STUDY OF THE INFLUENCE OF THE VITREOUS ENVIRONMENT ON THE BACTERICIDAL PROPERTY OF CERAMIC GLAZES

O. Ruiz[1], A. Tamayo[2], J. Rubio[2], F. Rubio[2], S. Burgui[3], R. Bueno[3], J. Palomares[4].

(1) Grupo Torrecid. Spain.

(2) Instituto de Cerámica y Vidrio – CSIC. Spain.

(3) Asociación Industria Navarra – AIN. Spain.

(4) Instituto de Ciencia de Materiales de Madrid – Spain.

1. INTRODUCTION

The technological innovation applied to ceramics in recent years has enabled the advent of new solutions and generated future trends capable of affording the best competitive edges and greater technical and aesthetic added value to ceramic products. Thanks to that, ceramic coatings are today a reference material in numerous construction and industrial applications.

However, new requirements are continually being demanded of ceramic surfaces, one such case being for them to have antimicrobial properties.

The antimicrobial potential of silver is well known at both micrometric and nanometric levels. In this sense, numerous studies have shown that the process through which silver acts as an antibacterial agent consists of an oxidation reaction in an aqueous solution exposed to air, which produces silver ions from the following reactions[1]:

$$4Ag + O_2 \rightarrow 2Ag_2O$$

$$2Ag_2O + 4H^+ \rightarrow 4Ag^+ + 2H_2O$$

Once the Ag^+ cation is formed, its antibacterial activity is thought to take place via three mechanisms[2,3,4,5]:

- The Ag^+ ions react with the thiol groups in the proteins responsible for respiration of the bacterium and its transport in the membrane, which disrupts the membrane's potential and permeability, thus leading to death.
- The Ag+ ions enter bacteria through ion channels and the membrane. Once inside, silver becomes complexed with DNA and RNA bases leading to a loss of replicating capacity.
- Increased production of reactive oxygen species that causes oxidative stress and consequent degradation and death in the bacteria.

The aim of this work was to study the changes in the vitreous environment of ceramic glazes and the oxidation state of silver, when silver is incorporated into the frit composition, as well as its relationship with bactericidal properties. For that purpose, two ceramic glazes for porcelain stoneware were selected, one with a gloss finish and one matt, formulated with frits containing different concentrations of silver.

2. EXPERIMENTAL AND RESULTS

The experimental procedure began with the preparation of two types of frit, one with a gloss finish and the other matt. Different concentrations of silver, ranging from 0.05% to 1% by mass, were added to each of them during firing. Once the frits had been produced, they were used to prepare formulations of both conventional porcelain glaze for spray-gun application, and digital porcelain glaze, to be deposited using inkjet technology. The purpose was to assess the influence of particle size on bactericidal performance in ceramic glazes. Finally, the glazed ceramic pieces were subjected to a porcelain firing cycle at a maximum temperature of 1200ºC. The table below shows the references of the various tiles produced, with the concentration of silver (% by mass) in the frit used in each of the glazes.

	% Ag mass in the frit					
Type of Glaze	0	0,05	0,1	0,25	0,5	1
Conventional gloss glaze	CB	CB1	CB2	CB3	CB4	CB5
Conventional matt glaze	CM	CM1	CM2	CM3	CM4	CM5
Digital gloss glaze	DB	DB1	DB2	DB3	DB4	DB5
Digital matt glaze	DM	DM1	DM2	DM3	DM4	DM5

***Table 1**. Glaze references.*

2.1 STUDY OF THE VITREOUS STRUCTURE AND OXIDATION STATE OF SILVER AND ITS RELATIONSHIP WITH BACTERICIDAL PROPERTIES

In order to understand the relationship between a glaze's bactericidal capability and its vitreous structure, the activation Energy (Ea) for the frits used was calculated. Structural relaxation or thermodynamic fragility was taken from the calculation of activation energy (Ea) in the frits, so that the lower the Ea, the higher the vitreous structure's ability to fracture and therefore with easier access to the silver cations. Experimentally, the method consisted of performing differential thermal analysis (DTA) of the frit to calculate its glass transition temperature (Tg) at a certain heating rate[6.7]. By taking different values for this heating rate and the corresponding Tg, and then applying the Kissinger equation, activation energy can be found. Specifically, in this paper, four DTA measurements for heating rates of 5, 10, 15 and 20 ºC/min were carried out.

