolites are incorporated into many commercial polymers, such as polyethylene, polypropylene, and polyamide (PA) at levels of 1–3%.[18] The use of commercial antimicrobial fillers (zirconium phosphate and silica gel as carriers for silver) in dental resin composites is reported as rendering inhibitory effects against Streptococcus mutans.[19] The report says that the mechanical properties of the resulting composites depend on the nature of the filler (silver/carrier). The Ag^+ ions avidly bind to negatively charged components in proteins and nucleic acids, thereby causing structural changes in bacterial cell walls, membranes, and nucleic acids that affect viability.[20] In particular, silver ions are thought to interact with a number of functions; a few of them have been identified as thiol groups, carboxylates, phosphates, hydroxyls, imidazoles, indoles, and amines.[21] Hence, silver ions that bind to DNA block transcription, and those that bind to cell surface components interrupt bacterial respiration and adenosine triphosphate (ATP) synthesis.[22]

A steady and prolonged release of the silver biocide (Ag^+) on interaction with water molecules provides the antimicro-

Correspondence to: H. Münstedt (e-mail: polymer@ww.uni-erlangen.de)
Contract grant sponsor: European Union (SPAN); contract grant number: G5RD-CT2001-00568

© 2005 Wiley Periodicals, Inc.

TABLE I. Details of Various Silver-Based Antimicrobials Fillers Used in Polyamide

Antimicrobials	Suppliers	Silver	(Carriers of silver)
Elementary silver	W. C Heraeus GmbH& Co. KG	99.9 %	
Alphasan RC 2000	Milliken Chemical	10 wt%	Sod.hyd.zir.phosphate
JMAC composites PG	Clariant	15 wt%	TiO_2
Irgaguard B 5000	Ciba	<20 wt%	Zeolites
Irgaguard B 7000	Ciba	<20 wt%	Zinc-Glass
AM 92289 (PA masterbatch)	Wells Plastics Ltd	<25 wt%	Zeolites

bial properties to the matrix polymer containing silver. Hence, the water diffusion characteristics of the emerging composites system are decisive parameters of the antimicrobial properties. In this case, the nature of the fillers plays a major role in the final performance of the emerging composites. The filler can improve the water permeation of composites either by reducing the crystallinity[23] of composites or by generating more free voids within the specimen to allow the entry of more water molecules. The silver ion release from the composites depends on the polarity of the matrix that favors water uptake,[24] crystallinity, and the presence of certain fillers that can improve the water diffusion.[17] To have an antimicrobial efficacy, the materials have to release silver ions in biocidal concentrations.[25] So the emerging composite specimens must possess a perfect balance of all the controlling parameters either associated with the matrix polymer or the filler. We have reported on the effect of concentration, time, and silver grain size on the silver ion release properties of PA/Ag composite systems.[26] It was seen that the Ag^+ release increases with time and concentration of the silver powder and decreases with an increase in the specific surface area (SSA) of the silver powder used.

The PA/Ag antimicrobial polymers (containing elementary silver powder) were generated to use for the fabrication of fishnet. When subjected to antimicrobial and antifouling tests, it was found that the composites were active only after 1 week in an aqueous medium.[26] One possible reason could be the poor release of Ag^+ by the composites during the first week. Efforts are made in this work to monitor the Ag^+ release properties of composites containing silver-based antimicrobial fillers (non-elementary) other than elementary silver powder. Various PA composites produced from a series of silver-based antimicrobial materials (commercial) besides the elementary powder were soaked in water (distilled and deionised) to determine their Ag^+ release potential in an aqueous medium.

This report mainly describes the silver ion release capability of various PA composites where the fillers composed of silver, either in elementary form or as silver ions confined to certain carrier materials. A comparison of their silver ion release efficacies is discussed with special reference to ionic release and antimicrobial properties within shorter periods. The novelty of the work lies in the comparison of the release characteristics of various antimicrobial materials in PA and a quantitative estimation of their Ag^+ ion release potential. The results of the release efficacies over shorter and longer periods will be helpful in the design of innovative antimicrobial products as the release behavior of each type of the filler is examined thoroughly. The observed difference in the release behavior of various silver-based antimicrobial materials in PA suggests the importance of choosing a filler alone or as a combination for a proper design of the products. This will help to accomplish a steady and continuous release of biologically relevant concentrations of silver ions over longer periods. Besides this, the performance of the PA/Ag composites where the silver filling was performed according to supercritical carbon dioxide ($scCO_2$) method is also discussed. The composite specimens were subjected to antimicrobial tests to check the agreement between the Ag^+ release experiments and the antimicrobial properties.

MATERIALS AND METHODS

Sample Preparation

PA (Ultramid C20 F) was mixed with various commercial silver-based antimicrobial fillers (Table I) and elementary silver powder (SSA of 0.78 m^2/g) in a kneader at 230°C in an atmosphere of N_2 gas. The mixes were then compression molded at 230°C into rectangular specimens with a dimension $20 \times 10 \times 1$ mm^3 (for voltammetric experiments) and cooled after the pressing time by circulating water through the press. Total mixing time was kept for 7 min so that the mixes achieved a steady torque within this time.

