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Synthesis and characterization of bioactive glasses functionalized with Cu nanoparticles and organic molecules

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Abstract

Bioactive sol gel glasses, based on the ternary system $15CaO{\cdot}5P_2O_5{\cdot}80SiO_2$, doped with Cu were synthesized and characterized in order to define the oxidation state of Cu as a function of thermal treatments. In particular, we were able to optimize the condition to obtain: (i) the reduction of Cu^{2+} to Cu^0 followed by nano-aggregation of metal nano-particles (MeNPs) into glass matrix; (ii) a mixed $Cu^{2+}/Cu^{+}/Cu^0$ NPs-containing glasses; (iii) a Cu^{2+}-containing glasses.

Successively, the surface of sol–gel glasses was functionalized by means of organic molecules (amino and mercapto alcohols) to give rise to a system that can interact with the functional groups of the drugs. The functionalization was carried out as a function of the sol–gel glass composition, organic molecules concentration and temperature of the process; the results indicate a preference for the amino groups. The bioactivity (formation of an apatitic layer after simulated body fluid SBF test) was verified for the functionalized sol–gel Cu^{2+}-containing glasses and they are still bioactive.

Keywords: Sol–gel glass; Copper; Nano-particles; Bioactive glasses; Functionalization

1. Introduction

In the field of traditional biomaterials, e.g. bioactive ceramics, the importance and use of bioglasses for various clinical applications has been studied for a long time. Bioglass® 45S5[1] is the first well studied glass and later other bioactive glasses were derived from 45S5 system. The peculiarity of these glasses is their bioactivity which means formation of an apatitic layer (constituted of apatite, HA, and/or hydroxycarbonate apatite, HCA) after chemical reaction(s) with the body fluids.[2] The main applications of these bioactive glasses in the clinical field are (i) the filling of osseous cavities, (ii) the reconstruction of maxillofacial defects, and (iii) the production of dental devices.

Bioactive glasses exhibit ideal characteristics of a drug delivery systems (DDS).[3,4] They can transport an active dosage of a drug molecules to the target site without any premature leaking and negative effect on other body areas.[5–7] The characteristics of a material could be tailored in order to confine a drug into a DDS and control its release. In general, high specific surface area and optimum porosity, depending on the size of the drugs, are the primary prerequisites. In addition, the presence of the drug on the surface of a biomaterial is controlled by the chemical nature of the surface.[8]

Gel bioactive silica-based glasses (e.g. based on the SiO_2–CaO–P_2O_5 composition) can be considered as good candidates for drug delivery activity[2] as they show high specific surface area with micro- and meso-porosity. These SiO_2-based biomaterials are constituted of silanol groups (Si–OH) that can interact with the functional groups of drugs and based on the strength of these interactions, the retention of drug molecules can be modulated.[9,10] Further, Si–OH groups are able to undergo chemical modifications with organic groups through functionalization process. The surface modifications can be performed depending on the functional groups of the drug molecules adsorbed on the surface.

A functionalization of a material can also be performed by means of metal nanoparticles (MeNPs, with Me = e.g. Au, Ag)[11,12] that can act as direct therapeutic agents and/or carrier of biological active molecules. In addition, copper nanoparticles (CuNPs)[13,14(and reference therein)] have also presented optical

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properties similar to those of AuNPs and AgNPs. Moreover, the usefulness of copper as antibacterial agent has been known for long time.[15–17(and reference therein)] Copper is an effective agent with low toxicity, which is especially important in the antibacterial treatment. Several methods[14] (radiation methods, microemulsion techniques, laser ablation, chemical vapor deposition) are proposed for the synthesis but they are sometimes expensive, long time consuming and cause aggregation of CuNPs leading to decrease of their chemical and antibacterial properties. The use of a supporting material can modulate the release of copper and improve the properties. In order to enhance chemical durability inorganic Cu-supported materials are preferred over organic ones.[18]

Recently, we have performed studies on bioactive material based on the ternary glasses $15CaO{\cdot}5P_2O_5{\cdot}80SiO_2$ containing gallium ions and AuNPs respectively and dealt with topics like DDS, metal nanoparticles and drug functionalization.[19,20] Bioactive sol–gel Ga-containing glasses were loaded with curcumin and it was found that gallium in glass matrix increases incorporation of drug and slows down their release in the biological medium. Moreover curcumin in solution stabilizes Ga^{3+} ions and prevents its precipitation. This system was proposed as a new type of DDS due to combined activity of therapeutic metal ions and drug molecules.

