A high-pressure/supercritical method to dry silica-based materials prepared by biomimetic aqueous sol-gel methods

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Abstract

Recently, we employed two new small bi-functional bioinspired catalysts for the hydrolysis and condensation of silica precursors in aqueous media and at ambient temperature and pressure: L-glutathione-reduced (GSH) and DL-methionine (Met). These biomimetic catalysts are non-toxic well-known biomolecules which are widely available at reasonable prices. Because of their different pH character in water solutions, GSH (acidic) and Met (near neutral) together with cysteamine (Cys, strongly basic) can be employed alone or titrated against one another for the facile and non-harsh aqueous formation of amorphous/mesoporous silica-based materials over broad and tunable ranges of pH, including neutral and near-neutral pH. These methodologies present evident advantages in terms of the obtained silica structure/stability, on the absence of potentially toxic substances and when the immobilization of thermo- and pH-labile biopolymers and bioactive molecules is intended. However, and after silica formation, water and catalyst residuals should be removed from these silica-based materials. Water may be removed by conventional freeze-drying and evaporation methods but this will not remove catalysts from the prepared materials. In addition, evaporation methods are known to exert a strong capillary pressure on the inorganic/organic pore walls that will promote the collapse of most part of the materials pore volume. Finally, calcination is not a valid option when silica-organic composite biomaterials are foreseen.
In this work, silica was prepared from TEOS, in aqueous solutions of different pH, and using the above referred biomimetic catalysts and strategies. Then, a "green" and combined/sequential high-pressure/SCF extraction/drying method (using water, ethanol and supercritical carbon dioxide) was employed in order to recover, purify and dry the obtained biomaterials, in a single unit operation and at moderate temperatures. Conventional freeze-drying and evaporation methods were also employed for comparison purposes. A supercritical solvent impregnation/deposition (SSI) method was also employed to load a bioactive molecule (dexamethasone) into the previously prepared/processed materials. Obtained silica samples were chemically and physically characterized using several analytical techniques. The effects of pH and of the employed catalysts and extraction/drying methods were evaluated and compared in terms of silica production yields and of some important silica physical and morphological properties. Preliminary results indicated that these green and biomimetic methodologies may have a great potential for the development and preparation of amorphous microporous/mesoporous silica and silica-based composite biomaterials, which can be used for several biomedical and hard tissue engineering applications.

INTRODUCTION

It is well known that silica-based materials can be employed in electronics, energy storage, insulation, coating, chemical reactions and separations as well as in some biomedical applications including prosthesis, implants, and drug carriers [1-5]. The synthesis of these materials through conventional sol-gel techniques usually requires

harsh reaction conditions which can include high pressures and/or temperatures, extremes in pH, and the use or generation of caustic chemicals. On the contrary and in nature, the production of amorphous bio-silica is usually accomplished under mild physiological and/or ambient conditions. For example, several marine organisms, including sponges and diatoms, can produce solid hierarchical silica structures with well defined nano-scale morphologies, at ambient temperature and near neutral pH, by protein mediated condensation of naturally occurring silicic acid [1, 6-8]. Researchers have already studied silica formation in the laboratory and developed biological mimics for silica condensation at ambient temperatures using small bi-functional molecules such as cysteamine, ethanolamine, and tromethamine [9,10].

The development of scaffolds for tissue engineering applications usually encompasses the formation of three-dimensional structures, based on synthetic or natural materials, the seeding of specific cells and the incorporation of bioactive species such as drugs, growth factors and proteins. One of the main purposes of this work is to use green and biomimetic strategies to prepare and to process silica-based materials that can be applied on the development of potential drug-eluting scaffolds with applications in hard tissue regeneration. Green and non-harsh sol-gel methods such as one-pot aqueous procedures at near room temperature/pressure and at near-neutral/neutral pH conditions were employed. Three bio-inspired molecules, cysteamine (Cys), DL-methionine (Met) and L-glutathione-reduced (GSH) were used to catalyze silica formation from tetraethyl orthosilicate (TEOS). While Cys has been previously reported as a catalyst for silica formation [9,10], to the best of our knowledge these are the first results using Met and GSH as bi-functional biomimetic catalysts for silica formation from TEOS. These substances are non-toxic and widely available biomolecules and, due to their different pH character in aqueous solutions, they can be employed alone or titrated against one another for the formation of silica over broad and tunable ranges of pH, including neutral pH. The proposed methodology can be also used to attain a desired final pH taking in consideration the most advantageous value in terms of the stability of the bioactive species to be incorporated. These procedures present clear advantages when compared to the conventional silica production methods (using HCl, p-TSA, ammonia, high temperatures/pressures) in terms of avoiding the use and the residual presence of organic/inorganic potentially toxic substances and when the immobilization of pH- and thermo-labile bioactive species is intended.

