ANTIBACTERIAL SiO_2–Ag SOL-GEL LAYERS ON FLAT GLASSES

HELENA HRADECKÁ, JOSEF MATOUŠEK

Institute of Chemical Technology Prague, Department of Glass and Ceramics
Technická 5, 166 28 Prague, Czech Republic

E-mail: Helena.Hradecka@vscht.cz

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Sol-gel method was used to prepare SiO_2 layers with addition of Ag on Float and Fourcault glass substrates. By modifying procedures described in literature and by optimizing conditions of layers preparation (time of sol ageing, temperature and time of layers temperature treatment) homogenous layers were obtained, either colorless or with various intensity of yellow coloring. Quality and homogeneity of the layers were investigated by means of optical microscopy while the color intensity was evaluated by spectrophotometry. Antibacterial properties of the layers were tested with the use of Escherichia coli bacteria. The results have shown antibacterial effect of the layers prepared on Float glass substrate. The layers prepared on the Fourcault glass substrate did not show any antibacterial capability. Chemical changes caused by interaction of the layers with the glass substrate during thermal treatment are discussed. Also chemical durability of the layers in distilled water was followed. It was found that the layers are attacked by distilled water (60 °C) after relatively short time of exposition.

INTRODUCTION

Silver is known as efficient means used for inhibition of aerobic metabolism of one-cellular organisms (bacteria, viruses, and mycosis) and for the ability to attack macrobiotic DNA and RNA which hinder replication of those organisms [1].Various antibacterial inorganic materials containing silver have been developed. In the last decade, sol-gel method has been frequently used for preparation of those materials. Kawashita at al [2] described antibacterial effect of silica glass pulver containing silver. The glass was synthesized by sol-gel method and was composed of SiO_2 and Al_2O_3 with addition of silver. It was found that glass powders with relation between the content Si/Ag 1/0.23 and Al/Ag $\geq$ 1 can be used as an antibacterial material for medical applications. This glass was colorless and chemically durable. Chemical durability enables to release silver ions into water at a controlled rate for a long period of time which guaranties long-time antibacterial efficiency. Catauro at al [3] studied antibacterial effect of glass pulver with molar composition $Na_2O{\cdot}CaO{\cdot}2SiO_2$ doped by silver. The glass was prepared by sol-gel synthesis by heat treatment of dried gel at 600°C. High antimicrobial effect of the glass pulver was found and also the formation of hydroxyapatite layer on the surface of the samples soaked in a simulated body fluid was detected. Hyung-Jun Jeon at al [4] investigated the formation of silver-doped glassy sol-gel silica thin films prepared by treatment at various temperatures. It was found that silver ions were completely trapped in the silica glass matrix and their reduction can be achieved at 600°C. The coated films had an excellent antibacterial performance. Kokkoris at al [5] found antibacterial activity of nanostructured SiO_2 layers containing silver. The composition of the layers was adjusted to the formula xAg – $(1-x)SiO_2$ where x = 0.05, 0.10, 0.13. Silver containing silica coatings were deposited on glass substrates and heated at 500°C. The antibacterial capability of as prepared coatings has been shown to be more profound than the one of coatings heated under oxidation and reductive atmospheres. The difference in the antibacterial activity was ascribed to the differences in the distribution of silver in the coatings as well as to formation of silver nanoparticles with a larger diameter. Formation and tailoring of silver nanoparticles in SiO_2 matrix was studied by Mitrikas at al [6, 7]. Bactericidal effect of Ag nanoparticles immobilized on surface of SiO_2 thin film was studied by Akhan and Ghaderi [8]. The synthesized Ag-SiO_2 thin films presented strong long life antibacterial activity with relative high rate of reduction of viable bacteria.

In this work Float and Fourcault glass were used as substrates to be coated by sol-gel SiO_2 layer enriched by silver. The glasses differ in chemical composition mainly due to Sn content on the “tin” side of the Float glass surface. Silver interaction with those two different types of glasses, namely its reaction with the tin ions during thermal treatment might be the reason for possible different level of bactericidity of both glasses. It is the aim of this work to compare antibacterial activity of SiO_2–Ag layers on the glass substrates and to determine conditions for preparation of layers with relatively stable high antibacterial activity. For this reason, the experimental work is also focused on determination of chemical durability of the layers.