The graphs below show how activation energy in the frits evolved at different concentrations of silver (% by mass) and with the two particle sizes used in the ceramic glazes. In this sense, the following results should be highlighted:

- In all cases, as the silver concentration increases, the value of Ea decreases.
- At the same concentration of silver in a frit, the value of Ea is greater in the particle size used for conventional glazes compared to digital glazes.
- At the same silver concentration and particle size, the matt frit has a lower Ea than the gloss frit.

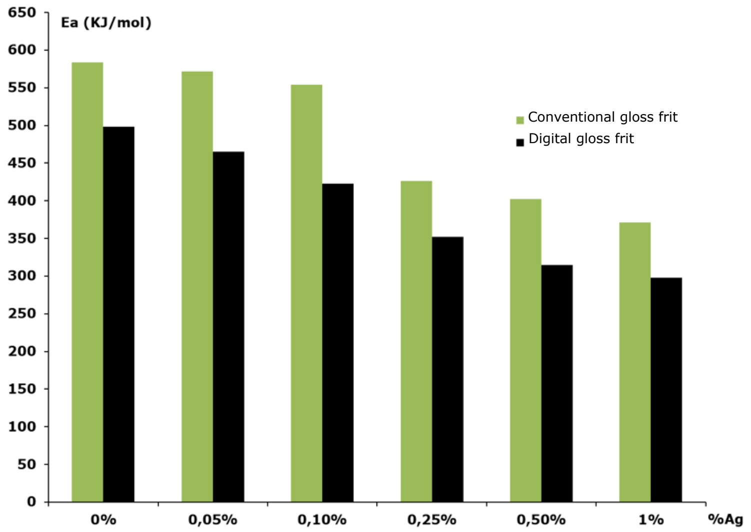

Figure 1. *Evolution of Ea in gloss frits at varying concentrations of Ag*

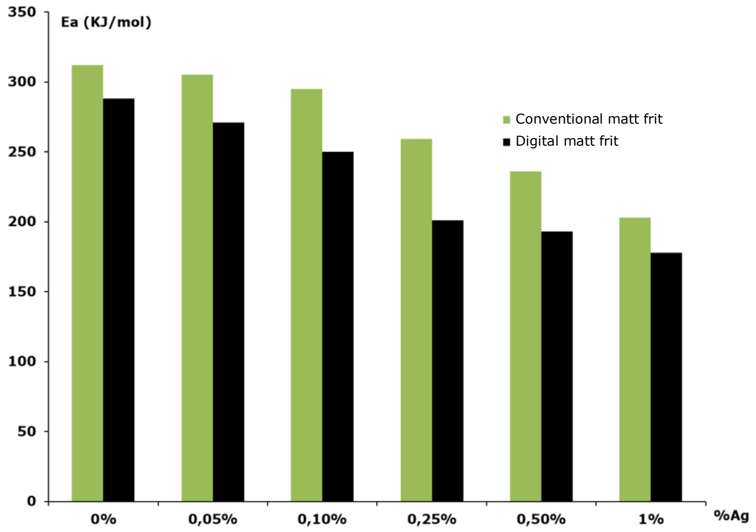

Figure 2*. Evolution of Ea in matt frits at varying concentrations of Ag.*

Furthermore, three techniques - scanning electron microscopy (SEM), X-ray photoelectronic spectroscopy (XPS) and Raman spectroscopy - were used to study the evolution of the silver oxidation state in the glazes. SEM observation of the glaze surface revealed the presence of silver particles, which seems to indicate that silver is in a state of zero oxidation in the glaze. In this sense, and by way of an example, the images below are of the digital glazes obtained from frits with 1% silver, and the resulting chemical analysis.

