OBTAINMENT OF BACTERICIDAL INKJET INKS BASED ON Ag NANOCOMPOSITES

M. D. Palacios[(1)], S. Mestre [(1)], A. Belda[(1)], V. Nos[(2)], J. Cabedo[(2)], J. Zaragoza[(2)]

[(1)] Instituto de Tecnología Cerámica (ITC). Asociación de Investigación de las Industrias Cerámicas (AICE). Universitat Jaume I. Castellón. Spain.

[(2)] ENDEKA CERAMICS, S.A.R. Vall d'Alba (Castellón). Spain

Keywords: bactericidal glazes; multifunctional inks; nanocomposite; silver; single firing.

1 Abstract

In this study, ceramic inks were formulated that are able to develop bactericidal properties and are appropriate for application by inkjet printing. These inks contain silver nanocomposites and are applied on to unfired glazed ceramic bodies, yielding single-fired tiles.
Silver nanocomposites were obtained from different precursors, which were made to react in order to obtain more stable structures that, in addition to incorporating bactericidal components, were able to reduce their reactivity and dissolution in the glassy matrix during the ceramic tile firing stage, avoiding the ensuing loss of properties.
The study also examined how the nature of the base glaze affected the reactivity of the resulting nanocomposites. The base glaze that enabled the bactericidal compounds to remain at the ceramic tile surface was selected as the optimum base glaze for the study.

2 Introduction

In recent years, research in ceramic tiles has focused on the development of functional surfaces. Such surfaces are preferentially obtained by means of thin layers of materials with special, electric, thermal, physical, or chemical properties. Among the last materials, particularly to be noted are materials with self-cleaning, bactericidal, and fungicidal properties.[1-3]
Generally speaking, a surface layer of active material on the tile can be obtained by either of two processes:

1. Application by immersion, spraying, or some other similar method of a preferentially liquid precursor on to an already fired glaze surface, followed by a gentle thermal treatment to fix the precursor.
2. Application of the precursor on to an unfired glaze followed by a single-firing treatment.

The advantage of the first method is that the layer of material is retained on the glaze surface. If the thermal treatment is appropriately controlled, a layer with the desired structure, mineralogy, and thickness can be obtained.
The second method only requires the single-firing stage, which would not entail any modification of the current ceramic tile manufacturing process. However, the difficulty lies in obtaining a material that is not attacked during the thermal treatment at high temperature. This requirement is met by ceramic pigments. These are already processed materials with an appropriate particle size that, either by their nature or by being duly protected, can withstand the single firing process.

Noble metals are biocidal substances that have been used for decades in fields such as medicine. Today with the development of nanotechnology, the nanoparticles of these metals are known to have different, usually better properties than those of the materials on a macroscopic scale. Although the mechanism that causes bactericidal activity is still not well understood, it has been demonstrated that silver nanoparticles exhibit this property when they have a certain size (below 100 nm), shape, and surface dispersion.[4]
However, the introduction of these nanoparticles into glaze compositions is a delicate issue, since silver nanoparticles would not withstand a single-firing treatment. A possible option would be to protect such nanoparticles with a refractory material before they are incorporated into the glaze to avoid thermal and chemical attack during firing.
This study was undertaken to develop an ink that contained silver nanoparticles and could be used by inkjet printing to generate glaze coatings with antibacterial properties, when fired by the usual ceramic tile single-firing cycles.

3 Experimental procedure

The following reagents were used in synthesis:

- As silver nanoparticle precursors: silver carbonate, Ag_2CO_3, a commercial suspension of silver (16 g/L concentration, average particle size 5.5 ± 1.2 nm and density 0.72 g/ml), and silver nitrate concentrations 0.1 M and 2.5% v/v.
- As protective layer precursors: tetraethyl orthosilicate (TEOS) and colloidal silica, with 50% solids content.
- The inks were prepared with a commercial solvent used in fabricating pigmented inks, a fluxing transparent frit, G40 quartz, and a dispersant.