However, and after silica aqueous formation, water and biomimetic catalysts residuals should be removed. Water may be removed by conventional freeze-drying and by other evaporation methods but this will not remove catalysts from the prepared materials. In addition, conventional evaporation methods are known to exert a strong capillary pressure on the inorganic/organic pore walls that will promote the collapse of most part of the materials pore volume. In addition, calcination is not a valid option when silica-organic composite biomaterials are foreseen.

As an alternative, an innovative “green” and sequential high-pressure/supercritical fluid extraction/drying method (using water, ethanol and supercritical carbon dioxide) was developed and employed in this work and in order to recover, purify and dry the obtained silica-based materials, in a single unit operation and at moderate temperatures. From a green perspective, supercritical fluids (SCFs), and namely supercritical carbon dioxide (scCO2), already proved to be excellent alternatives to replace VOCs and other harmful solvents in many processes (extraction, impregnation, reaction and organic/inorganic materials processing) [11]. SCFs also present unique properties which may offer innovative possibilities for the development of enhanced composite materials presenting better chemical, physical and thermo-mechanical properties [11]. Since SCFs have a null

surface tension, drying wet composites with SCFs prevents the formation of the liquid-gas interface which usually recedes during the emptying of the pores in wet gels and promotes the collapse of most part of the pore volume [12]. Moreover, SCF extraction can be employed to remove residuals and dry the generated composites, avoiding the use of high evaporation temperatures which may degrade the involved thermo-labile substances [11, 13-15]. Finally, the supercritical solvent impregnation/deposition (SSI) methodology usually permits to have previously prepared materials (or even articles/biomedical devices) and impregnate/deposit them later with a desired bioactive molecule, taking in consideration the envisaged therapeutic application and without interfering with the material/article/device manufacture and/or processing method [11, 16-20]. In conclusion, the use of SCFs and namely of $scCO_2$, to extract residuals, to process and/or dry obtained materials and to load a bioactive molecule in a particular solid matrix may present important advantages for the development of more efficient drug delivery systems and/or innovative biomaterials for biomedical applications.

In this work, silica was prepared from TEOS, in aqueous solutions of different pH, and using the above referred biomimetic catalysts and strategies. After synthesis, a combined/sequential high-pressure/SCF extraction/drying/impregnation method (using water, ethanol and supercritical carbon dioxide) was employed in order to recover, to purify, to dry and to load a drug (dexamethasone) into the obtained biomaterials.

Obtained silica samples were chemically and physically characterized using several analytical techniques. The effects of pH, of the employed biomimetic catalysts and of the used extraction/drying/impregnation methods were evaluated, discussed and compared in terms of silica production and dexamethasone loading yields, as well as in terms of some of the important silica physical and morphological properties.

MATERIALS AND METHODS

Tetraethyl orthosilicate (TEOS, purity > 99%), cysteamine (Cys, purity > 98%), DL-methionine (Met, purity > 99%), and L-glutathione-reduced (GSH, purity > 99%) were obtained from Sigma-Aldrich. Ethanol (purity > 99.5%) was obtained from Fisher Scientific and Panreac Química SA. Carbon dioxide (99.998%) was obtained from Praxair.

Concentrated solutions of Cys (~0.2 M, pH 9.45) and of GSH (~0.2 M, pH 2.28) were prepared in dH_2O and titrated until neutral pH was obtained (pH 6.97). Then, 4 mL of this Cys+GSH neutral pH solution was mixed with 1 mL of TEOS and the reaction was carried out in the shaker/incubator at 37 ºC (250 rpm). The same titration and synthesis procedures were followed to systematically produce silica under a variety of pH conditions that ranged from 2.28 up to 9.45 and for 96 hours. To isolate silica precipitates, samples were centrifuged, collected, washed several times with bi-distilled water (dH_2O) (in order to remove catalyst residues) and dried at ambient conditions until constant weight was obtained. When wet gel monoliths were obtained, they were recovered and dried at room temperature until constant mass was obtained. Dried monoliths were ground into fine powders, washed and dried.