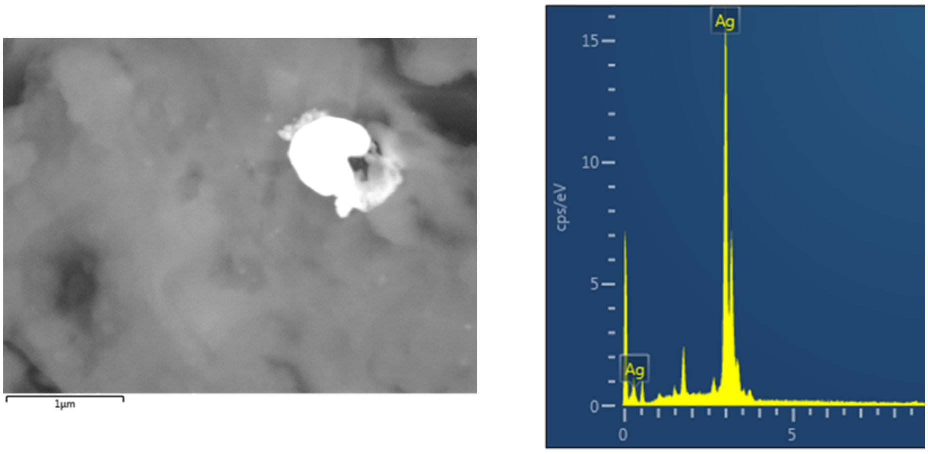

Figure 3. *SEM image and chemical analysis of the digital gloss glaze obtained from the gloss frit with 1% Ag.*

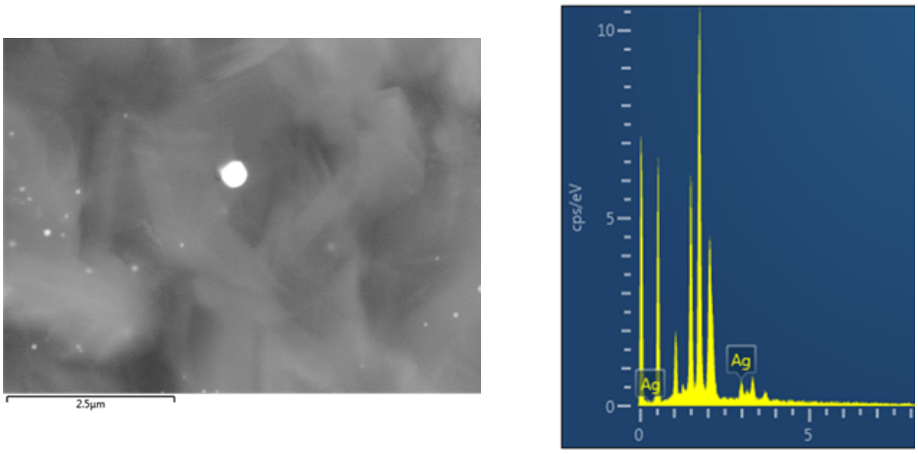

Figure 4. *SEM image and chemical analysis of the digital matt glaze obtained from the matt frit with 1% Ag.*

X-ray photoelectron spectroscopy (XPS) is based on matter and photons interacting due to the photoelectric effect. In this sense, when a sample is irradiated with photons with energy higher than that bonding electrons in the atoms, the electrons leave the sample with a kinetic energy equal to the excess of energy in the photons compared to the electron bonding energy. In principle, XPS spectroscopy enables the elements present at surface level, as well as the oxidation state[8] to be identified.

In this research, XPS readings were taken on glaze surfaces with a SPECS GmbH PHOIBOS 150 MCD spectrometer, using a monochrome magnesium X-ray source at 1253 eV, 200 W and 12 kV. An analysis of each spectrum was performed, establishing a step energy of 50 eV and 20 eV and an area of 5 x 5 mm^2. The position of each peak was calibrated to 284.6 eV using the C1s spectrum and analysed by a Non-linear Least Squares (NLS) method that fits to Gaussian-Lorentzian-type curves.

The XPS spectra study area focused on the photo-electric peaks of silver corresponding to levels $Ag3d_{3/2}$ and $Ag3d_{5/2}$. The graphs hereunder show the XPS spectra obtained for conventional and digital ceramic glazes with silver-free frits, as well as with frits containing 1% silver. In this regard, the presence of silver in the glazes under study was confirmed. However, due to an overlap of the signals, comprehensive analysis of the spectra does not enable the oxidation state of silver in the vitreous structure of the glazes to be discerned.