The ink was ground in a laboratory bead mill (SL-M, DISPERMAT). Grinding was performed at a power of 200 watt for 6 hours.
The inks obtained were applied by screen printing or spraying, controlling the quantity deposited on the test glazes (white, crystalline, and matt). The thermal treatments were appropriate for the type of glaze involved and are detailed in the results section.
The pieces obtained were characterised by scanning electron microscopy to determine the surface microstructure and nanoparticle size, when they were identified by EDX.
Tile antimicrobial activity was determined according to the procedure described in a Japanese standard.[5] This standard determines the antimicrobial activity and efficiency of antimicrobial products on surfaces using two bacteria: *Staphylococcus aureus* and *Escherichia coli*. These bacteria must be quantified and deposited on the antimicrobially treated surfaces, as well as on untreated control surfaces, in order to be able to establish the reduction in the existing bacterial charge on the surface. The following formula is applied to calculate the biocidal activity:

$$R = [\log (B/A) - \log (C/A)]$$

where R is the value of the antimicrobial activity, A is the average of the counts obtained of viable bacteria after deposition of the micro-organisms on the control surfaces (blanks), B is the average of the counts obtained of viable bacteria after incubation for 24 hours, and C is the average of the counts obtained of viable bacteria after incubation of the samples treated with the biocidal additive for 24 hours.

4 Results

4.1 Preliminary trials with Ag

With a view to evaluating the bactericidal activity of silver, a preliminary study was performed in which suspensions of silver compounds were directly applied as drops on to both unfired and fired tiles. The following three types of silver precursors were used as raw materials:

- A suspension of silver nanoparticles (10 g/L) in heptane.
- Silver carbonate solution (10 g/L) in 2-propanol.
- Silver nitrate 2.5% v/v.

The silver precursors were tested on three types of glazes, as indicated in Table 1: a crystalline, a zirconium white, and a matt glaze, both unfired and fired at different temperatures between 800 and 1100 ºC.

Table 1 References and conditions used in obtaining the test pieces prepared in the preliminary tests

		800 ºC	**1000 ºC**	**1100 ºC**
CRYSTALLINE	UNFIRED	C1	C2	C3
	FIRED	C4	C5	C6
WHITE	UNFIRED	B1	B2	B3
	FIRED	B4	B5	B6
MATT	UNFIRED	M1	M2	M3
	FIRED	M4	M5	M6

After a first visual assessment, the samples on the crystalline and matt glazes were discarded, because the silver precursor was observed to have dissolved in the glassy matrix. However, in the case of the white substrate the drops applied could be visually observed even at the tested peak temperature.

4.1.1 Microstructural characterisation of the test pieces

Before the bactericidal tests, the samples were characterised by scanning electron microscopy and EDX analysis to verify whether the silver had been retained on the tile surfaces.

Samples B3 (Single firing 1100 ºC), B4 (Double firing 800ºC), B5 (Double firing 1000 ºC), and B6 (Double firing 1100 ºC) were selected. In each case, the references -1, -2, and -3 refer to the suspension of metallic Ag nanoparticles, the silver carbonate solution, and the silver nitrate solution, respectively.

The results obtained are summarised below:

- B3 samples (application on unfired white glaze): no silver was observed on the surface of the fired glazes, independently of the precursor and the firing temperature.
- B4 samples (application on fired white glaze, followed by refiring at 800 ºC): spherical particles of silver of about one micron diameter were observed when the nanoparticle suspension or the silver nitrate solution was used, coarse aggregates being obtained when silver carbonate was used.

- B5 samples (application on fired white glaze, followed by refiring at 1000 ºC): spherical particles were observed with the three precursors, though in smaller amounts and larger sizes when silver carbonate was used.
- B6 samples (application on fired glaze, followed by refiring at 1100 ºC): isolated spherical particles of silver were observed using nanoparticles or silver carbonate. A larger quantity of silver spheres was observed with silver nitrate.

Summing up, it may be stated that isolated silver particles were only identified on the surface of the samples obtained by double firing and, when thermal treatment temperature increased, the quantity of surface silver decreased, as it dissolved in the glassy substrate. SEM photographs and the EDX analysis of some of the glazes are shown in Figures 1 to 4.