In the other case, we have synthesized glasses containing AuNPs[20] which present best characteristics possessed by a bioactive materials, i.e. high surface area, high amount of AuNPs, presence of micropores and large concentration of surface OH groups. Moreover,[21] these glasses were functionalized with small molecules carrying either/both amino ($–NH_2$) or/and thiolic (–SH) groups. The functionalization occurred only when Au was present and preferentially with amino groups at room temperature with a weak Au–N linkage. But on varying the temperature it was possible to obtain a strong Au–S interaction. This indicated that it is possible to obtain different bonds with different strengths and consequently, different release times in solution leading to a wide range of possible applications.

Finally,[22] we have prepared a novel bio-conjugate materials where soybean peroxidase was immobilized (by covalent linkage) on AuNPs containing bioglasses. This material is able to maintain its activity over time, decreased the oxidative stress when in contact with MG-63 cells and also does not inhibit bioactivity.

The literature data available so far indicates that not much work has been done on CuNPs as compared to Au and Ag.[23] Therefore, with our recent experience on metal nanoparticles in a glass matrix, this study is focused on the synthesis and physicochemical characterization of potentially bioactive copper-containing glasses. The glasses based on the ternary glass $15CaO{\cdot}5P_2O_5{\cdot}80SiO_2$ (referred as SG sample) and doped with Cu (referred as SGCu sample) are obtained through sol–gel route. Later, the surface of SGCu glass is functionalized by means of organic molecules (amino and mercapto alcohols). Our aims include defining the oxidation state of copper as a function of the thermal treatment and to obtain a system that can interact with the functional groups of the drugs. Finally, bioactivity test in a simulated body fluid (SBF) is performed on the functionalized SGCu containing glasses in order to verify their capability to form hydroxyapatite (HA) layer.

2. Materials and methods

2.1. Glasses synthesis

Two glass systems, with molar composition $15CaO{\cdot}5P_2O_5{\cdot}80SiO_2{\cdot}xCuO$ (with $x=0$ and 1; the copper amount is conventionally indicated in the oxidic form CuO), were synthesized using a sol–gel route and are referred as SG and SGCu, respectively.

The SGCu sample was prepared using tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), and copper(II) nitrate trihydrate ($Cu(NO_3)_2{\cdot}3H_2O$) as sources of SiO_2, P_2O_5, CaO, and CuO, respectively.

Specific amounts of $Ca(NO_3)_2{\cdot}4H_2O$ and $Cu(NO_3)_2{\cdot}3H_2O$ have been added to a 1:10 mixture of HCl (0.1 M) and H_2O (Milli-Q). This aqueous solution was added to 40 mL of ethanol in which TEOS and TEP had been sequentially added, in order to obtain the desired stoichiometric composition.

The same procedure, but without the addition of $Cu(NO_3)_2{\cdot}3H_2O$, has been used to prepare the corresponding SG sample. In all cases the $HCl + H_2O$/TEOS + TEP molar ratio was 8.

The solutions were then stirred for 1 h and cast in teflon containers. These containers were hermetically closed and kept for 1 day at room temperature for gelation. The obtained gels were then aged for 1 day at 60 °C and later calcined for 3 h in an open Pt crucible at increasing temperatures (600, 1050 °C). Finally obtained samples are referred to as SG_600 or SGCu_600, and SG_1050 or SGCu_1050.

The temperatures employed for different thermal treatments have been chosen so as to obtain (i) aged gels (60 °C), (ii) gel glasses (elimination of solvents and reagents residues, 600 °C), and (iii) glass ceramics (1050 °C).