For the high-pressure/supercritical fluid extraction/drying studies, other biomimetic catalysts aqueous solutions (GSH, Cys and Met) were prepared at different pH conditions (from 2.9 up to 9.8). Silica formation reactions were carried out following similar procedures as those above referred, but in this case for 5 days (for the GSH- and Cys-catalyzed systems) and for 12 days (for the Cys+GSH and for the Met-catalyzed systems), at 37 ºC and under stirring. In these studies, obtained samples were not recovered/washed/dried as previously indicated. Instead, they went through the sequential high-pressure/supercritical fluid extraction/drying method (HPSE, using

water, ethanol and $scCO_2$), freeze-drying and oven evaporation methods. For HPSE assays, the operational extraction conditions were: i) high pressure water extraction at 20 MPa and 40 ºC, for 1 h and at a flow rate of 4 ml/min; followed by ii) high pressure ethanol extraction at 20 MPa and 40 ºC, for 1.5 h and at a flow rate of 4 ml/min; and finally iii) $scCO_2$ extraction (2.7 ml/min), for 2 h at 40 ºC followed by a 3 h at 50 ºC period. This assay was designated as HPSE-catalyst (1/1.5/5 h). A second assay, which differs from the first by an increase of 30 min in the ethanol extraction period, was also performed and it was designated as HPSE-catalyst (1/2/5 h). All assayed samples were extracted/dried in triplicate. As referred, conventional freeze-drying (-48 ºC, 3-7 mbar, ~48 hours) and oven evaporation (1 bar, 40 ºC, ~72 hours) methods were also employed for the same silica samples and for comparison purposes.

After the extraction/drying experiments, obtained silica samples went through a $scCO_2$ supercritical impregnation/deposition process (SSI). Dexamethasone was the chosen model drug and experiments were carried out at 40 ºC and at 20.0 MPa, for 14 h and employing a 0.2 MPa min^{-1} depressurization rate. Drug-loaded amounts were spectrophotometrically determined by drug release studies in dH_2O.

Obtained silica samples were chemically and physically characterized using several analytical techniques (FTIR-ATR, helium pycnometry, nitrogen adsorption, thermal analysis (SDT) and scanning electron microscopy (SEM). Preliminary hemocompatibility tests were also performed in order to pre-evaluate the effects of catalyst residuals and materials morphology on blood (following the ISO 10993-4 and ASTM F756-00 standards).

RESULTS

Figure 1 shows the obtained silica formation yields as a function of initial aqueous solution pH. As can be observed, silica yields are higher for extremes in pH, namely when employing GSH or Cys alone. It is well known that reaction pH will strongly affect TEOS hydrolysis and condensation and, as it will be seen later, silica morphology and properties [21-24]. Highly acidic solutions (pH<3.5) will promote rapid TEOS hydrolysis and slow condensation rates. This usually leads to the formation of three-dimensional wet gels that, after drying and shrinking, are composed of dense solid particles having significant amounts of alkoxy and hydroxy groups and presenting smooth microporous surfaces [21,22]. This was observed for the GSH-catalyzed systems. In contrast, generally the rate of TEOS hydrolysis is low and the condensation rate is fast at basic conditions. These slow hydrolysis rates usually promote the formation of particle suspensions, typically showing monodisperse particle size distributions, or the formation of particle aggregates [21-24], such as those observed in this work for the Cys and Cys+GSH systems. At near neutral conditions, both hydrolysis and condensation rates are relatively slow and thus, for the employed reaction times (96 hours), silica yields are lower than at pH extremes. However, it was observed that silica yields at near neutral conditions approached those obtained at pH extremes when reactions were carried out for much longer periods of time (months).

Figure 2 presents the prepared silica materials compositions and removal efficiencies according to the employed catalytic system (GSH, Cys and titrated Cys+GSH solutions), to the used residues extraction/removal procedures (HPSE-catalyst (1/2/5 h), freeze-drying and oven evaporation) and by SDT analysis. The HPSE-catalyst (1/1.5/5 h) method (not presented in this work) was found to be inefficient, namely in terms of water removal. Therefore, the ethanol extraction period (and thus the total ethanol extraction volume) had to be increased - HPSE-catalyst (1/2/5 h).