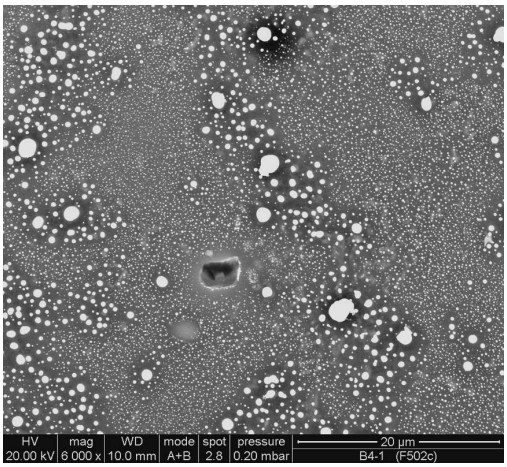

Figure 1 Appearance of sample B4-1.

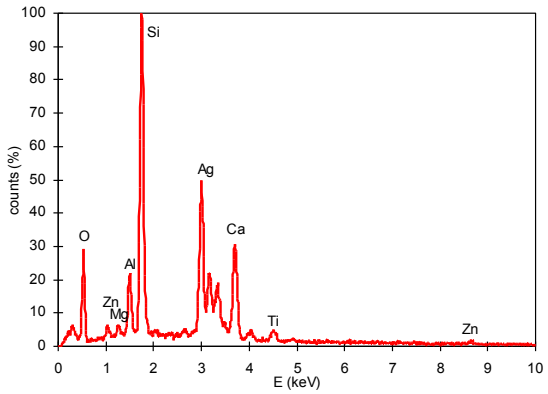

Figure 2 EDX analysis of the spherical particles.

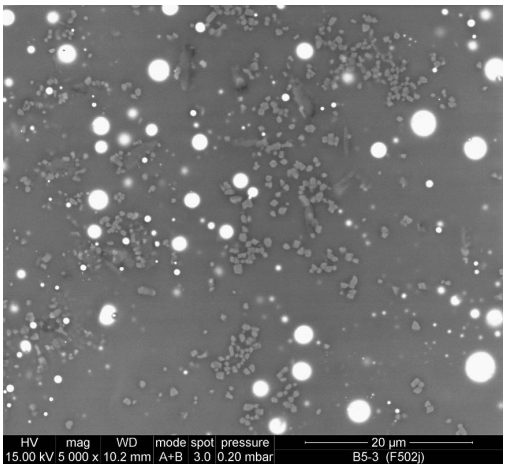

Figure 3 Appearance of sample B5-3.

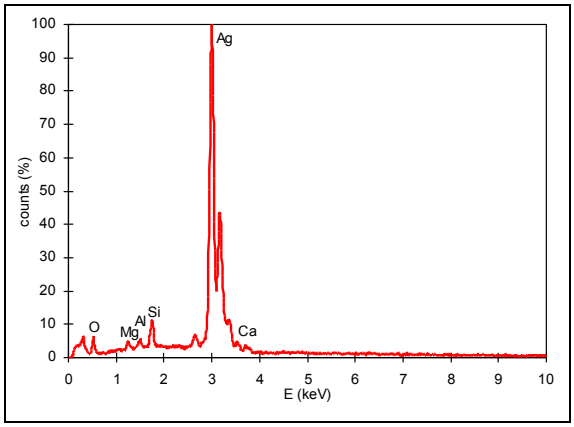

Figure 4 EDX analysis of the spherical particles

4.1.2 Study of antimicrobial activity

After these preliminary studies, two types of suspensions were prepared for application on to fired tiles:

- A mixture prepared from an inkjet ink and the silver nanoparticle suspension (reference A), as detailed in Table 2.
- The original silver nanoparticle suspension (reference H2).

Table 2 Preparation of mixture A.