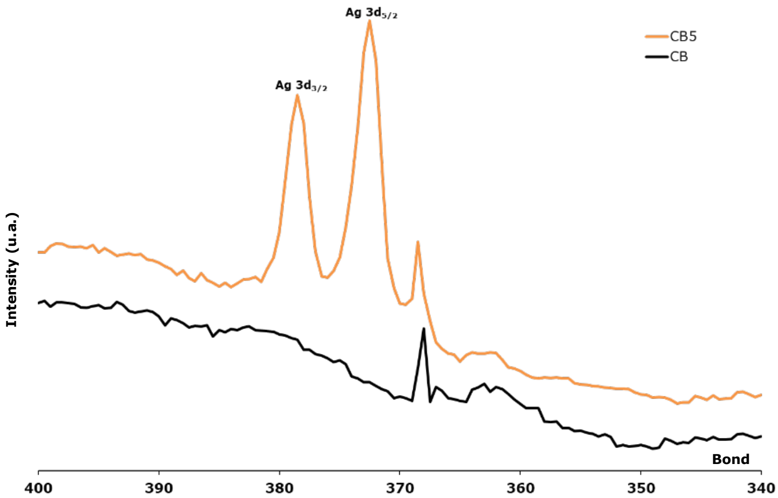

Figure 5. *XPS spectrum of the conventional gloss glazes, one Ag-free (CB) and one with 1% Ag in the frit (CB5).*

Figure 6. *XPS spectrum of conventional matt glazes, one Ag-free (CM) and one with 1% Ag in the frit (CM5)*

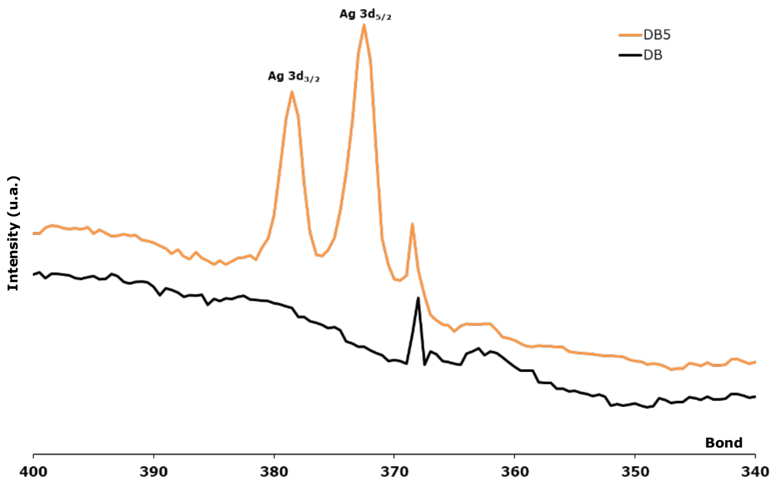

Figure 7. *XPS spectrum of digital gloss glazes, one Ag-free (DB) and one with 1% Ag in the frit (DB5)*

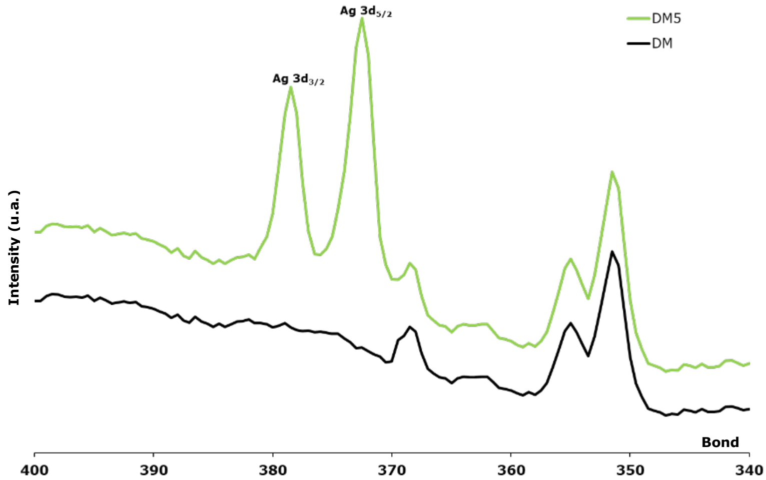

Figure 8. *XPS spectrum of digital matt glazes, one Ag-free (DM) and one with 1% Ag in the frit (DM5)*

In order to learn more about the oxidation status of silver in the glazes, the study was completed with a Raman analysis of the surface of the tiles. Raman spectroscopy is a complex technique that analyses vibration of the bonds between the atoms that make up a molecule due to excitation with a beam of light at a specific wavelength. In this case, the Raman readings were taken with a Renishaw inVia, irradiating the sample with an Ar laser at a wavelength of 514 nm and at 22.4 mW power, over an area of $1 \mu m^2$. The samples were focused with a Leica optical microscope using a 50x magnifying lens.