Another experiment (impregnation) was carried on the SG glasses in which 2 g of SG_600 were immersed for 1 h in 14 mL of 0.28 M $Cu(NO_3)_2{\cdot}3H_2O$. The obtained blue gel was aged at 60 °C followed by heat treatment at 700 °C in N_2/H_2 atmosphere for 2 h. This sample is referred as SGCu_700 (N_2/H_2). This procedure was optimized in order to obtain CuNPs into the glass matrix by varying the concentration of copper nitrate and soaking time of powders into the solution. While the thermal treatment at 700 °C was reported to be able to promote the Cu^{2+} reduction to Cu^0 and nano-aggregation of Cu metal NPs on silica surface.[14]

After thermal treatments at 600 °C and 1050 °C, Cu-containing powders were blue and grey coloured, respectively. While after 700 °C in N_2/H_2 atmosphere they were brown-red coloured.

The aged/calcined powders were ground in an agate mortar and sieved in order to isolate the fraction of particles with $Ø < 50\ \mu m$.

Table 1
Composition (mol%) of aged gels dried at 60 °C and calcined at 600 °C (±SD).

	SiO_2	CaO	P_2O_5	CuO
SG_600	80.2 ± 1.0	14.5 ± 0.5	5.3 ± 0.3	
SGCu_600	79.5 ± 1.0	14.6 ± 0.5	4.8 ± 0.3	1.1 ± 0.1

The experimental compositions, reported in Table 1, were determined after dissolution of the samples by means of Inductively Coupled Plasma (ICP).

2.2. Preparation of functionalized Cu-containing glasses

The functionalization process was carried out by dipping 200 mg of SG_600 into 40 mL of a 10 mM, 1 mM aqueous solution and also 0.5 M ethanol solution of the selected molecules: 2-amino-1-ethanol (referred as 2-NH_2), 6-amino-1-hexanol (referred as 6-NH_2), 2-mercapto 1-ethanol (referred as 2-SH), 6-mercapto 1-hexanol (referred as 6-SH), all provided by Sigma–Aldrich, 99% of purity). The reaction was stirred for 4 h and carried out at different temperatures: 4 °C, room temperature (RT = 20 °C) and 37 °C. The same procedure was utilized for SGCu_600 sample.

Successively, the particles were filtered, washed with a large amount of bidistilled water, and dried under gentle nitrogen gas flux.

The stability of the interaction deriving from the functionalization was investigated by dipping 250 mg of functionalized samples in 15 mL of bidistilled water and monitoring the release of the ligand after 1 and 7 days.

2.3. In vitro bioactivity studies

In order to evaluate bioactivity, we used the simulated body fluid (SBF) proposed by Kokubo et al.[24] SBF is an acellular aqueous solution with an inorganic ion composition almost equal to human plasma. The bioactivity response of the materials was evaluated on the surface of powder samples (250 mg) after being soaked in 50 mL of SBF for 7 days.

2.4. Characterization

The spectroscopic properties of the SG and SGCu samples, after different thermal treatments and functionalization steps, were obtained by the analysis of UV–vis spectra with a HP diode-array spectrophotometer (model 8452-A) in the 190–950 nm spectral range, by using the diffuse reflectance technique and a $BaSO_4$ plate as reflectance standard.

Surface morphology and its composition, before and after thermal treatments, was examined by means of environmental scanning electron microscopy (ESEM, FEI Quanta 200, Fei Co.), equipped with an energy dispersive spectroscopy (EDS) instrument (INCA 350, Oxford Instruments, UK). Data deriving from two different samples with the same nominal composition were obtained analysing four different areas of each sample.

XRD pattern were collected before and after the thermal treatments and SBF soaking in the 2θ range ($5° < 2\theta < 55°$) with a time step of 50 s and step size of 0.03°, by means of an X-ray diffraction apparatus (Panalytical X'PertPro), equipped with Ni-filtered Cu Kα radiation (λ = 1.54060 Å).