Component	% by weight
Metallic silver	7.2
Yellow ink	24.9
Heptane	67.9

Both suspensions were sprayed on to fired tiles sized 5x5.5 cm. The applied quantities are listed in Table 3. The pieces were then fired at 1100 ºC, with a residence time of 1 minute at peak temperature.

Table 3 Quantities of deposited suspension and silver.

Reference	g suspension /piece	g Ag/m^2
Treatment A	0.06	26.7
Treatment H2	0.40	100.0

The tests on bactericidal activity showed that the analysed glazes exhibited biocidal activity with relation to the *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739) bacteria. The test results are presented in Table 4.

Table 4 Results of the bactericidal activity tests

Micro-organism	Reference	Exposure time	Count (cfu/sample)	Value of antimicrobial activity
***Staphylococcus aureus* (ATCC 6538)**	Uncoated control	0	6.2×10^4	2.02
		24 h	1.8×10^4	
	Ref. A	24 h	$1.7.10^2$	
***Escherichia coli* (ATCC 8739)**	Uncoated control	0	1.3×10^5	4.07
		24 h	1.2×10^5	
	Ref. A	24 h	< 10	
Micro-organism	**Reference**	**Exposure time**	**Count (cfu/sample)**	**Value of antimicrobial activity**
***Staphylococcus aureus* (ATCC 6538)**	Uncoated control	0	6.2×10^4	2.53
		24 h	1.8×10^4	
	Ref. H2	24 h	53	
***Escherichia coli* (ATCC 8739)**	Uncoated control	0	1.3×10^5	4.07
		24 h	1.2×10^5	
	Ref. H2	24 h	<10	

The following conclusions were drawn from this first study:

- The deposition of silver particles of 1 micron or smaller on a glaze produced a surface with biocidal activity.
- The quantity of silver deposited on the glazes was quite high. The values obtained for glazes A and H2 were similar, though glaze H2 had a slightly

higher activity with relation to *Staphylococcus aureus*. Table 3 shows that the deposited quantity of H2 suspension (silver nanoparticles in suspension) was almost four times greater than that deposited of A. The reason that the values of both samples were so similar could be that, when the silver nanoparticles were applied as a suspension, they were less stable on the glaze surface, probably being more attacked by the glaze during the thermal treatment. Since the nanoparticles were contained in the ink in the A mixture, this made them more stable, leading to a similar concentration of silver on both surfaces.

- Finally, it should be noted that mixture A and suspension H2 were both applied on to fired glazes, which reduced attack by the molten glass to a certain extent during thermal treatment.

4.2 Synthesis of protected silver nanocomposites

The next step in the study was to obtain a protected silver nanocomposite that was:

- Capable of stabilising in an inkjet ink.
- Capable of withstanding attack by molten glass.

This would allow the ink with the silver nanocomposite to be applied on to the unfired glaze and, after a single-firing treatment, Ag nanoparticles to be obtained on the fired glaze surface.

To protect the Ag nanoparticles, it was decided to attempt to obtain a porous silica network in which the silver nanoparticles were dispersed. It was not desirable to obtain a capsule that was excessively impermeable because the silver would remain isolated within the silica. The ideal would be an envelope that would protect the Ag nanoparticles during thermal treatment to avoid dissolution in the molten glass, while allowing Ag nanoparticle contact with the surface at the end of the treatment.

To protect the nanoparticles, two approaches were used:

- Combining colloidal silica with silver nitrate, subjecting the mixture to an appropriate drying treatment to reduce the silver and to consolidate the capsule.
- Synthesising a functionalised silica precursor with groups associated with metallic silver, followed by addition of silver nitrate, and ending with chemical reduction of the metal.

Material from a mixture of colloidal silica and silver nitrate

Different mixtures of colloidal silica (50% solids content) and silver nitrate were prepared. Ammonia was added to the mixtures to maintain the base pH of the silica and carboxymethylcellulose (CMC) solution in order to prevent silver particle agglomeration, under stirring for 30 minutes. Drying yielded two pinkish-white solids that were calcined in a kiln at two temperatures, 300 and 1000 ºC, for 1 hour. The products were referenced M-01 and M-02, respectively.