PA/Ag Composites by the $scCO_2$ Method

The effect of an alternate processing technique (to incorporate silver in PA) on the Ag^+ release characteristics was also investigated by using the supercritical carbon dioxide ($scCO_2$) method[27] to incorporate silver in polymers. The $scCO_2$ method can generally penetrate a solid sample faster than liquid solvents because of its high diffusion rates and leaves less solvent residues present in the products. The $scCO_2$ method swells many polymers, especially nonpolar polymers, because of the affinity of the nonpolar polymers to CO_2. The method made use of $(C_5HO_2\ F_6)$ Ag (1)(C_8H_{12}) complex as the source of Ag.

Anode Stripping Voltammetry

Anode stripping voltammetry (ASV), an electroanalytical method of detecting metal ions in water, uses a two-step

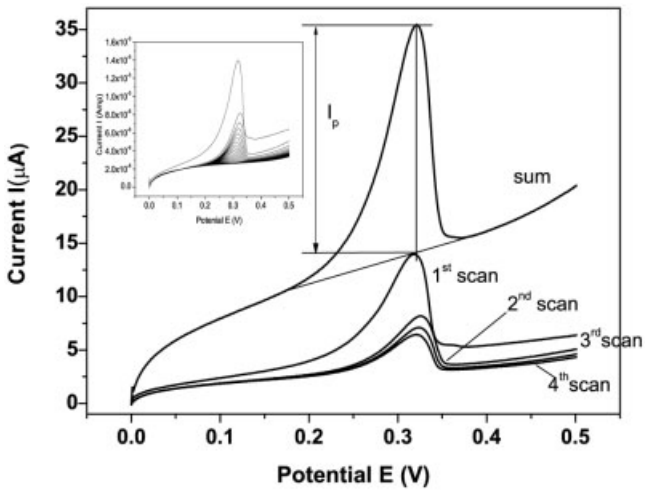


Figure 1. Voltammogram showing the stripping of silver ions and the peak height evaluation method (I_p = means peak height).

process of electrolysis and stripping. In the electrolysis step, metal ions in solution are deposited onto an electrode by using a negative voltage to the electrode for a predefined period of time under convective diffusion maintained by the rotating working electrode. During the stripping step, an oxidizing current is applied to the electrode, and the metals are stripped back into solution. Metals present in the solution are identified by their characteristic oxidation potential. ASV was used for the quantitative estimation of the silver ions released from the PA/Ag specimens. The voltammetric setup is of the Autolab/Eco Chemie (PG STAT 10) potentiostat systems. The rotating glassy carbon electrode (4-mm diameter) is used as the working electrode. This method is sensitive enough to quantify biologically relevant concentrations,[28] and a good level of agreement exists between the voltammetric setup and atomic absorption spectroscopic (AAS) measurements.[26]

Figure 1 (voltammogram) illustrates the procedure for the evaluation of silver ion concentration in the analyte. The potentials corresponding to the peak maximum give the value of the stripping potential. The baseline construction and the method of evaluation of the peak heights are also shown in Figure 1. The summation of all the scans provides an addition curve whose peak height (I_p) is directly proportional to the total concentration of the silver ions deposited and stripped out during the redox processes according to the modified Ilkovik equation[29]:

$$I_p \sim n^{3/2} A\, D^{1/2} \nu^{1/2} t\, C \qquad (1)$$

where I_p is peak current (μA), n is number of electrons transferred per molecule, A is electrode area (cm^2), D is diffusion coefficient (cm^2/s), ν is scan rate (volt/s), t is enrichment time (s is time during which the silver ions were deposited onto the electrode from the analyte), and C is concentration of the ions in the solution (g/L).

ASV measurements of various solutions with defined silver ion concentration led to a calibration curve. The calibration curve and the method of estimation of analyte concentrations were described in an earlier report.[26]

To monitor the silver ion release, the composites were stored in bottles containing water (distilled and deionized). The analytes collected from such storages were used for the voltammetric experiments to quantify the Ag^+ ion released by the composites at various time intervals.

Antimicrobial and Antifungal Tests

The antimicrobial tests were performed by using ASTM E 2149-01 method.[30] This dynamic test flask method was developed for routine quality control and screening tests to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials. The samples were tested against *Staphylococcus aureus* (ATCC 6538), *Escherichia coil* (ATCC 8739), and *Candida albicans* (CECT 1394) (all Spanish type culture collection). All the organisms were maintained according to good microbiological practice and checked for purity, by making streak plates and observing for a single species characteristic type of colonies. The sensibility of these microorganisms is determined by the logarithmic reduction showed after testing against the material with antimicrobial agent against each of them during a period of 28 days. The working bacterial solution containing the microbes in sterile ringer solutions grown at optimal growth conditions have a final concentration (after dilution) of $10^7 - 10^8$ CFU/mL. One gram of the material to be tested was admitted into three sterile wide-mouth bottles used for the antimicrobial tests. In each bottle containing the silver biocide sample, 1 mL from each bacterial inoculum having concentrations of $10^7 - 10^8$ CFU/mL was added. The samples (the bottles with materials and each inoculum) were maintained at room temperature and protected from light. During the exercise, the bottles were shaked. At different contact periods (3 h, 24 h, 7 days, and 28 days), 1 mL from each sample was withdrawn, and decimal dilutions (until 10^7) were prepared in sterile ringer solutions. One hundred microliters of the decimal solutions were spread onto agar media [tryptone soya and sabouraud dextrose (for *Can. albicans*)] and incubated on the plates. The colonies were counted (in CFU/mL). The activity values are expressed as log reduction (difference between the logarithms of viable cells on control and the silver-filled specimen). In another attempt, the antimicrobial properties were evaluated by using an alternate method (Japanese Industrial Standard JIS Z 2801), which is found as more reliable for silver-based antimicrobial materials.[31]