The amount of organic molecules (2-SH, 6-SH, 2-NH_2 and 6-NH_2) presents on the glass surface after the functionalization step was estimated by the evaluation of the % content of N, C, H and S by Elemental Analysis (CE Instrument, mod. EA1 110). The estimation of the quantity of organic molecules (mmol) present on 100 mg of sol–gel glass samples after the functionalization was performed using the %S for the thiol-alcohols and %N for the amino-alcohols species.

3. Results and discussion

The results are subdivided in two parts: the first one (Part A) is related to the synthesis and characterization of SGCu samples. This deals with the best synthesis procedure to define the oxidation state of copper and to verify the presence, shape and distribution of CuNPs.

The second one (Part B) is related to the functionalization of SGCu_600 sample compared with SG_600 one, as a function of type and concentration of organic molecules and temperature of the functionalization process.

3.1. Part A

3.1.1. UV–vis spectroscopy

In the Fig. 1 are reported the UV–vis spectra (range 200–850 nm) of SG_600, SGCu_600, SGCu_1050 and SGCu_700 (N_2/H_2). The sample SG_600 shows two bands in the 200–350 cm^{-1} range centered at around 220 and 310 nm, due to the ligand–metal charge transfer (LMCT) of O → Si. In the SGCu_600, SGCu_1050 samples, in the 200–350 nm range, is present broad band centred at around 270 nm probably due to the LMCT O → Cu^{2+}.[25] In addition, the SGCu_600 sample shows a band centered at

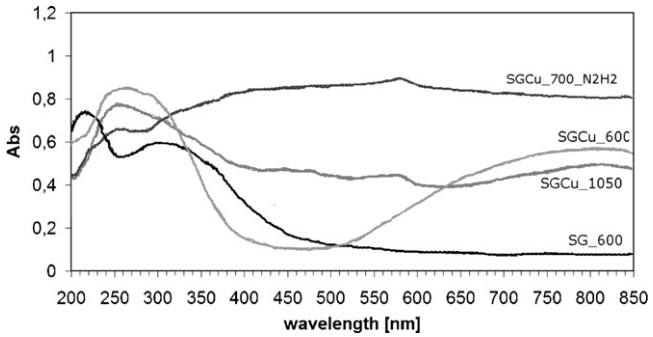

Fig. 1. UV–vis spectra of SG and SGCu samples after different thermal treatments.

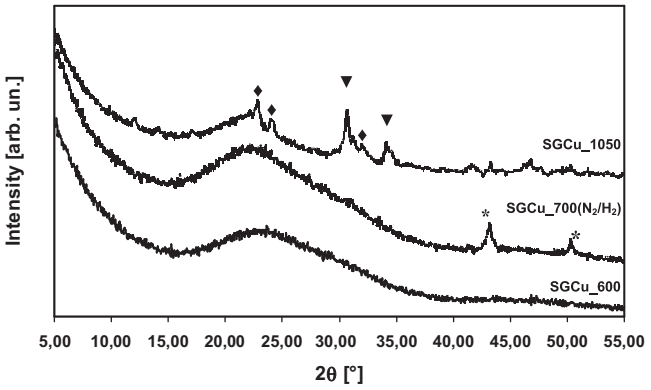

Fig. 2. XRD patterns of SGCu samples after different thermal treatments: symbols *, ▼, and ♦ indicate respectively Cu, $Ca_3(PO_4)_2$ and α-$Ca_3(PO_4)_2$ peaks position.

800 nm characteristic of *d–d* transitions of Cu^{2+} in octahedral coordination.[25]

The SGCu_700 (N_2/H_2) shows the characteristic plasmonic band of metallic Cu nanoparticles (CuNPs) at about 580 nm[14,26] accompanied with a drastic reduction of the bands in the 200–350 nm range, suggesting a reduction of Cu^{2+} to Cu^0. In the SGCu_1050 there are again the above mentioned bands and in addition a very weak band at about 480 nm due to charge-transfer metal to ligand (MLCT) $Cu^+ \rightarrow O$ (the oxygen anions are referred to that present in the glass matrix),[25] indicating that this thermal treatment does not reduce completely the Cu^{2+}ion to Cu^0.

