# SUPERCRITICAL CARBON DIOXIDE DEPOSITION OF DEXAMETHASONE ONTO BIOMIMETIC SOL-GEL BIOACTIVE GLASSES

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**Abstract.** Bioactive glasses have been widely used for hard tissue engineering applications mostly due to their physicochemical and bioactivity properties, which allow the development of an apatite-like layer when in contact with physiological fluids. Bioactive glasses (based on $SiO_2$, $P_2O_5$, $Na_2O$, CaO) and silica-based composites were prepared by a one-pot aqueous sol-gel method (at 310 K and atmospheric pressure) using two biomimetic catalysts (glutathione and cysteamine) and tetraethyl orthosilicate (TEOS) as the silica precursor. Other precursors, such as triethyl phosphate, calcium nitrate tetrahydrate and sodium nitrate, were also used for the preparation of these materials. In addition, synthetic calcium hydroxyapatite and a polymeric porogenic agent (chitosan) were employed for the preparation of some specific bioactive glasses samples. All prepared materials were freeze-dried and submitted to sinterization process (from 333 to 1273K). Obtained materials were characterized by nitrogen adsorption, helium pycnometry, mercury intrusion porosimetry, scanning electron microscopy, infra-red spectroscopy and X-ray diffraction. Prepared bioactive glasses and silica-based composites were loaded with dexamethasone (an osteogenic drug) by a supercritical carbon dioxide ($scCO_2$) deposition process at different $scCO_2$ density conditions (755-920 kg/m$^3$). Processing time (14 hours) and depressurization rate (0.5 MPa/min) were kept constant for all experiments. Porosity, surface morphology and drug release results proved the viability of the employed biomimetic sol-gel and of $scCO_2$ deposition process for the development of DXMT-loaded bioactive glasses and composite materials with potential uses for biomedical applications.

**Keywords** : Supercritical carbon dioxide deposition, bioactive glasses, biomimetic catalysts; glutathione; cysteamine

## 1. Introduction

Bioactive glasses (BGs) have been used as important biomaterials in implants, fixation devices and other hard tissue engineering applications [1,2]. Among several BG, the most used and best characterized is type Bioglass ®45S5, which is a silicate glass that contains 45% $SiO_2$, 24.5% $NaO_2$, 24.5% CaO and 6% $P_2O_5$ (w/w) [1,3] and that has been already employed in several FDA-approved implants and devices [2,4]. Bioactive glasses present favorable and adequate chemical, physical and mechanical properties as well as high bioactivities which will induce the development of a surface apatite-like layer when in contact with physiological fluids [1,5]. This will promote enhanced in vivo bone bonding and integration without causing inflammation, toxicity or forming fibrous tissues. Once the layer is formed, a firm connection between BGs and the bone structure (host bone) is allowed, owing to the similar mineral composition from the layer and bone and to the leaching of $Na^+$ and $Ca^{2+}$ cations to the surrounding fluids. These cations work as network modifiers leading to material bioactivity [1,2].

Bioactive glasses are usually prepared by melting and sol-gel methods. However, conventional sol-gel techniques usually require the use of harsh reaction conditions such as high pressures and/or temperatures, extremes in pH and the use or generation of caustic chemicals. On the other hand, the production of amorphous biosilica can be accomplished under mild conditions as it happens in nature [6,7].

Recently, new biomimetic catalysts for tetraethylorthosilicate (TEOS) hydrolysis and condensation: L-glutathione-reduced (GSH) and cysteamine (Cys) were investigated. Because of its acidic character in aqueous solutions, GSH can be employed alone or titrated against Cys (basic catalyst already reported in literature) for the formation of silica-based materials over broad and tunable ranges of pH (including neutral pH). These strategies can be used to achieve the most favorable pH conditions that should be employed for the production of silica-based materials and in terms of the desired final biological properties (like biocompatibility, cell toxicity and biodegradability) and mechanical/morphological properties (such as stability, porosity, nano-rugosity and surface area).