RESULTS AND DISCUSSION

Effect of Filler Types on the Ag^+ Release

In a previous report, the silver ion release properties of PA/Ag composites containing elementary silver powder were

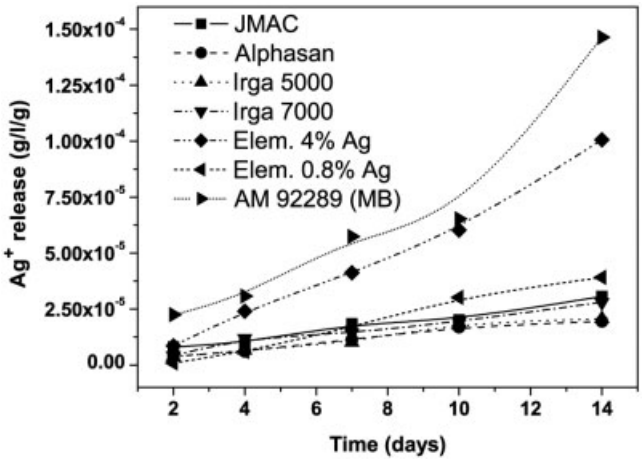


Figure 2. Total Ag^+ release from PA/Ag composites containing various commercial silver based antimicrobial fillers (filler content 4 wt %).

discussed in a detailed manner using the release evidence from stripping voltammetry and atomic absorption spectroscopic experiments.[26] It was found that the Ag^+ release showed only a marginal increase during the first 4 days. Later on, the release attained almost a constant value (up to 7 days), followed by an abrupt rise after 1 week especially for composites containing higher silver concentrations. For the silver ion release, the water molecules have to enter the specimens to oxidize the metallic silver powder. In the first few days, the release occurs at the expense of the silver particles confined to the surface layers. This can be instantaneous, because the water molecules need not diffuse well into the specimens. The sudden increase after one week was attributed to plasticization of the specimens after one week of continuous diffusion. The lower release rate observed during the first week is reflected in the antimicrobial properties. The antimicrobial property tests showed the composites as efficacious only after 7 days.[26] So the absence of antimicrobial properties in the first week could be related to the poor silver ion release by the specimens during this time interval. To understand whether the inhibition of a higher release during the first week is due to the time requirement for the water diffusion to inner layers, some commercial silver antimicrobials were used. These commercial materials (fillers) rely on certain carrier materials for silver ions, which can accelerate the diffusion of water molecules into the specimen and the migration of silver ions through the composites to the aqueous medium within shorter periods. In these cases, it is possible that there is less surface barrier for the water molecules to diffuse into the composite specimens because of the reduced diffusion barrier provided by the carrier materials.

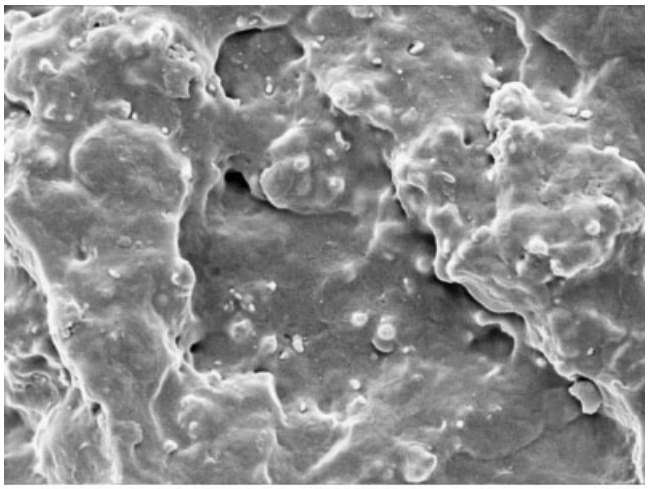
The Ag^+ release efficacies of PA/Ag composites containing different types of the silver antimicrobials were tested by using voltammetric experiments. Five different types of silver-based antimicrobial fillers are used in PA besides the elementary silver powder. All the fillers were incorporated in PA under the same processing conditions. The commercial sources other than the elementary silver powder contain certain basic materials such as titanium dioxide, sodium hydrogen zirconium phosphate, or some zeolites as carriers of silver. Table I provides some information about these materials. To compare the potential Ag^+ release properties, all the composites containing 4 wt % of a particular type of the filler were soaked in the same volume of water. The silver (silver in carrier) content in most of the non-elementary silver fillers is $<20\%$ [i.e., silver content $< 0.8\%$ (20% of 4 wt %) in the composites].