Material from a functionalised silica precursor and silver nitrate

This involved synthesis in two steps. First, a functionalised silica was synthesised from sodium silicate and 3-aminopropylethoxysilane as described in the literature.[5] The second step consisted of mixing the functionalised silica with a silver nitrate solution and subsequently reducing the Ag (I) ion to Ag (0) with $NaBH_4$. After drying, the resulting solid was referenced M-03.

4.2.1 Microstructural characterisation of the solids

The synthesised materials were characterised by scanning electron microscopy. EDX analyses were performed to identify the silver particles in the samples.

A photograph of the M-01 material is shown in Figure 5. A compact solid with some silver particles of different size can be observed.
A photograph of the M-03 solid is presented in Figure 6, which shows the formation of a sponge-like solid, containing embedded silver particles measuring between 50 and 100 nm.

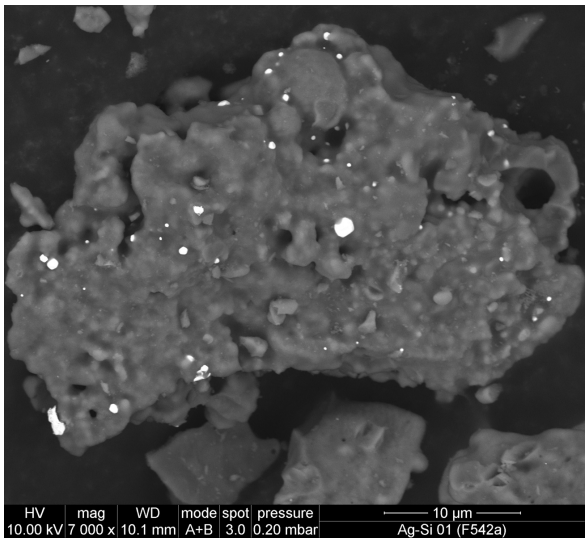

Figure 5 Sample M-01

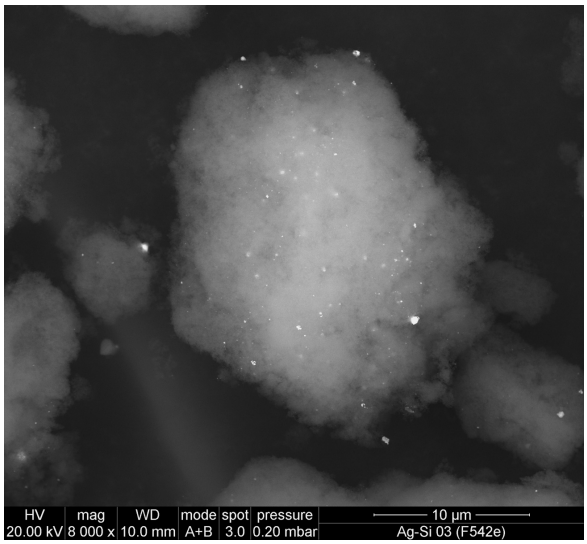

Figure 6 Sample M-03

4.2.2 Applications of nanocomposites on glazes

Suspensions were prepared with nanocomposites M-01 and M-03 from a screen printing vehicle and a 10% addition of each solid, respectively. These suspensions were deposited by spraying on to unfired white glazes, with a quantity of 0.31 g/cm^2. The test pieces were fired using a standard floor tile cycle with a peak temperature of 1120 ºC and residence time of 3 minutes at peak temperature.
The pieces displayed a slightly yellowish tone and were quite rough, owing to the presence of a great quantity of silica in the applied screen printing inks.
The surfaces of both pieces were characterised by SEM. In the case of the glaze with the M-01 solid (Figure 7), silver particles were observed to appear in different regions of the surface, with particle sizes between 60 and 200 nm.
However, the surface of the glaze with the M-03 solid (Figure 8) exhibited no silver at the surface, either as particles or in a comprehensive EDX analysis. On the other hand, the M-01 particles exhibited better integration into the glaze.