To further improve the osteogenic properties of implanted BGs, several bioactive molecules such as specific growth factors and drugs as dexamethasone (DXMT) can be incorporated into these materials. The supercritical carbon dioxide ($scCO_2$) deposition method allows drug deposition of $scCO_2$-soluble bioactive molecule onto previously prepared materials without interfering with processing methods. This method can be performed avoiding high temperatures (which leads to substance degradation) or the use of organic solvents [8-10].

The aim of this work was to prepare bioactive glasses as well as silica-based composite samples with biomimetic catalysts in order to load them with osteoinductive drug by a supercritical carbon dioxide deposition process. All materials were submitted to chemical, physical and biological characterization in order to develop materials to incorporate on a resorbable hydrogel for bone tissue regeneration.

## 2. Materials and methods

Glutathione (GSH - Sigma-Aldrich, >99.0%) and Cisteamine (Cys - Sigma-Aldrich, >98.0%) were used as biomimetic catalysts. The following chemical were used as precursors for the sol-gel 45S5 synthesis: tetraethyl orthosilicate (Aldrich, >99.0%), triethyl phosphate (Aldrich, >99.8%), sodium nitrate (Sigma-Aldrich, >99.0%) and calcium nitrate tetrahydrate (Sigma-Aldrich, >99.0%). Hydroxyapatite (HA, Agoramat), chitosan (medium molecular weight, Sigma-Aldrich), dexamethasone (Sigma-Aldrich, >98.0%) and carbon dioxide (99.998%, Praxair, Spain) were also used.

Bioactive glasses (based on $SiO_2$, $P_2O_5$, $Na_2O$, CaO) and silica-based composite (CP) materials were prepared using a one-pot aqueous sol-gel method at 310K and at atmospheric pressure. Biomimetic catalysts aqueous solutions were prepared: GSH (0.15M, pH 2.6) and Cys (0.15M, pH 9.1). The catalyst solution at neutral pH (GSH+Cys, pH 7.1) was prepared by the titration of the previously prepared GSH and Cys solutions until neutral pH was attained. TEOS, $H_2O$, GSH, Cys, TEP, CaNT and $NaNO_3$ were employed at the following proportion 1:2.39:0.11:0.03:0.09:0.66:0.45 (w:w). Additional silica-based composite samples were obtained by replacing TEP, CaNT and NaNO3 by synthetic HA powders. For other samples, CHIT was added (2% w/v) to the GSH solution and mixtures were stirred overnight until complete CHIT dissolution. The final pH of this solution was pH 3.1.

Reactions were carried out for 25 days, for all the employed catalysts. Obtained materials were freeze-dried, they were grinded and submitted to the sinterization process at 333K (72h), 473K (40h), 873K (5h) and 1270K (2h) [2], at heating rate of 8ºC/min.

Obtained solid materials were later loaded with dexamethasone by a $scCO_2$ deposition process. Obtained BG or CP powders (~90 mg) were placed into sealed dialysis bags and then introduced in a high pressure

view-cell which was previously loaded with DXMT sample proportion of 1:2.65 (w:w). Drug loading yields were changed by controlling $scCO_2$ densities (755-920 $Kg/m^3$), i.e., by performing experiments at 313K/13.6 MPa, and at 313 K/32.0 MPa. Employed depressurization rate was 0.5 MPa/min and processing time was 14 hours.

DXMT-loaded BG and CP powders (~20 mg) were placed into sealed dialysis bags and immersed in 20 mL of MilliQ water at 310 K on a continuous release system. At pre-determined time intervals, samples were analyzed in a UV-Vis spectrophotometer at 242 nm.