EXPERIMENTAL

Preparation of the layers

The procedures described in the literature [9-11] for preparation of SiO_2 layers containing silver were found to be not suitable for obtaining homogeneous layers with a good adhesion to the surface of glass substrates used in this work. For this reason, a modified procedure for sol preparation was elaborated starting from the literature data by looking for optimal composition of starting sol. Tetraethoxysilan (TEOS), ethanol, distilled water and nitric acid were the main compounds used for preparation of the sol. The compounds were mixed at room temperature for 90 minutes and after adding silver nitrate for the next 30 minutes. Sols containing silver nitrate and TEOS in various molar ratios ($AgNO_3$: TEOS = 0.12, 0.14, 0.17, 0.24) were prepared in order to follow the influence of sols composition on the homogeneity of the layers. Also the influence of the sol ageing on the quality of the layers was followed by using sol immediately after preparation or after ageing. Two ageing conditions were examined: one week of ageing at room temperature and 4 h ageing at the temperature of 70°C.

Composition of microscopic slides used for coating was determined by RTG fluorescent analysis (RTG spectrometer ARL 9400XP). The content of main components of both glass substrates is summarized in Tab 1. The tin content on the side 1 of Float glass can be explained by diffusion of tin ions into glass surface which was in contact with the tin bath.

Table 1. Chemical composition of Float and Fourcault glass substrates (only main component included).

Components	Float side 1 (mass %)	Float side 2 (mass %)	Fourcault side 1, 2 (mass %)
SiO_2	71.6	72.0	71.3
CaO	8.9	8.7	7.2
Na_2O	13.3	13.4	15.0
MgO	4.2	4.3	3.9
Al_2O_3	0.7	0.7	1.9
K_2O	0.3	0.3	0.2
SnO_2	0.7	0.0	0.0

Dip-coating technique was used to apply the sol on washed (by detergent, water, distilled water, ethanol) and dried (60°C, 30 minutes) glass substrate. The substrate was dipped into the sol (velocity of 200 mm/min) and drawn from the sol (60 mm/min), dried in the drying kiln (30 min, 60°C) and heated in an oven (350-550°C, time 2 and 6 hours). The layers prepared on the substrate were colorless on the "air" side (side 2) but exhibited yellow color on the "tin" side (side 1) of the Float glass substrate. No color was observed after heat treatment of the Fourcault glass substrates. Optical microscopy was used to evaluate quality and homogeneity of the layers and UV-VIS spectrometer was applied to appreciate intensity of the yellow color.

Spectrophotometry

The UV-1201 Shimadzu spectrophotometer was used to determine absorbance of the coated samples in the range of 300 to 600 nm. The data were evaluated by means of Spectrum program on PC connected with UV-1201.

Determination of layers antibacterial effect

Bactericidity of the layers was followed by immersing of coated substrates into a solution containing bacteria Escherichia coli. Starting concentration of the bacteria ranged from 10^8 to 10^{11} cells/ml. The actual starting concentration in the testing suspension was established by cultivation determination of the suspension applied on nutrient agar. The testing suspension was placed into sterilized polypropylene vessels. After immersion of coated substrates into the suspension and closing of the vessels annealed on 37°C, concentration of the survived bacteria was determined as a function of time (7, 24, 48 hours). Blanc experiment was carried out using uncoated glass substrates. The results were expressed as colony forming units /ml (CFU/ml). Two S/V (surface to volume) relations were proved by applying one (S/V= 0.71 cm^{-1}) or two (S/V= 1.42 cm^{-1}) samples immersed into testing suspension. The results presented in this work represent average value of two or three independent measurements of bactericidal effect using the same sample.

Determination of chemical durability of the layers

Chemical durability of coated and uncoated surface of glass substrate was proved using leaching tests based on determination of sodium and silicium concentration in the solution after treatment of samples in distilled water by the temperature of 60 ^{0}C. Polypropylene vessels filled by 30 ml distilled water were used for corrosion tests. Three glass samples were simultaneously immersed in the distilled water for the 1, 3 and 7 days respectively.