Figure 2 shows the results of the concentrations of the silver ion released by the composites (PA containing various elementary and non elementary silver) in water as a function of time. Composites containing the elementary and the AM 92289 master batch showed a silver ion release, which is distinctly higher than that from the other composites. It is important to note that the entire 4 wt % of filler in composites containing elementary silver powder is constituted by the silver entities. Other commercial fillers contain (about 80%) components other than silver. Hence, for a meaningful comparison, PA/Ag composite with 0.8 wt % of elementary silver powders also was subjected to silver ion release experiments and discussed (Figure 2). Considering the amount of silver present in AM 92289 (1 wt %) and its silver ion release potential, it can be understood that among the different types of the silver-based antimicrobial fillers, AM 92289 has more susceptibility to release silver ions during the entire period of analysis. This finding could be due to the improved water diffusion characteristics (due to the presence of hygroscopic zeolite) possessed by the specimens containing AM 92289, which would have facilitated easier water transport toward the interior part of the composite specimens within shorter periods. The release potential of composites containing other commercial silver-based antimicrobial materials are not appreciable compared with AM 92289. But they released higher concentrations in the first week than the elementary composition containing the same proportion of silver (0.8 wt %).

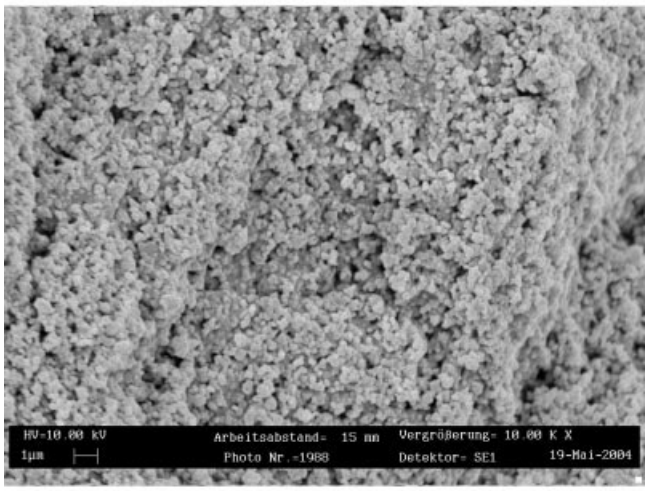
The total water uptake (in molar percentage by the composites from JMAC and AM 92289 were determined (Table II). The value observed for the composites containing AM 92289 after 14 days are distinctly more than that for the composites from the elementary silver. These higher water diffusion characteristics could have generated higher concentrations of silver ions from composites containing AM 92289. The percentage crystallinities of these composites were also

TABLE II. Water Uptake and Crystallinity of Elementary Silver, JMAC, and AM 92289 Based Polyamide Composites (Filler Content 4 wt %)

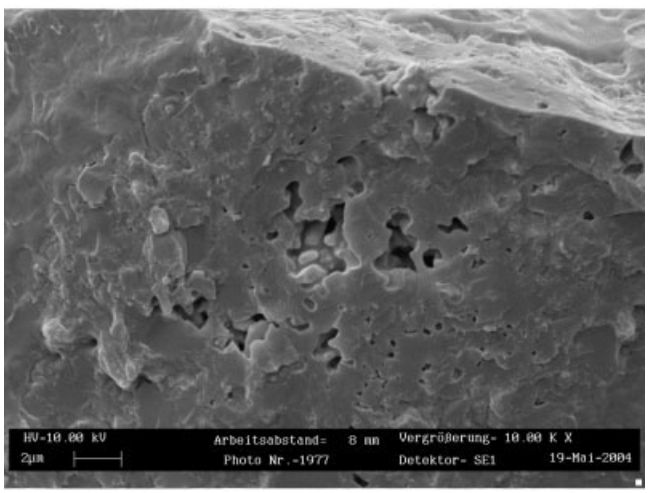
Materials	Water Uptake (mol %)	Crystallinity (%)
PA/AM 92289	0.73	9.48
PA/JMAC	0.58	8.32
PA/Elem. silver	0.55	7.18
PA (pure)	0.65	12.62



(a)



(b)



(c)

Figure 3. SEM showing the morphology of the PA composites containing (a) elementary silver (b) JMAC, and (c) AM 92289.

evaluated by using DSC because crystallinity influences the water uptake behavior (Table II). It is evident from the DSC experiments that the crystallinity of pure PA decreases on filling the antimicrobial fillers. Among the composites, the highest crystallinity was shown by the AM 92289 and the lowest by the elementary compositions. Despite the lowest crystallinity, the elementary-filled samples have less water uptake. The higher water uptake of AM 92289 despite its higher crystallinity compared with elementary composites can be due to the porous nature of the carrier materials present in AM 92289. In this case, rather than crystallinity, the voids could have facilitated the water diffusion more into the bulk of the specimen.

Figure 3(a–c) shows the SEM micrographs of the PA/Ag composites containing elementary, JMAC, and the AM 92289, respectively. It is possible to see dispersed silver powder in the PA matrix [Figure (3a)]. The micrographs (b and c) exhibit many tiny pores confined to the carrier materials, which would have permitted the entry of the water molecules into the composites. These pores are inherent channels situated in the carrier materials used in the production of these silver-substituted materials (JMAC and AM 92289). These channels may facilitate the diffusion and migration of the chemical entities (H_2O and Ag^+) within the matrix. Composites containing the elementary silver (0.8%) also release silver ions in higher concentrations, but only after 1 week of time. The lower release potential of the elementary composites (0.8%) in the first week of immersion (Figure 2) in water can be attributed to the surface barrier for the diffusion of water molecules. On the other hand, for the commercial materials, the presence of the carrier materials would have reduced the water diffusion barrier. However, the elementary compositions were found to be more capable of releasing the ions later on.