All obtained materials were chemically and physically characterized. Mercury intrusion porosimetry (Autopore IV, Micromeritics, USA) was performed to determine pore size distribution, while real density was determined by helium pycnometry (Accupyc 13330 model, Micromeritics, USA). Surface area, pore diameter and pore volume were determined nitrogen adsorption (ASAP 2000 V2.04, Micromeritics, USA). Processed samples were also characterized by scanning electron microscopy (Phillips XL30, The Netherlands), by FTIR (ATR and transmission modes) spectroscopy (4200 Spectrometer from Jasco, Inc., Japan) and by X-ray diffraction (XRD) (Philips X'Pert diffractometer, The Netherlands).

Some samples were also submitted to a surface treatment for apatite formation obtained by soaking them into simulated body fluid (SBF) using an *in vitro* procedure described in the literature [11]. Samples were immersed in 0.1gr sample per 10ml SBF proportion for 14 days at 310 K], with medium renewal, and prior to hemocompatibility analysis samples were freeze dried.

Hemocompatibility assays were performed according to the International Standard Organization (ISO 10993-4), and hemolysis tests were assayed according American Society for Testing and Materials (ASTM – F756-00) standard using 0.2 g/ml of samples. Rabbit venous blood (Prognóstica, Belas, Portugal) was diluted in a cyanmethemoglobin hemolytic reagent (modified Drabkin solution). A direct contact test was conducted in which samples were placed on modified Drabkin solution and were measured spectrophotometrically at 540 nm (since the cyanmethemoglobin absorbance is directly proportional to hemoglobin concentration in the blood). Assays were carried out in duplicate.

## 3. Results and Discussion

Bioactive glasses prepared by sol-gel method using biomimetic catalysts present a similar chemical composition as those obtained by conventional catalysts [2]. FTIR confirmed the presence of characteristic silica bands such as hydrogen-bonded water stretching bands (3470-3450 $cm^{-1}$), adsorbed water molecules deformation vibrations (1653-1634 $cm^{-1}$) and surface silanol groups and silicon-oxygen covalent bonds vibrations (1200-1000 $cm^{-1}$). Si-O-Si symmetric stretching vibration (800 $cm^{-1}$) and the corresponding bending mode (469-466 $cm^{-1}$) are also present [2]. To confirm the presence of bioactive glasses samples, decomposition of two nitrates during sinterization treatment (1042, 820 and 744 $cm^{-1}$ bands, Figure 1) were detected. A crystalline phase assigned to the P-O deformation modes in crystalline phosphate is also detected (622 and 617 $cm^{-1}$ bands) in sinterized samples obtained from GSH+Cys and from Cys (Figure 1). On the other hand, the symmetrical Si-O-Si stretching vibration on $SiO4^{4-}$ of crystalline silicate is detected after sinterization and for samples prepared by using GSH (729 and 697 $cm^{-1}$ bands).

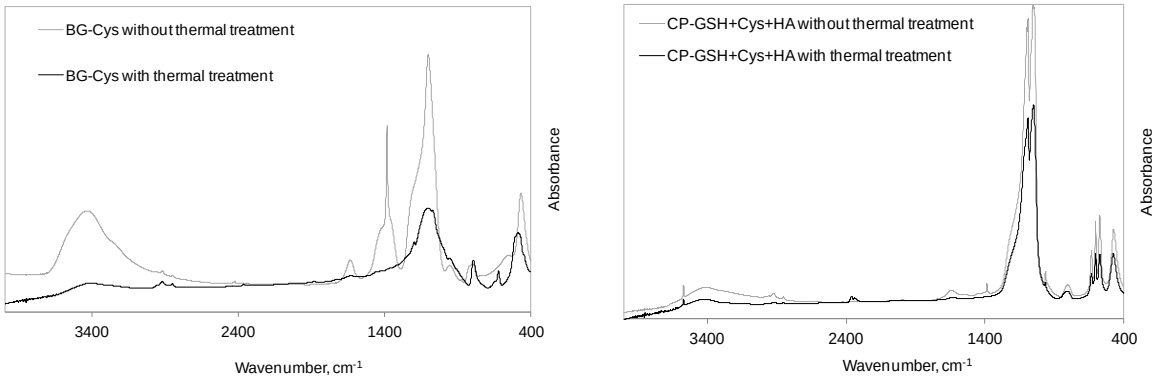


**Figure 1**. FTIR spectroscopy of bioactive glasses (on the left) and silica-based composite samples (on the right) before and after thermal treatment.