Uncoated original glass sample was also exposed to corrosion using the same procedure. Concentration of Na, Si and Ag in the leached solution was determined by the AAS method. The concentration data concerning Float glass substrates are to be considered as a sum of these elements extracted both from the "tin" and "air" side of all three samples treated simultaneously.

RESULTS AND DISCUSSION

Optimization of preparation conditions

Optimal molar relations of sol components are summarized in Table 2 together with the data published earlier [6-8].

Any change of main components ratio ($AgNO_3$: TEOS) in the range of 0.12-0. 24) does not affect homogeneity and quality of the layers. With rising value of the ratio only intensity of yellow coloration of the "tin" side of the Float glass substrate is increasing. In this work, the highest value of $AgNO_3$ content was used to enhance antibacterial effect of the layers.

The appearance and homogeneity of the layers was strongly influenced by the time of sol ageing. Optimal time of ageing was found to be one week at the room temperature. Ageing time can be reduced if the temperature of ageing is increased. At the temperature of 70°C, 4 hours ageing sols enabled to prepare layers with practically the same homogeneity. The sols were stable for two weeks after their preparation. Inhomogeneous layers were prepared if the sol was used immediately after preparation without any ageing.

Results of spectrophotometric measurements

Time of heat treatment of the coated Float glass (2 or 6 hours) did not pronounce any significant influence on the quality of the layers except for a mild increase of yellow color of the "tin" side of the samples (Figure 1) The enhancement of intensity of color was detected by small increase of maximum values of peaks of the absorption curves. The main factor with respect on layers coloration was found to be temperature of heat treatment. Yellow coloration of the layers was noticed starting from the temperature of 450°C. Intensity of the coloration is clearly rising with temperature of heat treatment. Deep yellow color was found for the layers heated at the temperatures of 500°C and 550°C (Figure 2).

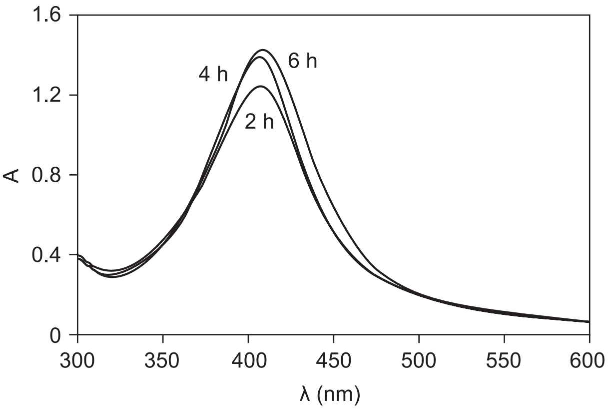

Figure 1. Dependence of absorbance of coated Float glass substrates on time of heat treatment (temperature 550°C).

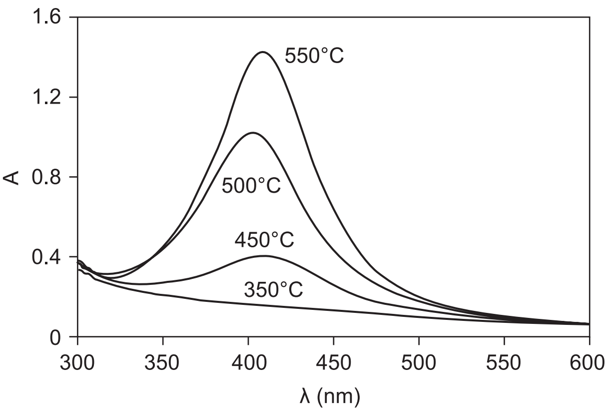

Figure 2. Dependence of absorbance of coated Float glass substrates on temperature of heat treatment (time 6 h).

Table 2. Conditions of sols and layers preparation.