Spectra acquisition time was 10 seconds, with a total of 50 scans per test, covering a spectral range of 100 cm^{-1} to 1500 cm^{-1}. Our study of the Raman spectra revealed an increase in band intensity between 100 cm^{-1} and 200 cm^{-1}, which, according to the literature, is due to the presence of micro or nanoparticles of metallic silver in the vitreous environment of the glazes under study[9]. As an example, the Raman spectra shown below compare silver-free glazes against glazes with 1% silver in the frit.

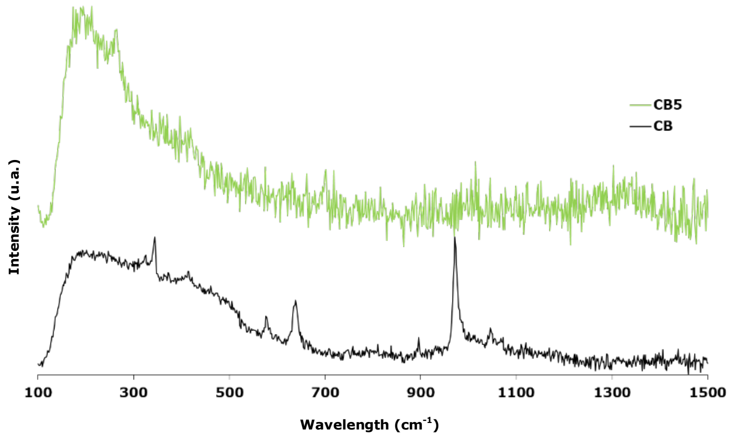

Figure 9. *Raman spectra of conventional gloss glazes, one Ag-free (CB) and one with 1% Ag in the frit (CB5)*

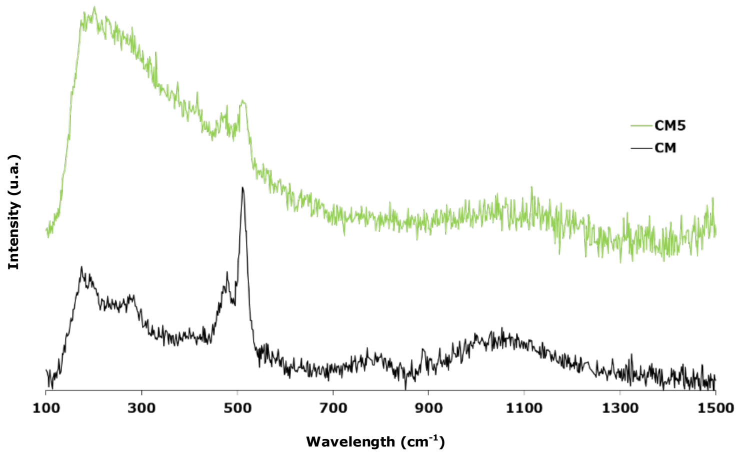

Figure 10. *Raman spectra of conventional matt glazes, one Ag-free (CM) and one with 1% Ag in the frit (CM5)*