Silica-based composites were also analyzed by FTIR (Figure 1) that confirmed the presence of characteristic bands at 1089, 962, 601, 571 $cm^{-1}$ corresponding to $PO_4^{3-}$ and 631 $cm^{-1}$ corresponding to $OH^-$ group, as confirmed by literature [12]**,** as well as the presence of silica bands [2]. XRD spectra (data not shown) of prepared BGs (obtained using biomimetic catalysts) show different crystalline phase, which could differ from samples prepared with different biomimetic catalysts used. For BG prepared with GSH (BG-GSH), $Na_2Ca_2Si_3O_9$ crystalline phase could be identified (Figure 2). On samples prepared from Cys and GSH+Cys mixtures a different crystalline phase ($Na_2Ca_3Si_6O_{16}$) and a specific $SiO_2$ high-temperature polymorph (cristobalite) could be detected. On BG-Cys samples $Na_3PO_4$ phase could also be achieved [2].

Mechanical strength and material biodegradability comes from the crystalline phase formation [1,2] due to sinterization process. By temperature increase, nitrates will also be removed making materials stabilized and biocompatible [5]. The bioactivity associated to sinterization treatment could also be attributed to the presence of network modifiers as $Na^+$ and $Ca^{2+}$ leading to silanol group formation at the surface which leads to reactivity increase and a bioactive behavior. Stability on BG is conferred by the presence of components such as MgO and CaO as well as the formation of a covalent $SiO_2$ network which forms a tetrahedral unit [$SiO_4$], forming thus a structured unit [2, 4].

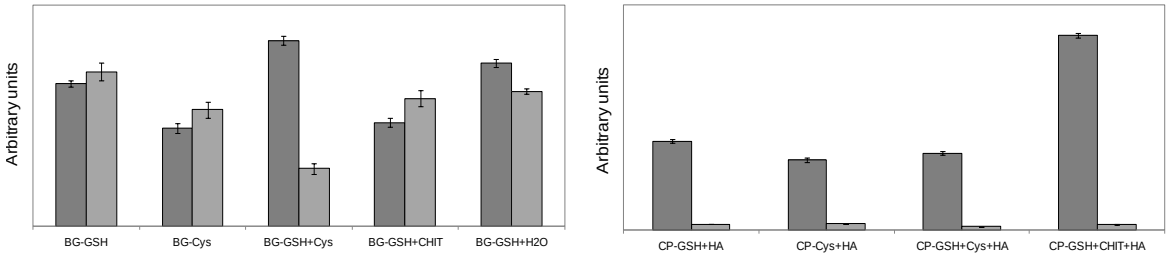


**Figure 2**. Selected morphological characterization results of prepared bioactive glasses and silica-based composite samples with different catalysts: ■ surface area ($m^2/g$) and ■ real density ($g/m^3$).

Higher surface area and lower real density can be achieved for BG prepared from GSH+Cys mixture (Figure 2). This behavior can also be found on CP, which is probably related to the presence of calcium hydroxyapatite particles once there is no HA shrinkage during sinterization treatment. Besides differences referred previously, real density is quite similar in all samples, in bioactive glasses and silica-based composites. Surface values obtained for BG are extremely low when compared to silica systems prepared with same catalysts. This may be due to sinterization treatment, which leads to a significant decrease on surface area [5]. Sinterization temperature increase cannot induce, by its own, pore size reduction, but can enhance silica condensation which leads to a pore diameter decrease with material mechanical properties increase [4]. The presence of porogenic agent on sample (like biopolymers) could lead to porosity increase once it could be burnout during sinterization process. All obtained materials could be classified as mesoporous materials due to their pore diameter values, once are between 1.4 and 57nm. These characteristics are extremely important due to final material application, bone tissue engineering, once it should be in contact with bone tissue cells and should allow and facilitate cell proliferation, migration and own osteoinductive properties to assure physiological functions [1,5].