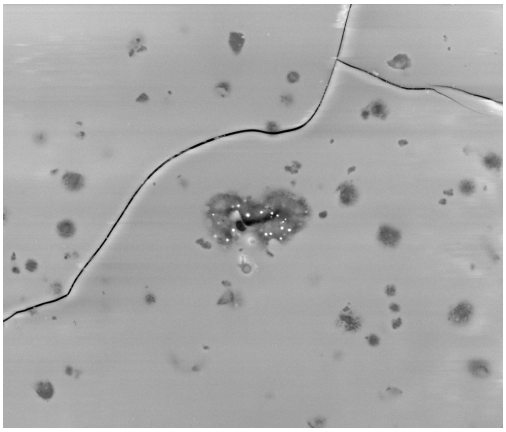

Figure 7 Fired glaze with M-01.

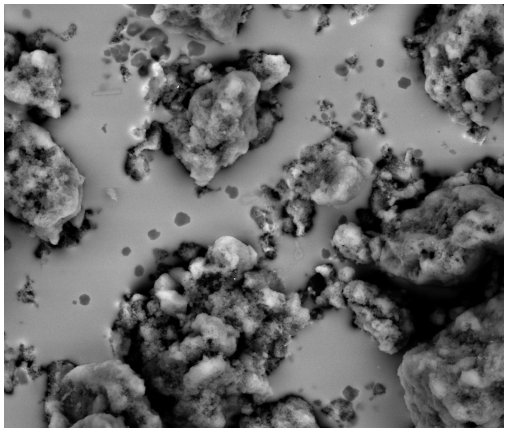

Figure 8 Fired glaze with M-03.

4.3 Obtainment of an ink

After observing the silver in the glaze treated with the sample M-01 suspension, an ink was prepared with the mixture calcined at 1000 ºC (M-01) in order to verify the behaviour of the nanocomposite on being subjected to a process similar to that of inkjet ink preparation.

The vehicle used was a commercial product customarily used in the preparation of this type of ink. Finally, a dispersant was added to stabilise the inks.

The inks were prepared with the materials detailed in Table 5. The percentage of silver by weight in the ink was 0.75%.

Preparation consisted of mixing the quantities indicated by magnetic stirring of all the ingredients and subsequent transfer to a bead mill, where the mixture was milled for 6 hours at 200 W.

Table 5 Proportions of the materials used in preparing ink T3.

	M-01	Silica	Frit	Vehicle	Dispersant
mass (g)	22	118	60	200	6
% by weight	5.4	29	14.8	49	1.5

The ink was applied on to pieces of glaze of the zirconium white type, measuring 5x5 cm. The quantities of ink deposited on the pieces were 22 g/m^2 and 44 g/m^2, equivalent to 0.17 and 0.33 g Ag/m^2, respectively, as indicated in Table 6. The pieces were fired using a standard floor tile cycle with a peak temperature of 1120 ºC and residence time of 3 minutes at peak temperature.

Table 6 Quantities of applied ink and silver

Reference	g ink/m^2	g Ag/m^2
T31	22	0.17
T32	44	0.33

Figure 9 shows a SEM photograph of a region of the fired glaze. Isolated particles of silver identified by EDX can be observed. The characterisation is difficult owing to the presence of zinc and zirconium in the glaze, which may be confused because of the similarity of their tone and gloss to those of silver. In addition, the roughness of the sample did not allow clear images to be obtained and it is not possible to determine with absolute certainty whether the particles lie at the surface or in a lower layer.

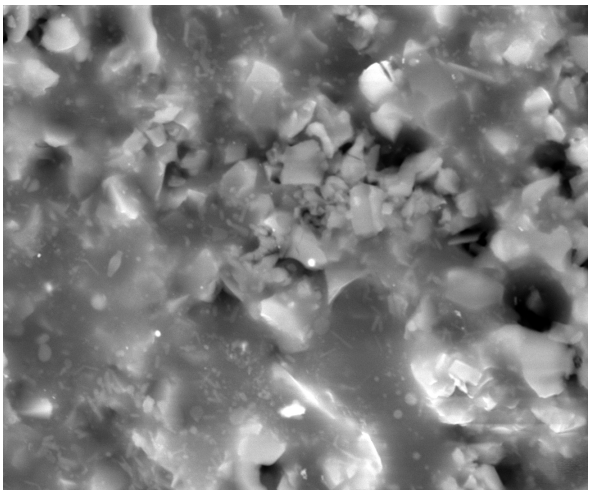

Figure 9 Sample Fired glaze T31.