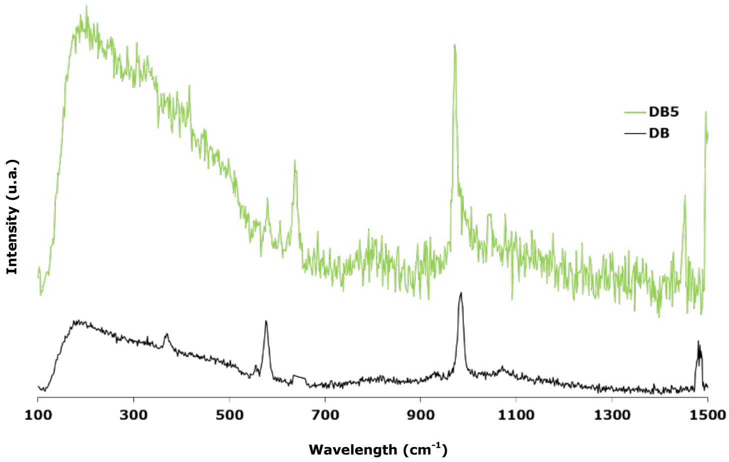

Figure 11. *Raman spectra of digital gloss glazes, one Ag-free (DB) and one with 1% Ag in the frit (DB5)*

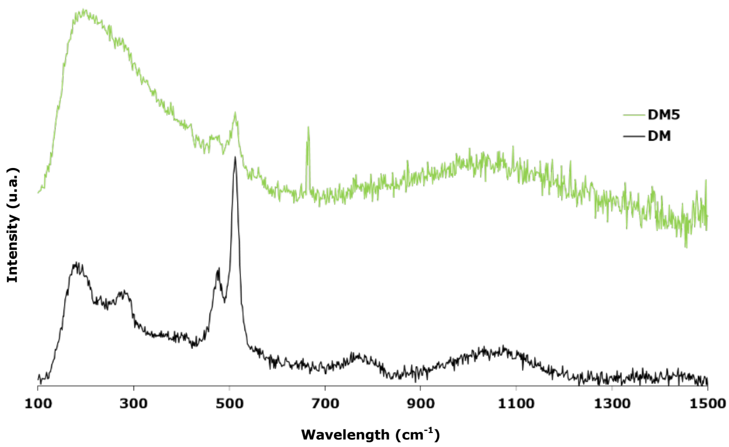

Figure 12. *Raman spectra of digital matt glazes, one Ag-free (DM) and one with 1% Ag in the frit (DM5)*

2.2 DETERMINATION OF THE INFLUENCE OF SILVER CONCENTRATION ON THE BACTERICIDAL PROPERTIES OF CERAMIC GLAZES

Finally, the bactericidal activity in the resulting glazes was evaluated. To do so, the American ASTM E3031-20 standard was used, which describes a method to determine the antibacterial activity of antimicrobials on a ceramic surface. What makes this standard more singular compared to others, such as ISO 22196 or E2180, is that it takes into account the ceramic glazes' hygroscopic capacity.

To do that, the glaze surface is subjected to hydration for 24 hours prior to the test starting. That way, the number of false positives is reduced, making for more accurate readings of the antimicrobial surface's ability to inhibit growth. In addition, the standard states that antibacterial activity should be tested with the *Escherichia coli* bacteria. The process consisted of preparing five 50 x 50 mm samples of the tile to be evaluated, as well as another five 50 x 50 mm samples of borosilicate glass, which acts as the reference surface. Once all the pieces have been sterilised in an autoclave at 120ºC for 1 hour, they are deposited on a petri dish containing a cloth saturated with water and allowed to incubate for 24 hours at 35ºC. Subsequently, a pre-defined amount of bacteria is inoculated onto each specimen and covered with a polyethylene film to ensure the inoculum is in contact with the glaze surface. The pieces are then left in the incubator at 35ºC for 24 hours. After that time, as the standard requires, the film and ceramic tile are placed in a container and the population of inoculated bacteria is recovered. Then, the recovered inoculate is incubated at 35ºC for 24 hours and, finally, the bacteria count is taken. From that count, the study determines bactericidal activity as the degree (%) of bacteria elimination on the surface with the antibacterial component compared to the reference surface.

The graph below shows how the percentage of bacterial reduction varies depending on the concentration of silver in the frit in each type of glaze under study.