3.1.2. X-ray diffraction

XRD pattern (see Fig. 2) of SGCu_600 exhibits a broad envelope centred at $2\theta \approx 22\,^\circ$characteristic of the amorphous phase. Similar pattern is observed in SGCu_700 (N_2/H_2) along with typical peaks corresponding to Cu^0 are detected (JCPDS 04-0836: (1 1 1) $I/I_0 = 100\%$, $d = 2.09$ Å, $2\theta = 43.21^\circ$; (2 0 0) $I/I_0 = 28\%$, $d = 1.81$ Å, $2\theta = 50.35^\circ$)[27] confirming previous results.

The XRD pattern of SGCu_1050 (Fig. 2) is characteristic of a more crystalline sample in which the peaks are belonging to those of $Ca_3(PO_4)_2$ (JCPDS 86-1585: (2 2 1), $I/I_0 = 100\%$, $d = 2.91$ Å, $2\theta = 30.69^\circ$; (1 1 0) $I/I_0 = 40\%$, $d = 2.62$ Å, $2\theta = 34.17^\circ$) and of α-$Ca_3(PO_4)_2$ (JCPDS 29-0359: (0 3 4), $I/I_0 = 100\%$, $d = 2.91$ Å, $2\theta = 30.69^\circ$; (1 3 2) $I/I_0 = 51\%$, $d = 3.88$ Å, $2\theta = 22.86^\circ$; (3 3 5) $I/I_0 = 41\%$, $d = 2.86$ Å, $2\theta = 31.27^\circ$; (2 6 1) $I/I_0 = 28\%$, $d = 3.70$ Å, $2\theta = 24.02^\circ$).[27]

These are the most important crystalline phases but it is also possible to identify Cu^0 (JCPDS 04-0836: (1 1 1) $I/I_0 = 19\%$, $d = 2.08$ Å, $2\theta = 43.42^\circ$; (2 0 0) $I/I_0 = 10\%$, $d = 1.81$ Å, $2\theta = 50.27^\circ$).[27] The peaks of Cu^0 are not so intense and well resolved as in the case of SGCu_700 (N_2/H_2) which indicates incomplete reduction from Cu^{2+} to Cu^0.

3.1.3. Scanning electron microscopy

A morphological and compositional characterization reveals, only in the case of SGCu_700 (N_2/H_2) (Fig. 3, sections a–c), the presence of spherical particles (section a: white particle; section b: corresponding EDS) in the range of 150–400 nm of dimension and mainly constituted of Cu. These particles are distributed onto the glass surface (section a: darker area and section c: corresponding EDS) that is constituted of Si, Ca, P and O.

These results were very similar to that reported in our previous study[20] on the formation of AuNPs onto the glass surface. This evidence put in light that the synthetic route in controlled/reduction (N_2/H_2) atmosphere allowed to obtain a complete reduction of Cu^{2+} to Cu^0 with a consequent formation of CuNPs (nano-aggregation) on the glass matrix surface. However, the presence of CuNPs in the bulk material cannot be excluded.

The morphological and compositional analysis of SG_600, SGCu_600 and SGCu_1050 samples (data not reported for shake of brevity) showed a homogenous morphology and composition.

All these results are in agreement with each other and indicate that it is possible to define in a glass matrix the oxidation state of Cu as a function of thermal treatments.