We have evaluated the long-term Ag^+ release (LTR) characteristics (which is a unique property of the silver-based antimicrobial materials) of the two types of the composites (elementary and the AM 92289). For this purpose, the samples that had been in water for longer duration (3 months) were transferred to fresh water after 3 months. Their Ag^+ release characteristics for 2 days (after 3 months and after transference to fresh water) in fresh water were monitored.

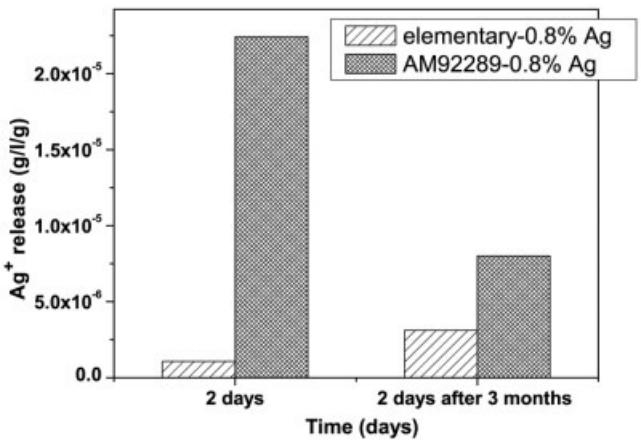


Figure 4. Long-term silver ion release characteristics of PA/Ag composites containing elementary silver and AM 92289.

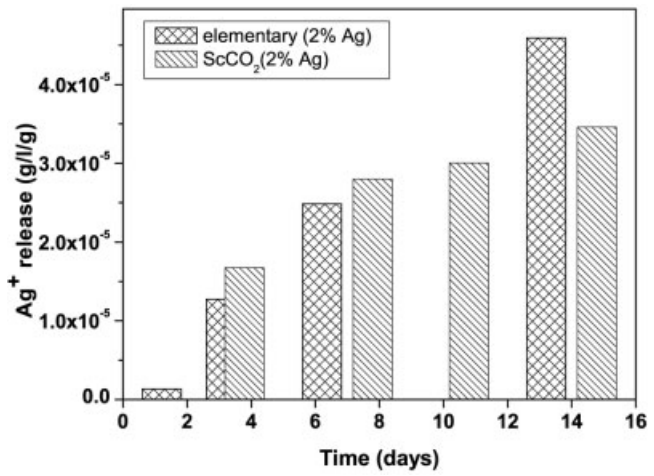


Figure 5. Comparative silver ion release efficacies of PA composites containing elementary silver powder and silver impregnated using $scCO_2$ method.

The results from Figure 4 show that the elementary composition has more consistency in releasing Ag^+ ions than the commercial masterbatch (AM 92289) in PA, over longer periods. It is interesting to find that even after 3 months of continuous release, the composites containing the elementary silver release more Ag^+ in 2 days than their capacity to release the ions during the first 2 days since its first immersion in water. This could be due to the entirely different matrix (PA) structure and properties prevailing in the composite specimen due to 3 months of continuous water diffusion and Ag^+ migration processes. On the other hand, after 3 months, the release potential decreases in the composite containing AM 92289. It is possible that the silver gets exhausted in this case because of the higher initial rate of Ag^+ release (Figure 2) of composites containing AM 92289. For a steady and long-term Ag^+ release, it is important to have a proper balance between the amount of silver within the carrier materials and the entry of water. It is necessary to optimize the matrix properties of the composites so that the silver ion elution to the aqueous medium is consistent and within the biocidal concentration level, for longer duration. One possible inference can be that for short-term antimicrobial efficacy, commercial varieties having a carrier material are promising, and elementary compositions can be a better option for long-term antimicrobial requirements. The results of the antimicrobial tests (discussed later) also support this finding.

On the basis of the above observation, a promising antimicrobial PA/Ag composite can be constituted by the enrichment of a silver antimicrobial material having a carrier in the surface layers and elementary silver embedded in the inner layers. In such a design, silver particles within the inner layers, which are largely responsible for the release over a longer period of time, can oxidize and generate the ions with the progress of diffusion. Because of the long-term water diffusion, the matrix gets plasticized fully, and hence, the Ag^+ migration could occur from the inner layers as well.

PA/Ag Composites by the $scCO_2$ Method

Figure 5 shows the results of the silver ion release measurements performed by using the PA/Ag samples produced according to the $scCO_2$ method. It was found that for shorter duration, the release of Ag^+ was slightly higher in the samples where the silver filling was performed by using the $scCO_2$ method. But with increasing leaching time, the elementary compositions dominates the $scCO_2$ compositions. The use of $scCO_2$ did not produce appreciable effects in PA possibly because of the comparatively lesser effectiveness of $scCO_2$ in polar polymers. Another possible reason for the discrepancy can be the size mismatch of the silver entities used. The elementary silver powder used for the melt compounding has an average particle size of about 2 μm, and the silver used for the impregnation using $scCO_2$ is in the nano level. This experiment also supports elementary silver-filled composites as an option for long-term antimicrobial properties.