Authors	Sol composition (mol relations)							Burning temperature (°C)	Time of burning (h)
Goutam at al [6]	TEOS	$AgNO_3$	H_2O	HNO_3	propanol	i-butanol			
	1	0.12	12	0.2	6	7		550	6
Zayat at al [7]	TEOS	$AgNO_3$	H_2O	HNO_3	ethanol	EG	TCAA		
	1	0.12	11.9	0.2	7.9	2	0.04	600	3
Novotný, Matoušek [8]	TEOS	$AgNO_3$	H_2O	HNO_3	ethanol				
	1	2.6	29.7	0.5	7.8			550	6
this work	TEOS	$AgNO_3$	H_2O	HNO_3	ethanol				
	1	0.24	12	0.2	13			350-550	2-6

Bactericidity of the layers

Results of bactericidity measurements are presented on Figures 3 and 4 for the Float glass sample substrates coated at the temperature of 500°C both on "tin" and "air" surface. Two different S/V values were applied to determine antibacterial effect. In the case of S/V value 0.71 (Figure 3), time of layers heat treatment has only a small effect on their bactericidity. The difference between coated and uncoated substrates effect is evident and confirms antibacterial capability of layers containing silver. If the S/V relation was 1.42 (two coated samples immersed in the tested suspension), the death rate of bacteria was much higher and independent on the time of layers heat treatment. In this case, total deterioration of all bacteria was achieved after 7 hours (Figure 4).

Antibacterial activity was also measured on SiO_2–Ag layers prepared on the Fourcault glass substrate. The results are summarized in the Figures 5 and 6. The antibacterial effect of these samples was negligible and very close to uncoated glass surface. It can be deduced that reduction of Ag^+ ions into Ag atom and following formation of Ag nanoparticles play important role in antibacterial effect of the sol-gel SiO_2 layers containing silver.

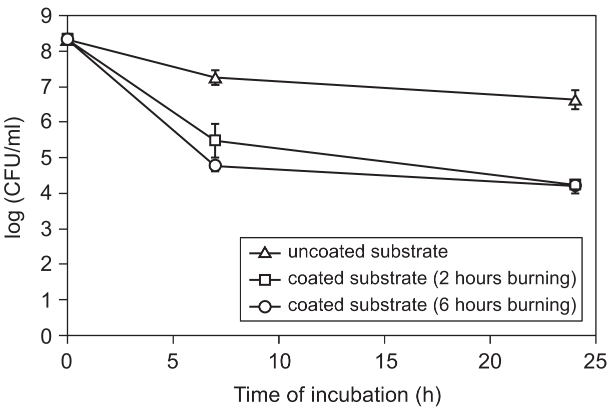

Figure 3. The dependence of log E-coli bacteria concentration on time of incubation (substrate Float, S/V= 0. 71 cm^{-1}).

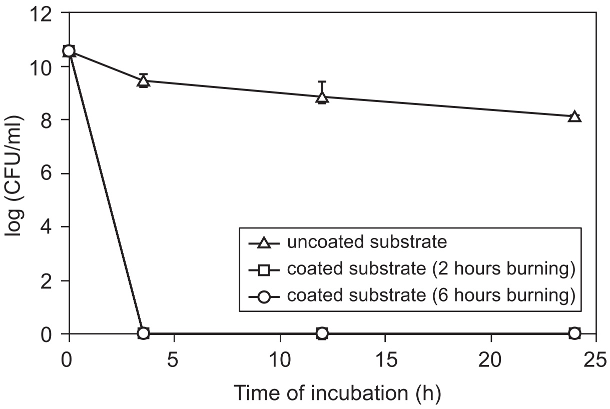

Figure 4. The dependence of log E-coli bacteria concentration on time of incubation (substrate Float, S/V =1. 42 cm^{-1}).

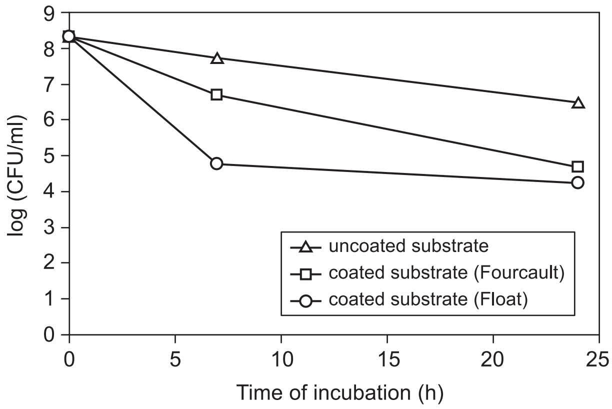

Figure 5. The dependence of log E-coli bacteria concentration for layers on Float and Fourcault glass substrates (S/V = 0.71 cm^{-1}).