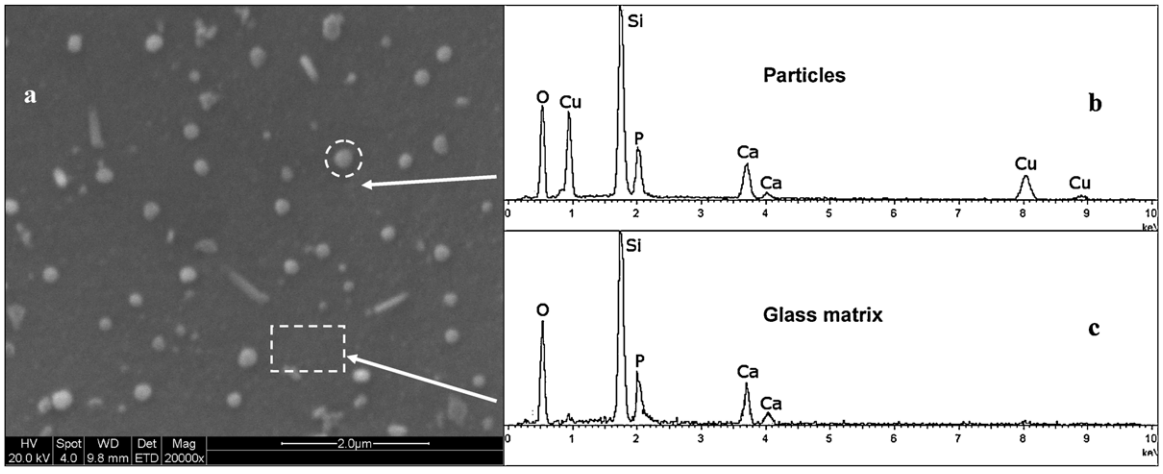

Fig. 3. SEM micrograph (a) and corresponding EDS spectra (b and c) of SGCu_700 (N_2/H_2).

Table 2
Millimole of alcohols loaded on the 100 mg of SGCu_600 °C as a function of concentration and temperatures determined on the basis of elemental analysis.

	4 °C	20 °C	37 °C
SGCu_600 (2-NH_2) 0.01 M	0.013	0.011	0.007
SGCu_600 (2-NH_2) 0.1 M	0.035	0.043	0.061
SGCu_600 (2-NH_2) 0.5 M (EtOH)	0.106	0.098	0.072
SGCu_600 (6-NH_2) 0.01 M	0.019	0.018	0.021
SGCu_600 (6-NH_2) 0.1 M	0.033	0.043	0.035
SGCu_600 (6-NH_2) 0.5 M(EtOH)	0.053	0.050	0.037
SGCu_600 (2-SH) 0.01 M	0.000	0.000	0.000
SGCu_600 (2-SH) 0.1 M	0.005	0.000	0.001
SGCu_600 (2-SH) 0.5 M (EtOH)	0.009	0.027	0.017
SGCu_600 (6-SH) 0.01 M	0.019	0.027	0.027
SGCu_600 (6-SH) 0.1 M	0.051	0.033	0.070
SGCu_600 (6-SH) 0.5 M (EtOH)	0.006	0.009	0.009

3.2. Part B

3.2.1. Elemental analysis

Table 2 summarize the results of elemental analysis carried out on SGCu_600 glass as a function of temperature, type and concentration of molecules.

In the case of samples with the same concentration and alkyl chain, the amino-groups interact more easily with the glass with respect to thiol-groups. At low concentration (0.01 M), for the amino-alcohols, the long chains (6-NH_2) are favoured with respect to the shorter (2-NH_2). On the other hand, just the opposite was observed for the other concentrations (0.1 M and 0.5 M). At low and medium concentration (0.01 M and 0.1 M), for the mercapto-alcohols, the long chains (6-SH) are favoured with respect to the shorter (2-SH) and vice versa for 0.5 M.

In general, with an increasing of the functionalization molecules concentration we found an increment of molecules linked on the sample surface. An inverse behaviour is detected in the case of 6-SH (0.5 M) which is probably due to the dimerization of thiols with formation of S–S bond that inhibits the functionalization.

Finally, considering the effect of temperature, it may be noted that the amino-groups are favoured at 4 °C, while thiol-groups at 20 °C or 37 °C.

Instead, results deriving from Table 3 report the effect of the presence of Cu in the glass: thiol-groups interact only with SGCu and amino groups with SG and SGCu but preferentially with SG. The amino groups give rise to a hydrogen bond with OH groups of the glass, while thiol-group can interact only with Cu^{2+} ions. As reported previously,[28] the affinity of Cu^{2+} for N is higher than for S and this explain the higher amount of amino-ligand linked with SGCu.