Bioactive glasses prepared with biomimetic catalysts (Figure 3) assume an irregular shape and shrinkage due to thermal treatment which have a similar morphology to the one obtained for Bioglass® prepared with conventional catalysts [2]. For BG-Cys and BG-GSH+Cys a well-defined structure can be achieved assuming a coral-like structure. When compared to silica-based composite samples, these ones present higher surface rugosities (for all employed catalysts), but with no shrinkage associated. Similar structures were found in literature for silica-based materials using Cys as catalyst [7].

After processing, materials could be impregnated with drugs to improve its characteristics in order to achieve a faster regeneration process. Conventional techniques for drug impregnation assume soaking procedure in which drug solubility depends on solvent nature [8,10]. However, this could lead to slow diffusion inside matrix, or to a structural change due to drying procedure. As alternative, a supercritical carbon dioxide deposition process was used once it is a dry solvent, naturally abundant, and quite often used due to its characteristics once is non-toxic, non-flammable, easy to recycle, has relatively low critical pressure (7.38 MPa) and temperature (304.1 K) and could also be eliminated upon depressurization once is a gas under ambient conditions. These characteristics allow a good diffusion onto the matrix and the main advantage, working at mild conditions, and loading hydrophobic drugs/substances [10,13]. As an initial burst can be

observed in most of the kinetic profiles, it may be indicative that most of the drug is deposited on the surface of the bioactive glass, and could also be associated to a diffusion or dissolution process [14].

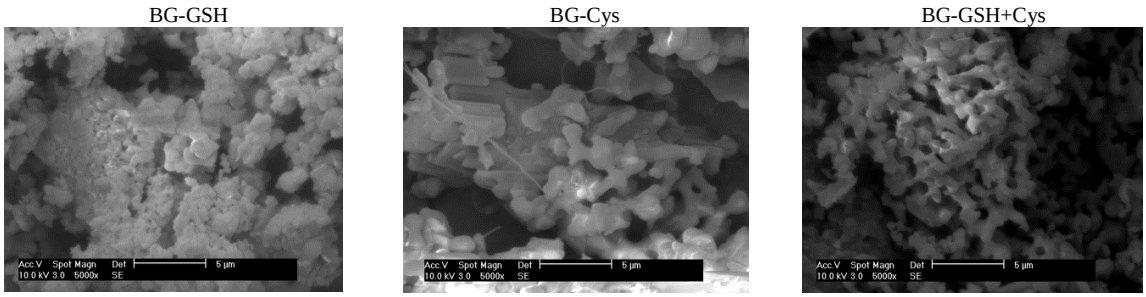


**Figure 3**. SEM microscopy of prepared bioactive glasses using different catalysts.

As an anti-inflammatory agent with osteoinductive properties, dexamethasone (DXMT) was used to lead to faster bone tissue regeneration. Dexamethasone kinetic behavior could be influenced by different morphological characteristics from obtained materials, in which large surface areas expose –OH groups from materials surface leading to weak bonding from surface to drug molecules. Another feature that is also important in drug loading is the material porosity. It is known that drug adsorption occurs inside pores and characteristics as pore diameter and size selective process are crucial to determine the size and the amount of guest molecule to be introduced into the matrix as well as the rate of drug release [14].

It is also expected high solubility for high pressure conditions which leads to substance micronization, leading thus to high release rates due to diffusion through pores of the material, which is evident on BG-GSH release profile (Figure 4), in which can be seen that for higher $CO_2$ density there is a higher amount released.