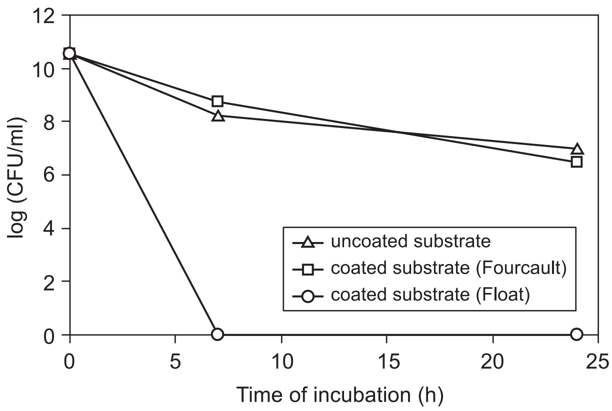

Figure 6. The dependence of log E-coli bacteria concentration for layers on Float and Fourcault glass substrates (S/V = 1.42 cm^{-1}).

Chemical durability of the layers

Corrosion tests were carried out only on the layers prepared on the Float glass substrates which exhibited high level of antibacterial activity. Results of the corrosion tests are summarized in Figures 7-9.

Following information can be derived from these figures:

- concentration of Si and Na in leached solutions is growing up with the time of leaching for all samples tested;
- the highest concentration values of Na ions were found in the coated glass sample prepared by temperature treatment for 2 hours;
- nearly the same concentration values of Si and Na were found for all three samples after leaching for 7 days;
- for the short times of leaching (1 and 3 days) the lowest Si and Na concentration values were detected in case of uncoated glass sample;
- silver is much easer extracted from the layers prepared by the short time (2 hours) of temperature treatment.

This information has to be discussed with regard to the real chemical composition of the surface layers of coated and uncoated glass samples. The chemical composition of the layers is formed during the high temperature treatment which is the last stage of surface layers formation. The most important process determining the chemical composition of the layer appears to be diffusion mainly Na^+ ions from glass substrate into SiO_2 layer and Ag^+ ions from the layer into the glass substrate. As a result of this interdiffusion process, the surface layers are composed of silicium and sodium oxides with a small amount of Ag, while glass substrate is enriched by silver. Concentration distribution of Si, Na and Ag across the layer-glass substrates interface determined by SIMS confirms this assumption [12]. Silver is partially trapped in the glass substrate matrix and it is released into water by relatively low rate which ensure long life antibacterial effect. This assumption was supported by repeated leaching of the layers in the bacterial suspension (Figure 10). Relatively small decrease in antibacterial activity was found in case of repeated leaching of the samples.

The diffusion process is much more rapid in case of the layer prepared by temperature treatment for the shorter time (2 h), because the layer formed by these conditions is not sintered completely and therefore is much more porous than the layer tempered for 6 hours. Also dissolution of SiO_2 is easer in case of porous layer. With rising time of leaching, the concentration of Si and Na ions in the surface of all three samples approached similar level and their amount in the leach did not depend on the temperature of layers heating.

It is well known that a thin surface of the uncoated glass substrate is depleted of sodium ions [13]. The depletion appears to be the reason of the low amount of extracted Si and Na ions in the leach. If the time of leaching was sufficiently long (in our case 7 days), the exchange reaction $Na^+ \leftrightarrow H^+$ and total dissolution of SiO_2 lattice proceeds in deeper parts of the glass where the chemical composition is practically the same for all samples examined. In such a way the rate of corrosion is almost the same for all glass samples (both coated and uncoated) exposed to seven days corrosion in distilled water.

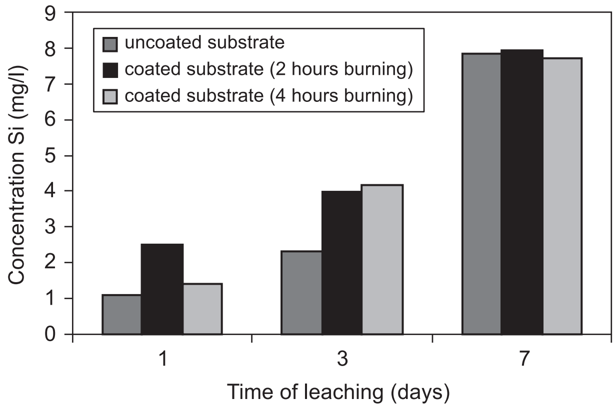

Figure 7. Silicium concentration in the leach after corrosion of uncoated and coated Float glass samples in distilled water at 60°C.