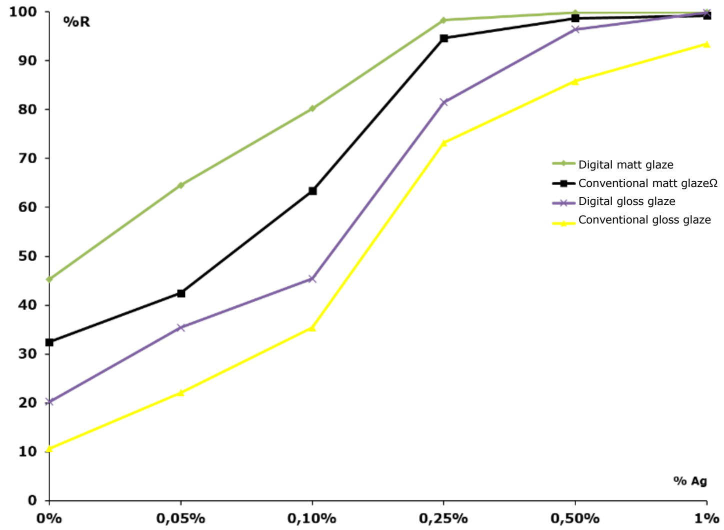

Figure 13. *Varying degree of bacterial reduction in the glazes depending on the concentration of Ag (% by mass) in the frit.*

From the graph, the following results can be highlighted:

- The conventional matt glaze has a bacterial reduction capability equal to or greater than 99% with a 1% concentration of silver in the frit, while the conventional gloss glaze does not reach that level of reduction.

- In both digital glazes, 99% of bacterial reduction is achieved. In the case of the matt digital glaze, it is achieved at a lower silver concentration in the frit (0.5%) than its glossy frit counterpart, which needs 1% silver.
- Digital ceramic glazes have a bacterial reduction capability equal to or greater than 99% at lower concentrations of silver in the frit than conventional glazes.

3. CONCLUSIONS

From the results presented, the following conclusions can be drawn:

- In all the frits studied, Ea decreases as the concentration of silver increases.
- At the same concentration of silver in the frit, Ea is greater in the particle size commonly used for conventional glazes than the size used for digital glazes.
- With the same silver concentration and particle size, the matt frit has a lower Ea than the gloss frit.
- SEM observation of the surface of the ceramic tiles shows the presence of silver particles, which indicates it is in a state of zero oxidation in the vitreous environment in the glaze.
- XPS spectroscopy confirms the presence of silver in the glazes studied. However, it is not possible to determine the state of oxidation in the vitreous environment of the glazes, due to overlapping of signals.
- Raman spectroscopy enabled the presence of metallic silver to be confirmed in the vitreous structure of both gloss and matt fired glazes.
- Conventional matt glaze has a bacterial reduction capability equal to or greater than 99% with a 1% concentration of silver in the frit, while conventional gloss glaze is unable to attain such a level of reduction.
- In both digital glazes, 99% bacterial reduction is achieved. In the case of matt digital glaze, it is achieved with a lower concentration of silver in the frit (0.5%) than for its glossy counterpart, which calls for 1% silver.
- Digital ceramic glazes have a bacterial reduction capability equal to or greater than 99% at lower concentrations of silver in the frit than conventional glazes.

4. REFERENCES

[1] Liu, J.Y. et al. Environ. Sci. Technol., 2010, 44 (6), 2169-2175.

[2] K. Mijnendonck et al. Antimicrobial silver: uses, toxicity and potential resistance. Biometals. 2013; 26, 609-621.

[3] J. L. Hobman et al. Bacterial antimicrobial metal ion resistance. J. of Medical Microbiology. 2014, 64, 471- 497.

[4] W.K. Jung et al. Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli. Applied and Environmental Microbiology, 2008, 74, 2171-2178.

[5] H. J. Park et al. Silver-ion-mediated reactive oxygen species generation affecting bacterial activity. Water Research, 2009, 43, 1027-1032.

[6] Lithium rich phosphate glass: Crystallization kinetics, structural and conduction characteristics. Prashant D. et al. Journal of Non-Crystalline Solids, 358 (2012) 252-260.

[7] A comparative study of crystallization kinetics between conventionally melt quenched and mechanochemically synthesized $AgI-Ag_2O-CrO_3$ superionic system. Anshuman D. et al. Materials Science & Engineering, B103 (2003) 162-169.

[8] Espectroscopía fotoeléctrica de rayos X. J. M. Campos. Instituto de Catálisis y Petroleoquímica (CSIC).

[9] Raman spectroscopic investigations on UV irradiated phosphate glasses with high content of silver or sodium. A. I. Sidorov et al. Optical Materials, 102 (2020) 109816.