4.4 Study of antimicrobial activity

The bactericidal activity of the pieces referenced T31 and T32 was determined. The results are detailed in Tables 7 and 8.
The samples displayed an important antimicrobial activity with relation to both bacteria, including the samples with a lower antimicrobial agent concentration. Indeed, no significant differences in antimicrobial activity were noted between the different tested samples.
The antimicrobial effect was more pronounced with relation to *E. coli*, so that a treatment with good activity in regard to the Gram (-) bacteria is probably involved. However, confirmation of the activity with relation to a particular species and/or strain requires further testing.

Table 7 Results of sample exposure on the *E. coli* inoculum.

	***Escherichia coli* ATCC 8739**		
		Count (cfu/sample)	Value of antimicrobial activity
Control sample	Time 0 h	2.5×10^6	
	Time 24 h (B)	6.4×10^6	
Sample T31	Time 24 h (C)	$< 4.0 \times 10^2$	>4.21
Sample T32	Time 24 h	$< 4.0 \times 10^2$	>4.21

Table 8 Results of sample exposure on the *S. aureus* inoculum.

	***Staphylococcus aureus* ATCC 6538**		
		Count (cfu/sample)	Value of antimicrobial activity
Control sample	Time 0 h	8.1×10^6	
	Time 24 h (B)	5.9×10^6	
Sample T31	Time 24 h (C)	8.2×10^2	3.89
Sample T32	Time 24 h	$< 4.0 \times 10^3$	>4.21

5 Conclusions

A series of trials were carried out to evaluate the biocidal activity of ceramic glazes with silver as bactericidal agent. The preliminary tests with silver precursors of the nanoparticle or salt type showed that activity was achieved with glazes processed by double firing, while the glazes synthesised with single-firing treatments exhibited no activity.

In order to obtain single-fired glazes with biocidal activity, materials were prepared in which the silver was protected by a silica capsule, a refractory material, to prevent the silver nanoparticles from dissolving during thermal treatment while maintaining the connection with the surface.

Using these silica-silver nanocomposites, inkjet inks were prepared that were applied on to unfired glazes and subjected to a single-firing cycle.

The results of the biocidal tests evidenced the bactericidal activity of the glazes treated with the ink prepared from a silica-silver nanocomposite.

6 References

1) RAVINDRA, S.; MOHAN, Y. M.; REDDY, N. N.; RAJU, K. M. Fabrication of antibacterial cotton fibres loaded with silver nanoparticles via "Green Approach". *Colloids and Surfaces A: Physicochem. Eng. Aspects* 367, 31-40, 2010.
2) TOLAYMAT, T. M.; EL BADAWY, A. M.; GENAIDY, A. *et al*. An evidence-based environmental perspective of manufactured silver nanoparticle in syntheses and applications: A systematic review and critical appraisal of peer-reviewed scientific papers. *Science of the Total Environment* 408, 999–1006, 2010.
3) KASSAEE, M.Z.; AKHAVAN, A.; SHEIKH, N.; BETESHOBABRUD, R. γ-Ray synthesis of starch-stabilized silver nanoparticles with antibacterial activities. *Radiation Physics and Chemistry* 77, 1074–1078, 2008.
4) GUZMAN, M.; DILLE, J.; GODET, S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomed-Nanotechnol* (in press).
5) JIS Z 2801A1:2006 Antimicrobial products. Test for antimicrobial activity and efficacy.
6) QUANG, D. V. SARAWADE, P. HILONGA, A. *et al*. Facile route for preparation of silver nanoparticle-coated precipitated silica. *Applied Surface Science* 257, 4250–4256, 2011.