Table 3
Millimole of alcohols 0.01 M loaded at 20 °C on 100 mg of SG_600 °C and SGCu_600 °C.

SGCu_600 (6-NH_2)	0.018 mmol
SG_600 (6-NH_2)	0.033 mmol
SGCu_600 (6-SH)	0.027 mmol
SG_600 (6-SH)	0.000 mmol

Table 4
Millimole of alcohols loaded on 100 mg of SGCu_600 °C after 1 and 7 days of water treatment at 37 °C.

	0	1 day	7 days
SGCu_600 (2-NH_2) 0.5 M (EtOH)	0.106	0.051	0.014
SGCu_600 (2-SH) 0.5 M (EtOH)	0.009	0.000	0.000

3.2.2. UV–vis spectroscopy

These results confirm elemental analysis results and here are reported some of the most significant spectra. In the case of amino-alcohols, Fig. 4a indicates a shift from 800 nm to about 760 nm due to the d–d band transitions of Cu^{2+} ions. This is due to the presence of N-ligand with respect to O-ligand. The band on the metal–ligand charge transfer N → Cu^{2+} falls around 300 nm and it is difficult to detect it because of the presence of MLCT of O → Si in the same range.[25] Fig. 4b reported the spectra after functionalization with mercapto-alcohols. The main difference between these spectra and that of SGCu_600 (Fig. 1) is the comparison of the band due to the charge transfer metal–ligand S → Cu^{2+} which falls between 350 and 400 nm[25] indicating functionalization has occurred. The metal–ligand charge transfer band S → Cu^{2+} is more evident for the 6-SH chain and this is in agreement with elemental analysis results.

Finally, Fig. 5a and b report the effect of the temperatures where amino-groups interact better with the glass when the uptake is carried out at 4 °C and thiol-groups when is carried at 20° C or 37 °C.

The stability of functionalization was assessed as a result of leaching tests in water (Table 4) and for the same alkyl chain; amino-groups release is slower than the mercapto-groups. In fact amino groups can interact with both Cu^{2+} and OH^- ions of the glass, while mercapto-groups can only interact with the Cu^{2+} ions.

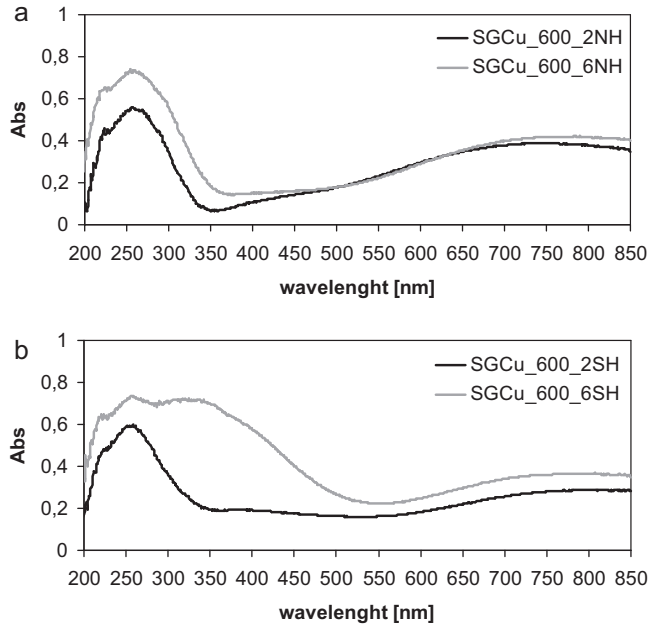

Fig. 4. UV–vis spectra SGCu samples after functionalization with amino-alcohols (a) and mercapto-alcohols (b).

Table 5
Characteristic peaks of HA (JCPDS 9-432) identified after 7 days in SBF.

	2θ	d (Å)	I/I_0	Counts	2θ	d (Å)	I/I_0	Counts
SGCu_600 (2-NH_2)	31.6	2.83	100	153	25.5	3.48	69	106
SGCu_600 (2-SH)	31.6	2.83	100	239	26.1	3.41	56	133

This different release behaviour can be utilized in the biomedical field. If the compounds with pharmacological effects must be released gradually in the body it can be linked to amino-alcohols functionalizing molecules, as it is characterized by a slow release over time and vice versa for mercapto-alcohols.