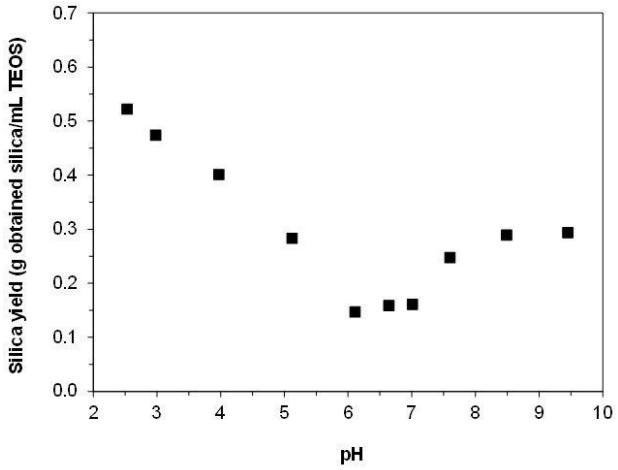
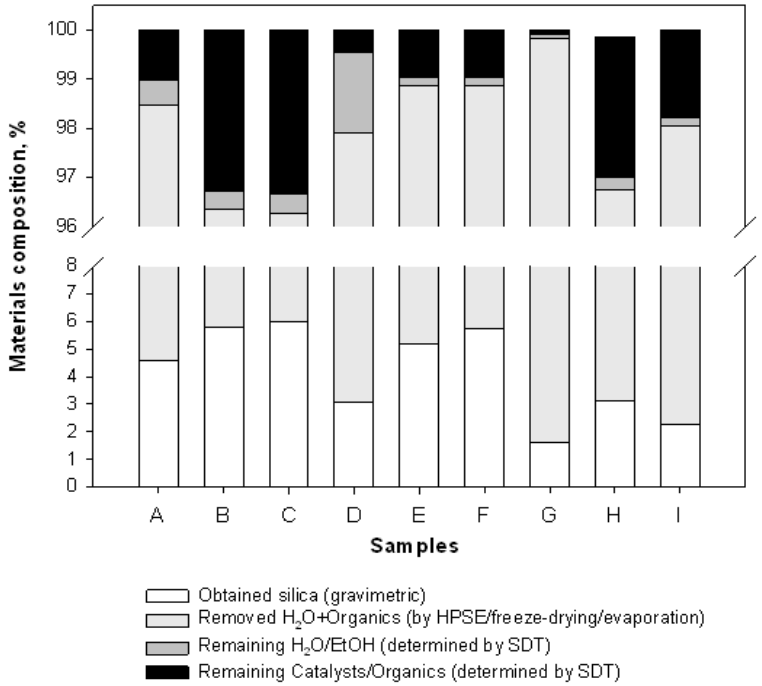


Figure 1. Silica formation yields as a function of initial aqueous solution pH. Reaction aqueous solutions were prepared by titrating solutions of Cys (~0.2 M, pH 9.45) and of GSH (~0.2 M, pH 2.28) until a desired pH is achieved. Reactions performed at 37 ºC, 250 rpm, for 96 hours.

