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Strontium-releasing fluorapatite glass-ceramics: crystallization behavior, microstructure and solubility

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Abstract

The purpose of the present work was to investigate the effect of strontium partial replacement for calcium on the crystallization behavior, microstructure and solubility of fluorapatite glass-ceramics. Four glass compositions were prepared with increasing amounts of strontium partially replacing calcium. The crystallization behavior was analyzed by differential scanning calorimetry and x-ray diffraction (XRD). The microstructure was investigated by scanning electron microscopy. The chemical solubility was quantified according to ISO standard 10993-14. The amount of strontium released in solution after incubation in TRIS-HCl or citric acid buffer was measured by atomic absorption spectroscopy. XRD analyses revealed that partially substituted strontium-fluorapatite and strontium- β -kermanite crystallized after strontium additions. The lattice cell volume of both phases increased linearly with the amount of strontium in the composition. Strontium additions led to a reduction in crystal size and an increase in crystal number density. The chemical solubility and amount of strontium released in solution increased linearly with the amount of strontium present in the composition in both TRIS-HCl and citric acid buffers. Total amounts of strontium released reached a maximum of 547 ± 80 ppm in TRIS-HCl and 1252 ± 290 ppm in citric acid buffer for the glass composition with the highest amount of strontium. For all strontium-containing compositions, the amount released in TRIS-HCl continued to increase between 70 and 120h, indicating sustained release rather than burst release.

Keywords

Fluorapatite; β -kermanite; glass-ceramic; strontium; solubility

Introduction

A significant amount of research has been conducted in the past decade on strontium-containing bioactive glasses for bone regeneration^{1–5}. These compositions were developed for the production of glass or glass-ceramic scaffolds^{6–9}, glass coatings on metal implants¹⁰ or incorporation into bone cement formulations^{11, 12}. The interest for strontium additions into bioactive glass or glass-ceramic compositions stems from earlier work demonstrating

the dual effect of strontium on bone formation, with enhancement of osteoblastic cell replication combined with inhibition of osteoclastic activity^{13–15}. These findings led to the development of strontium-containing drugs such as strontium ranelate for the prevention of osteoporosis-related bone fractures and currently available in Europe^{16–18}. However, although strontium ranelate is still authorized for use in the European Union, its indications are now restricted to severe cases of osteoporosis, due to increased occurrence of cardiac ischaemic events associated with strontium ranelate treatment^{19, 20}. This explains the heightened interest towards *in situ* strontium delivery, made possible through the incorporation of strontium in the composition of bioactive glass and glass-ceramic scaffolds used as bone graft substitutes^{21–23}.

Substitution of strontium for calcium in silicate glasses is associated with a slight expansion in the glass network leading to a weakening in network connectivity²⁴. This is attributed to the lower charge to size ratio of strontium, compared to calcium²⁵. Further work revealed that strontium for calcium substitution in bioactive glasses increased solubility and was associated with the release of therapeutically active strontium ions^{1, 3, 26}. In addition to its effect on the glassy matrix, strontium may also substitute for calcium in the apatite crystalline structure²⁷. Numerous studies have examined crystallographic and physical aspects of this substitution in both the hydroxyapatite and the fluorapatite lattices and demonstrated the formation of continuous solid solutions in both systems^{28–31}. Importantly, it was concluded that strontium for calcium substitution was associated with the creation of lattice defects, vacancies and distortions strongly affecting surface properties through hydration layers and surface charges. This was shown to result in an increase in solubility attributed to a destabilization of the apatite crystal structure due to the slightly larger size of the strontium ion compared to calcium³².

Our previous work focused on the development of fluorapatite-based glasses and glass-ceramics in the $\text{SiO}_2\text{--Al}_2\text{O}_3\text{--P}_2\text{O}_5\text{--MgO--Na}_2\text{O--K}_2\text{O--CaO--CaF}_2$ system^{33–35}. It was shown that glass-ceramics in this system promoted differentiation of human mesenchymal stem cells into an osteoblastic lineage³⁶. Moreover, we successfully developed a glass composition in this system leading to a unique microstructure composed of flower-shaped submicrometer fluorapatite crystals^{35, 37}, which was further selected for the production of glass-ceramic scaffolds for bone regeneration. Finally, we recently reported on the production of fully dense fluorapatite glass-ceramics scaffolds by rapid vacuum sintering (RVS)³⁸. As mentioned previously, an extensive amount of literature is available on strontium-containing bioactive glasses and glass-ceramics^{3, 4, 25, 26, 39, 40}. However, few of these studies consider the impact of strontium for calcium substitution on microstructure, including crystal size and morphology of the final glass-ceramic product, which are critical to *de novo* biological apatite nucleation and growth on glass-ceramic scaffolds for bone graft substitutes.

The aim of the present work was to investigate the effect of strontium additions on the crystallization behavior, microstructure, chemical solubility and ion release behavior of fluorapatite glass-ceramics in the $\text{SiO}_2\text{--Al}_2\text{O}_3\text{--P}_2\text{O}_5\text{--MgO--Na}_2\text{O--K}_2\text{O--CaO--CaF}_2$ system. We hypothesized that partial replacement of strontium for calcium would lead to

crystallization of strontium fluorapatite, increase solubility and enable strontium ion release in solution.