Bioactivity test in SBF indicate that the functionalized glasses are bioactive. XRD patterns reveal the characteristic peaks of HA (JCPDS 9-432)[27] (Fig. 6). The resolution, in terms of counts number for each peak, is better for the mercapto-alcohol with respect to the amino-alcohol (see Table 5), indicating of a little more crystalline HA.

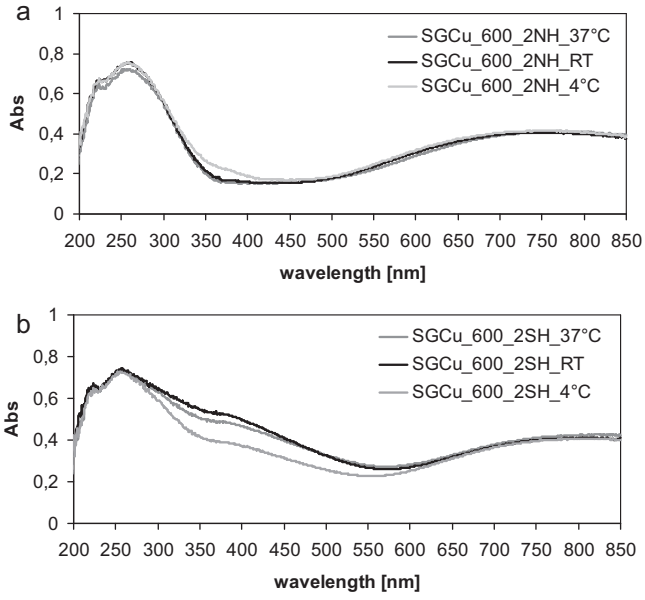

Fig. 5. UV–vis spectra SGCu samples after functionalization with amino-alcohols (a) and mercapto-alcohols (b) at different temperatures.

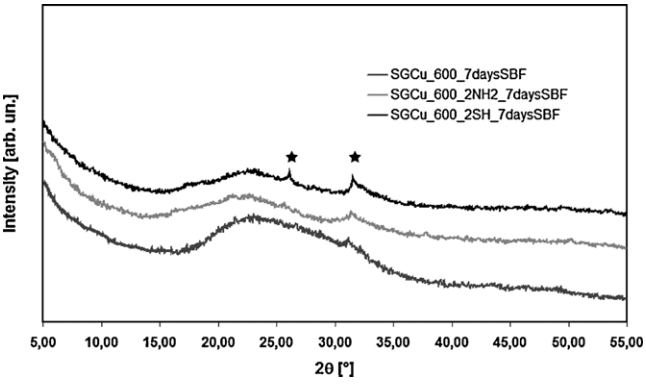

Fig. 6. XRD patterns of SGCu samples (with and without functionalization) after 7 days of soaking in SBF: symbol ⋆ indicates the peaks position of HA.

4. Conclusion

In the present study we successfully achieved our set goals. In fact, on the basis of the different thermal conditions it is possible to have glasses with different oxidation states of copper. In particular we obtained Cu^{2+}-containing glasses at 600 °C, CuNPs of spherical shape into glass matrix at 700 °C (controlled atmosphere) and a mixed $Cu^{2+}/Cu^{+}/Cu^{0}$ NPs-containing glasses at 1050 °C.

The functionalization of SGCu_600 glass indicate a preference for the amino groups due to higher affinity of Cu for N and the hydrogen bond between amino groups and OH^{-} ions of the glass. On the other hand, mercapto-groups can only interact with the Cu^{2+} ions. In addition, these functionalized glasses are bioactive, in term of HA formation.

These results are important in the context of biomaterials and allow us to assert that these systems can be proposed as DDS with a modulation of the drug kinetic release.

Further studies are needed to verify the possibility to functionalize the SG containing CuNPs in order to better understand the behaviour of these glasses.

Acknowledgments

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