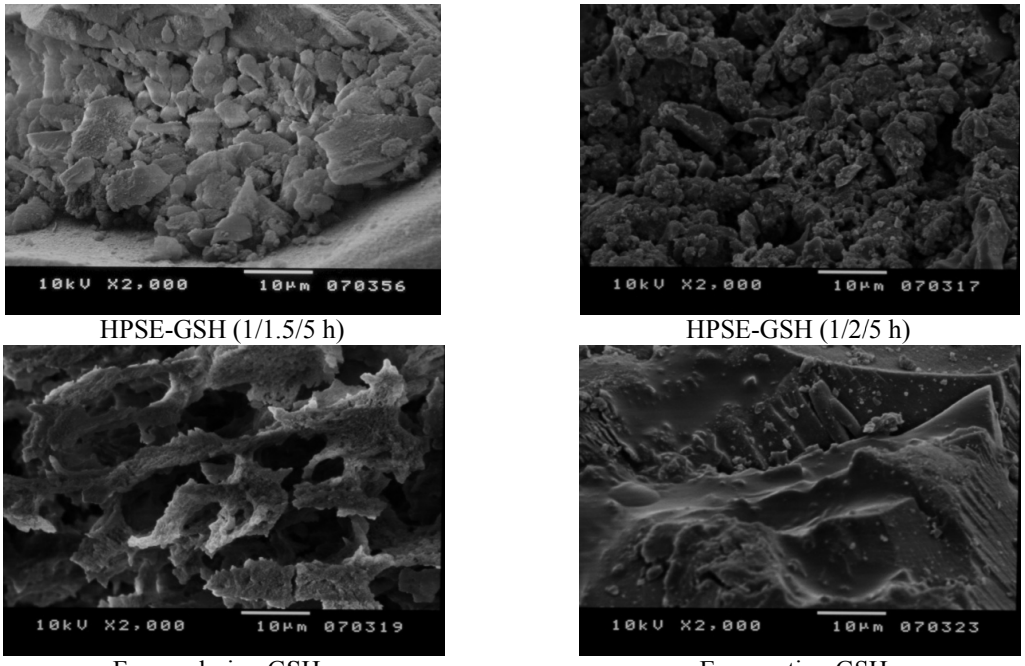


GSH	A – HPSE	**Cys**	D – HPSE	**Cys+GSH**	G – HPSE
	B – Freeze drying		E – Freeze drying		H – Freeze drying
	C – Evaporation		F – Evaporation		I – Evaporation

Figure 2. Prepared silica compositions and removal efficiencies according to the employed catalytic system (GSH, Cys and titrated Cys+GSH solutions), to the used residues extraction/removal procedures (HPSE-catalyst (1/2/5 h), freeze-drying and oven evaporation) and by SDT analysis.

As can be seen and although all the employed extraction/drying methods were capable to remove water and TEOS/ethanol residues from silica formation reactions, the combined high-pressure/supercritical extraction/drying method was clearly the best procedure since it was the only method able to remove most of the catalysts employed at reactions. This was achieved for all employed catalytic systems (GSH, Cys and Cys+GSH) and for both different types of obtained silica samples: wet gel monoliths (low pH, using GSH) and particle suspensions (near neutral pH, Cys+GSH, and basic pH, Cys). Some water ethanol residues are still present in final materials (as determined by SDT) but the efficiency removal of these substances can be easily improved in the

future by increasing process pressure, temperature, and/or ethanol and $scCO_2$ extraction periods and flow rates. Finally, the HPSE method was performed in just a single operation step, and it took much less time and involved less sample manipulation than the freeze-drying and the evaporation ones.
Moreover, and as it can be seen in Figure 3 (for GSH-catalyzed systems), the HPSE method led to different silica macroscopic morphologies, namely from those obtained with the conventional evaporation method. Similar macroscopic differences were also observed for the Cys-, Met- and Cys+GSH-catalyzed systems (data not shown).



HPSE-GSH (1/1.5/5 h) HPSE-GSH (1/2/5 h)

Freeze-drying GSH Evaporation GSH

Figure 3. SEM micrographs for silica prepared with the GSH-catalyzed system and extracted/dried by HPSE, freeze-drying and evaporation.

However, the measured real densities (obtained by Helim pycnometry) as well as the specific surface areas, pore volumes and average pore diameters (obtained by nitrogen adsorption) did not reveal a clear influence of the employed extraction/drying methods on the above referred properties (for a specific catalytic system). In particular, it was expected that the evaporation method led to much higher densities and to much lower surface areas, pore volumes and average pore diameters. This was probably due to the "mild" evaporation conditions that were employed (40 ºC, atmospheric pressure).
On the contrary, the employed catalytic system seems to have a great effect on these properties. Microporous materials (average pore diameters ≤ 2-3 nm) of large specific surface areas (500-800 m^2/g) were obtained for the GSH-catalyzed systems. On the other hand, mesoporous materials (13 nm ≤ average pore diameters ≤ 17 nm) or relatively high specific surface areas (150-200 m^2/g) were obtained for the Cys-catalyzed systems. The observed difference on the obtained specific surface areas between these two catalyzed systems may indicate that GSH originated a material having a quite large number of micropores. Curiously and for the Cys+GSH-catalyzed system (carried out at neutral pH conditions, pH 7.0), the obtained silica materials presented a bimodal behavior in terms of average pore size diameters distribution, i.e., a combination of a microporous and a mesoporous material (average pore diameters ≤ 2-3 nm and 20 nm ≤ average pore diameters ≤ 50 nm). However, these materials presented quite low specific surface areas (30-65 m^2/g) when compared to those obtained for the