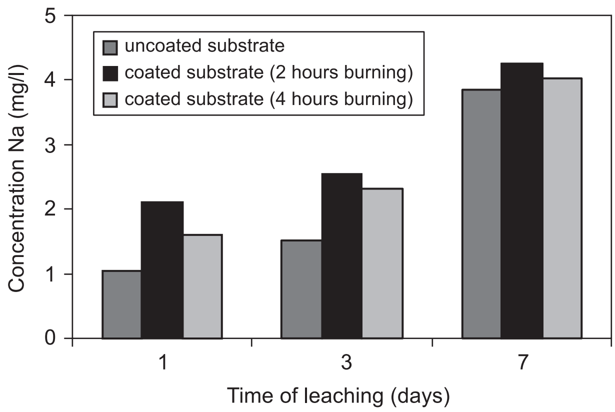

Figure 8. Sodium concentration in the leach after corrosion of uncoated and coated Float glass samples in distilled water at 60°C.

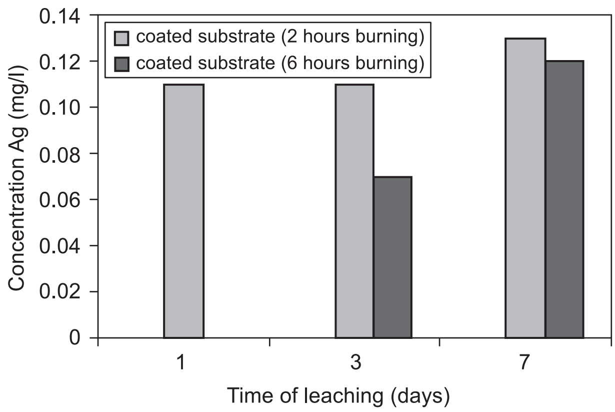

Figure 9. Silver concentration in the leach after corrosion of coated Float glass samples in distilled water at 60°C.

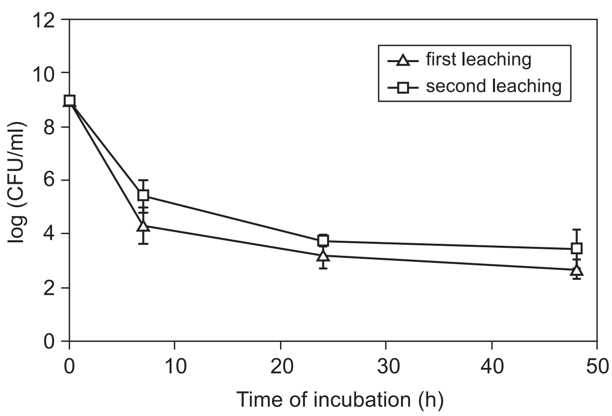

Figure 10. The dependence of log E-coli bacteria concentration for layers on Float glass substrates after first and second leaching in bacterial suspension (S/V = 0. 71 cm^{-1}).

CONCLUSION

Homogeneous SiO_2 layers containing small addition of silver can be prepared if the starting sol is composed of components in the molar ratio as follows: TEOS : ethanol : distilled water : HNO_3 : $AgNO_3$ = 1 : 13 : 12 : 0.2 : 0.24. The optimal conditions of the layers preparation on the surface of the glass sample are following:

- molar relation $AgNO_3$: TEOS = 0.24;
- time of sol ageing by room temperature 7 days;
- speed of substrate drawing from the sol 60 mm/min;
- temperature of final temperature treatment 500-550°C;
- time of final temperature treatment 6 hours.

The layers prepared under these conditions on the Float glass substrate display a good bactericidity (100 % mortality of Escherichia coli bacteria after 7 hours of incubation). It was found that the layers are attacked by distilled water (60°C) after relatively short time of exposition. Si, Na and Ag ions were detected in the leach after the corrosion. The amount of silver in the leach is controlled by diffusion of Ag^+ ions from the layer-substrate system in the water. The layers prepared on the Fourcault glass substrate did not show any antibacterial capability.

Acknowledgement

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