TABLE III. Antimicrobial Properties of Polyamide Composites Containing Elementary Silver Powder and AM 92289 Fillers Tested According to ASTM and JS Methods

	Antimicrobial Activity (Log Reduction in UCF/mL)											
Pathogens	PA Control				PA/Ag (Elementary)				PA/AM 92289			
Time (hour/days)	24h	7d	14d	28d	24h	7d	14d	28d	24h	7d	14d	28d
EU-Ph	3	—	—	NR	3	—	—	NR	3	—	—	NR
Log Reduction*												
ASTM Method												
E coli**							1	2		1	2	3
St aureus**				—			>1	3			>1	3
Candida Albicans***				—			1	2		1	1	2
JIS Z 2801 Method												
E coli**						1	2	3	3			
St aureus**						1	2	3	3			

* Criteria of acceptance imposed by the European Pharmacopoeia.
NR = not required.
Incubation time and temperature: **24 h and 37°C; ***48 h and 20–25°C.

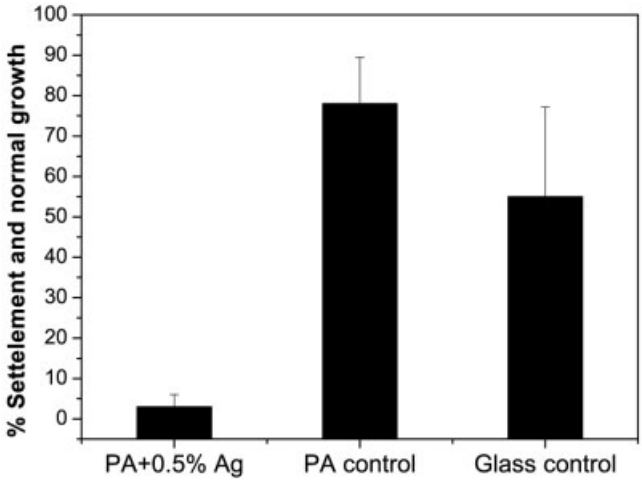


Figure 6. Settlement and development of *Spirorbis spirorbis* on test materials.

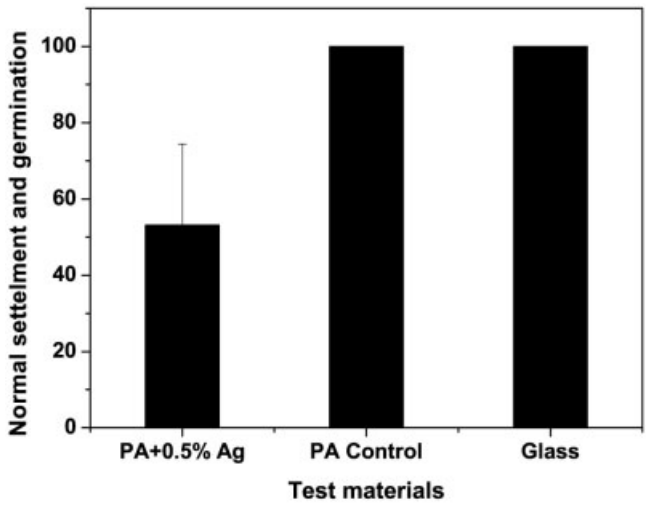


Figure 7. Relative success of *Ulva intestinalis* zoospore settlement and germination.

Antimicrobial and Antifungal Properties

The composite specimens were analyzed to detect their antimicrobial and antifungal efficacies. Initially, PA/Ag composites containing 4 wt % of elementary silver powder and AM 92289 were subjected to the antimicrobial tests according to the ASTM procedures. A good level of agreement between the silver ion release measurements and the biocidal activity decides the feasibility of the emerging composites for the fabrication of bioactive products. The results (Table III) indicate the PA/Ag composites containing elementary silver powder and the AM 92289 as efficacious against the tested microbes. The PA specimens containing the AM 92289 master batch showed early efficacies, which is in perfect agreement with the silver ion release experiments. The AM 92289-based composites dominated the elementary counterparts in its release capabilities during the first 14 days since the first immersion in water (Figure 2). It can be seen from Table III that the elementary fractions achieve similar antimicrobial efficacies with the AM 92289 in PA after 28 days. This finding shows the increased release of Ag^+ with the progress of time (i.e., effective over longer time) from elementary silver-based PA/Ag composites.

Japanese test method (JIS Z 2801), an alternative test method, also was used for the antimicrobial property evaluation. This is because JIS has been found more reliable for testing the biocidal activity of materials where the biocides are not confined to the materials surface (i.e., those that are releasing to the medium such as silver ions in this case). In this case, the tested composites contained even lower amounts of silver in PA. The concentrations of elementary silver and AM 92289 were 2 wt % in the test specimens. It was found that the Japanese test methods proved the samples more active than the results from the ASTM method (Table III results after 24 h).

Antifungal tests were performed only on the elementary silver-based PA/Ag specimens. The antifungal tests using the PA/Ag specimens containing elementary silver proved their antifouling characteristics. The results of the comparative analyses between PA + 0.5%Ag and PA control, described in Figures 6–8, show 1) that PA + 0.5% Ag is highly effective against settlement of larvae of the tubeworm *Spirorbis spirorbis* and ascidian *Ciona intestinalis* and 2) partial efficacy against germination of spores of the alga *Ulva intestinalis*. Figure 9(a–c) shows the settlement and germination of ulva intestinalis on the PA/Ag (0.5% Ag), PA control, and glass control. The results show that the PA/Ag composites prevented normal spore germination and growth from occurring compared with the control materials.