GSH- and the Cys-catalyzed systems. Therefore, it seems that reaction pH (acidic, neutral or basic) is not playing the only role on the silica formation process and that the simultaneous use of these two biomimetic catalysts (GSH and Cys) are in fact catalyzing silica formation from TEOS by two different mechanisms, leading to a material having a combination of the morphological properties that would be obtained when the two catalysts were employed alone (and on pH extremes, acidic or basic). These results are quite surprising and are presently being confirmed and verified by Mercury intrusion porosimetry.

Figure 4 presents a dexamethasone (DXMTA) release profile into dH_2O from SSI-processed silica samples (at 40 ºC, 20.0 MPa, for 14 h and employing a 0.2 MPa min^{-1} depressurization rate). Initial samples were prepared using the Cys-catalyzed system and extracted/dried by the HPSE method. These preliminary results show that it was possible to load DXMTA by SSI into previously prepared/processed silica particles. In addition and despite an initial burst release can be observed (due to surface and near-surface drug deposition), some control on the DXMTA release can be achieved until ~100 hours of release. This controlled release behavior is probably due to silica porosity (in this case, mesoporous silica), to pore tortuosity, and to some specific interactions that may be established between DXMTA and silica surface silanol groups.

In conclusion, this work shows that silica obtained by biomimetic aqueous sol-gel methods can be successfully processed by pressurized and supercritical "green" solvents (such as water, ethanol and $scCO_2$) in order: i) to remove the silica formation residual substances and to dry the prepared materials; and ii) to load a $scCO_2$-soluble bioactive substance (such as a drug) into the previously prepared/processed silica materials. Despite these are just preliminary results, the proposed processes can be further developed and improved by the optimization of some of the operational conditions such as pressure, temperature, extraction/drying/impregnation/deposition contact time, solvent flow rates, use of co-solvents and system depressurization rates.

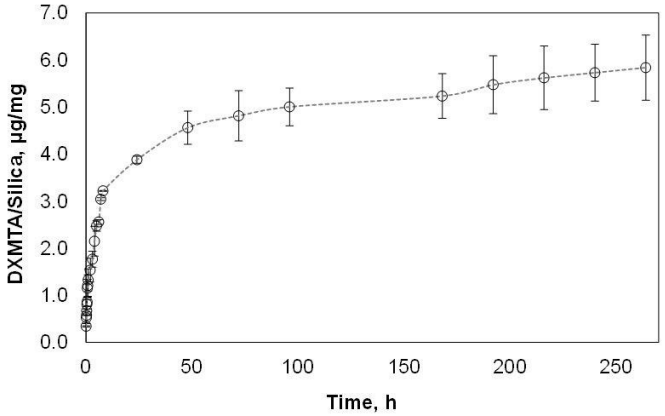


Figure 4. Dexamethasone released from SSI-processed silica samples. Samples were prepared at basic conditions (Cys-catalyzed system), extracted/dried by HPSE and loaded with Dexamethasone by SSI.

Finally, the performed preliminary hemocompatibility tests showed that all the obtained silica materials were hemolytic with the exception of those obtained by the Cys+GSH system and extracted/dried by HPSE. This is clearly due to the remaining catalysts amounts still present inside silica materials (in fact, there is a direct correlation of the hemolytic index with these remaining amounts, see Figure 2). Moreover, and after calcination, all obtained materials (by all methods(catalysts) were found to be non-hemolytic. Nevertheless and despite some operational conditions should be yet optimized, the HPSE method proved again to be the best extraction/drying method for these materials.

CONCLUSIONS

The obtained preliminary results indicated that the employed green and biomimetic synthesis and processing methodologies may have a great potential for the development and preparation of amorphous microporous and mesoporous silica materials and even of silica-based composite biomaterials, which can be used for biomedical applications. More work is being carried out on the proposed methodologies and on the search for the most promising materials in terms of their obtained and envisaged functional properties.

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