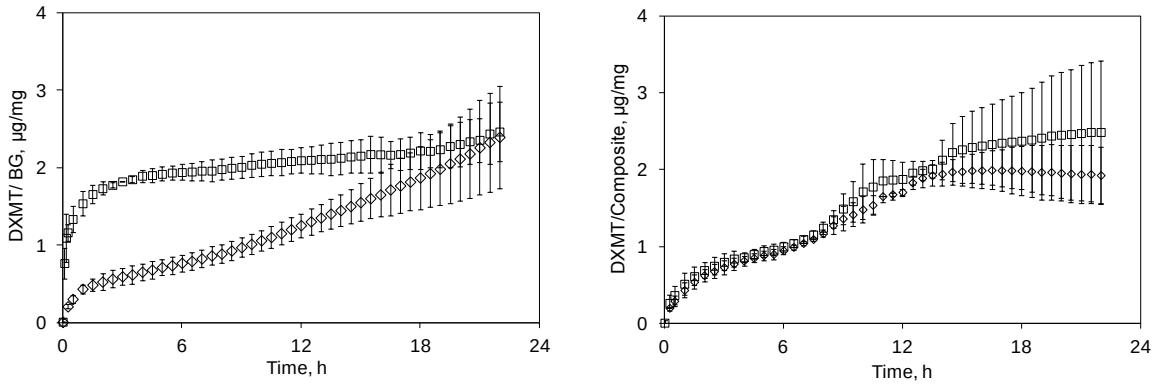


**Figure 4**. Release profile of DXMT deposited on BG-GSH (on the left) and CP-GSH+HA (on the right) at different pressure conditions: ◇13.6 MPa and □ 32.0 MPa.

On silica-based composite samples (Figure 4) there are no significant differences depending on catalysts used, and it verifies a pronounced step-like release profile suggesting that first there is the release of drug deposited on surface followed by the release of DXMT incorporated into the matrix [15,16]. Nonetheless, it is expected that BG and silica-based composites, known for their bioactive effects, combined with a small amount of the osteogenic and anti-inflammatory incorporated drug, may facilitate new bone regrowth, which is the ultimate goal for hard-tissue application.

The hemolytic behavior observed for BG samples (data not shown) is probably due to the presence of silanol groups at surface materials [17] in which hemolytic activity of amorphous silica is proportional to the concentration of surface silanol groups of these materials. All the samples prepared with Cys had a reduced hemolytic index, which can be explained by lower surface area on those samples, and thus a reduced silanol group exposure. However, samples immersed in simulated body fluid (SBF) induced changes at the surface covering material silanol groups with apatite formation. In some cases, like for BG-GSH, the hemolytic index was reduced by approximately 70% after SBF immersion. For composites there is no difference between samples after the SBF immersion, probably due to the HA nature that overlaps the effect of the silanol groups

of silica. Considering characteristics such as surface area, porosity and the known compatibility of BG and CP, the final application could be the conjugation of these materials within a resorbable hydrogel matrix to apply directly into the bone defect, as injectable material, through the minimum invasive surgery [18].

## 4. Conclusions

Silica formation using pH conditions was obtained using biomimetic catalysts (GSH, Cys and GSH+Cys mixture) achieving different pH conditions (from acidic, to basic conditions). Morphological and mechanical properties from bioactive glasses and silica based composites could be affected by the addition of porogenic agents (e.g. chitosan), in which different morphologies lead to different behaviors on drug release profiles. Supercritical $CO_2$-assisted deposition method could be employed on DXMT loading method. These results demonstrated the feasibility and the advantages of using these materials for hard tissue engineering prepared by an aqueous sol-gel method and supercritical carbon dioxide deposition process. This loading method proved also to be crucial for the development of dexamethasone loaded bioactive glasses and silica-based composites with potential application in tissue engineering.

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