CONCLUSIONS

The silver ion release efficiency depends on the type of the silver used for the generation of the composite specimens. In

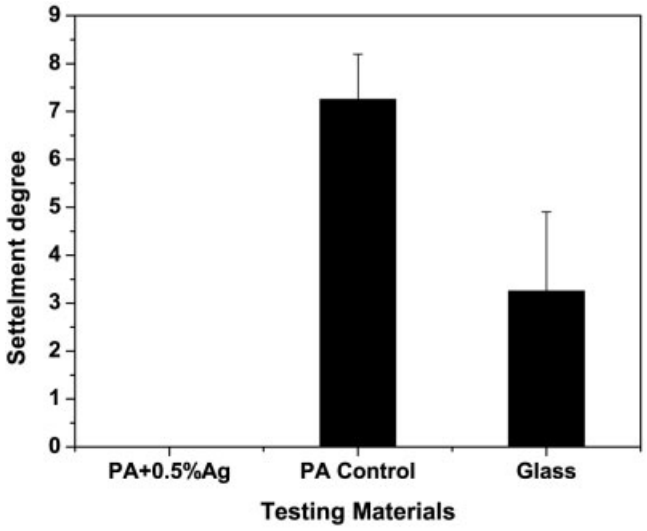


Figure 8. Degree of settlement of *Ciona intestinalis* on test materials.

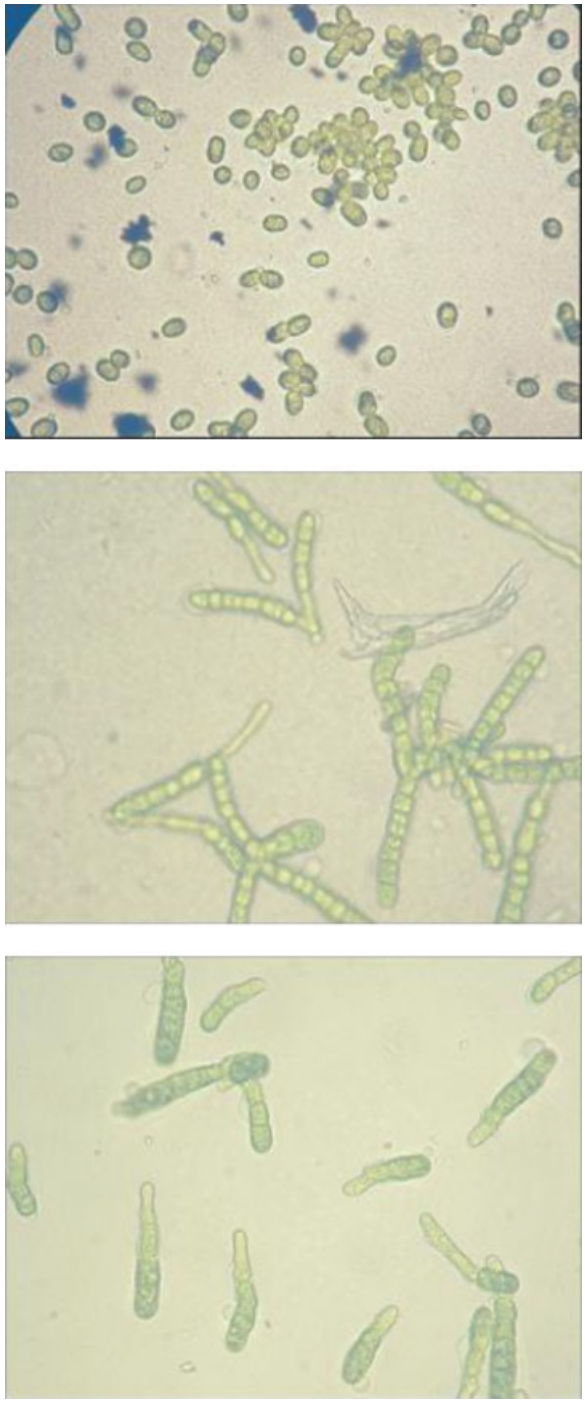


Figure 9. Settlement and germination of *Ulva intestinalis* on PA + 0.5% Ag (a), PA control (b), and glass control (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

some cases, the carriers used in the commercial silver-based antimicrobials made notable assistance in the silver ion release properties of the composites. All composites in which the fillers contain carrier materials seldom produced much effect on the release within the period of analysis. Elementary silver-based composites are found to be efficient for the requirement of long-term efficacy. The absence of antimicrobial property within shorter period for elementary compositions can be attributed to the higher surface barrier for water diffusion during this period, leading to poor Ag^+ release. Silver filling in PA using $scCO_2$ produced promising composites considering their Ag release efficacy during shorter periods. Both types of the antimicrobial tests revealed the potential efficacy of the composites, which support the silver ion release data. PA/Ag composite (0.5% of elementary silver) was effective against many fungi. PA/Ag composite containing lower concentration of elementary silver powder is an effective biomaterial when considering its excellent Ag^+ release and antimicrobial and antifungal properties. The experimental data suggest that a combination of elementary and commercial silver in polymers can constitute effective antimicrobial biomaterials for a variety of promising applications.

The authors thank Gaiker Technology Centre, Technology Park, Bilbao, Spain, for the antimicrobial tests and North Atlantic Fisheries College (NAFC, Scotland) for the antifouling tests. We thank the European Union for financial assistance.

REFERENCES

1. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for gram-negative bacteria. J Colloid Interface Sci 2004;275:177–182.
2. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E, Domann E, Schnettler R. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. Biomaterials 2004;25:4383–4391.
3. Samuel U, Guggenbichler JP. Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. Int J Antimicrob.Agents 2004;23:75–78.
4. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. J Biomed Mater Res 2000;53:621–631.
5. Son WK, Youk JH, Lee TS, Park WH. Preparation of antimicrobial ultrafine cellulose acetate fibers with silver nanoparticles. Macro Rap Commun 2004;25:1632–1637.
6. Blaker JJ, Nazhat SN, Boccaccini AR. Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications. Biomaterials 2004;25:1319–1329.
7. O'Neill MAA, Vine GJ, Beezer AE, Bishop AH, Hadgraft J, Labetoulle C, Walker M, Bowler PG. Antimicrobial properties of silver containing wound dressings: a microcalorimetric study. Int J Pharm 2003;263:61–68.
8. Grier N. Silver and its compounds in disinfection, sterilization and preservation. In: Block S, editor. Philadelphia: Lea & Febiger; 1983. p 380–428.
9. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compound. FEMS Microbiol Rev 2003; 27:341–353.
10. Hidalgo E, Bartelome R, Barrosso C, Moreno A, Dominguez C. Silver nitrate: antimicrobial activity related to cytotoxicity in cultured human fibroblasts. Skin Pharmacol Appl Skin Physiol 1998;11:140–151.
11. Simonetti N, Simonetti G, Bougnol F, Scalzo M. Electrochemical Ag^+ for preservative use. Appl Environ Microbiol 1992; 58:3834–3836.
12. Powell J. Our mightiest germ fighter. Sci Digest 1978;57–60.

13. Berger T, Spadaro JA, Chapin SE, Becker RO. Antifungal properties of electrically generated silver ions. Antimicrob Agents Chemother 1976;10:856–860.
14. Olson ME, Wright JB, Lam K, Burrell RE. Healing of porcine donor sites covered with silver-coated dressings. Eur J Surg 2000;166:486–489.
15. Wassall MA, Santin M, Isalberti C, Cannas M, Denyer SP. Adhesion of bacteria to stainless steel and silver-coated orthopedic external fixation pins. J Biomed Mater Res 1997;36:325–330.
16. Blaker JJ, Nazhat SN, Boccaccini AR. Development and characterisation of silver-doped bioactive glass-coated sutures for tissue engineering and wound healing applications. Biomaterials 2004;25:1319–1329.
17. Wöhrmann RMJ, Hentschel T, Münstedt H. Thermoplastic silver filled polyurethanes for antimicrobial catheters. Adv Eng Mater 2000;2:380–386.
18. Appendini P, Hotchkiss JH. Review of antimicrobial food packaging. Innovat Food Sci Emerg Technol 2002;3:113–126.
19. Yoshida K,Tanagawa M, Atsuta M. Characterization and inhibitory effect of antimicrobial dental resin composites incorporating silver-supported materials. J Biomed Mater Res 1999;47: 516–522.
20. Grier N. Silver and its compounds. In: Block S, editor. Disinfectants, sterilisation and preservations, 3rd ed. Philadelphia: Lea & Febiger; 1983.
21. Trevors JT. Survival of escherisia coli donor recipient and transconjungent cells in soil. Water Air Soil Pollut 1987;34: 409–414.
22. Shearer AEH, Paik JS, Hoover, DG, Haynie SL, Kelly MJ. Potential of an antibacterial ultraviolet-irradiated nylon film. Biotechnol Bioeng 2000;67:141–146.
23. Fornes TD, Paul DR. Crystallization behavior of nylon 6 nanocomposites. Polymer 2003;44:3945–3961.
24. Shintani H. Modification of medical device surface to attain anti-infection. Trends Biomater Artif Organs 2004;18:1–8.
25. Wohrmann RMJ, Munstedt H. Zur bestimmung der freisetzung von silberionen aus silbergefülltem polyurethan. Infection 1998; 26:49–52.
26. Kumar R, Münstedt H. Silver ion release from antimicrobial polyamide–silver composites. Biomaterials 2005;26:2081–2088.
27. Barry JA, Gidda HS, Scotchford CA, Howdle SM. Porous methacrylate scaffolds: supercritical fluid fabrication and in vitro chondrocyte responses. Biomaterials 2004;25:3559–3568.
28. Kamenev AI,.Lushov KA. Determination of silver by stripping voltammetry at composite pyrocarbon–glass ceramic electrodes. J Anal Chem 2001;56:380–383.
29. Fraunhofer JAV, Banks CH. Potentiostat and its applications. London: Butterworth; 1972. p 154–155.
30. Approved Standards Action (ASTM) E committees, E35 on pesticides. Test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions. New Standard 2001;11.05:E2149–01 October 2001.
31. Japanese Industrial Standard/Antimicrobial products. Test for antimicrobial activity and efficacy (Japanese standard association 4-1-24), Akasaka Minato-ku, Tokyo, 107–8 440 Japan.