Materials and methods

Glass preparation

Four glass compositions in the $\text{SiO}_2\text{--Al}_2\text{O}_3\text{--P}_2\text{O}_5\text{--MgO--Na}_2\text{O--K}_2\text{O--CaO--CaF}_2$ system, with increasing amounts of strontium oxide from 0 to 24 mol. %, in partial replacement for calcium (Table 1) were prepared by mixing adequate amounts of reagent grade oxides and carbonates and labeled G Sr-0, G Sr-12, G Sr-18 and G Sr-24, according to the amount of strontium in the composition. Glasses were twice melted at 1525°C for 3 hours in platinum crucibles and cast into cylindrical ingots (12 mm diameter; 60 mm in length). The density was measured on glass ingots ($n=3$ per group) using a gas displacement helium pycnometer (Accupyc II 1340, Micromeritics Instrument Corporation).

Crystallization behavior

Differential thermal analyses were performed on powdered specimens under nitrogen gas flow to determine glass transition and crystallization temperatures (Q600 DTA/TGA, TA Instruments). Specimens were powdered using an agate mortar and pestle. Analyses were performed at a heating rate of 40 °C/min. up to 1100°C. Aluminum oxide (99.99%) powder served as reference standard. Disc-shaped specimens were cut from the ingots and heat treated at temperatures ranging from 775 to 900°C for one hour at a heating rate of 2°C per minute. This temperature range was selected according to the DTA results. Crystalline phases were characterized by x-ray diffraction (XRD) on powdered specimens. Scans were performed in the two-theta range 10–90°, at 40 kV and 44 mA in Bragg-Brentano configuration (SmartLab, Rigaku Americas, Inc.). Peak positions were determined using PDXL-2 analysis software (Rigaku Corporation) after calibration using silicon powder standard (NIST, 640d). Lattice parameters were determined by Rietveld refinement^{41–43} and compared to published values and powder diffraction files to assess the amount of strontium incorporated in each crystalline phase.

Microstructural characterization

The microstructure was characterized by scanning electron microscopy (Hitachi S-4800 field emission SEM) on heat treated specimens. Routine SEM preparation techniques including fine polishing through a series of abrasives ending with 1 micrometer diamond paste, chemical etching with 1% hydrofluoric acid in aqueous solution and gold coating were used to study the microstructure. The mean crystal size as a function of strontium content and crystallization temperature was measured on digital micrographs ($n=4$ per group, minimum 100 particles per micrograph) using publicly available image analysis software (NIH Image J 1.48v⁴⁴).

Chemical solubility, ion release

The chemical solubility of the glasses and glass-ceramics ($n=3$ per group) was determined according to ISO standard 10993-14⁴⁵. One set of experiments was therefore conducted in citric acid buffer at pH 3.0 to test extreme conditions⁴⁶. A second set of experiments was

performed at pH 7.4 in buffered saline solution (Tris-HCl) to simulate physiological *in vivo* conditions. Untreated glasses and glasses subjected to a simulated sintering heat treatment at 775°C for 1h (SG) were powdered using an agate mortar and pestle and sieved to a particle size less than 275 µm but greater than 130 µm. Both sets of experiments were carried out at 37°C for 120 h with a sample mass/solution volume ratio kept constant. Containers were agitated at 2 Hz using a circular movement. The pH of the buffer solutions as well as the mass of the specimens were monitored before and after incubation. Upon test completion, specimens were dried at 80°C until no further weight loss was observed, the percent weight loss was then calculated for each composition and each buffer.

Strontium concentrations present in solution during and after completion of chemical solubility tests were measured by flame atomic absorption spectrophotometry (Perkin-Elmer 2380) using standard analytical techniques. Relevant dilutions were freshly prepared from certified standards for strontium. Aliquots were collected at 72 and 92 and 120 hours. These time points were specifically chosen to investigate whether strontium was released in a sustainable manner past 72 hours, the assumption being that sustained release capability is critical for long term action on bone regeneration.

Statistical analyses

Results were analyzed by ANOVA and Tukey's test to detect statistically significant differences. A *p*-value of less than 0.05 was considered statistically significant.

Results

The results from density measurements are summarized in Table 2. The mean glass density increased linearly ($R^2=0.985$) with the amount of strontium in the glass composition from 2.927 ± 0.008 g/cm³ for the strontium-free base glass (GSr-0) to 3.353 ± 0.021 g/cm³ for the glass containing 24 mol.% strontium oxide (GSr-24).

Crystallization behavior

Results from differential thermal analyses are displayed in Figure 1. Characteristic temperatures are summarized in Table 2. A broad and asymmetric exothermic peak was present for the strontium-free glass (GSr-0; Figure 1A), while two exothermic peaks were observed for all other compositions (Figure 1B through 1D). The broad exothermic peak observed for the strontium-free composition was successfully deconvoluted into two exotherms, results are listed in Table 2. The temperature difference between the two exotherms increased linearly with the amount of strontium in the composition ($R^2=0.95$). XRD analyses on powdered specimens confirmed the presence of fluorapatite ($\text{Ca}_5(\text{PO}_4)_3\text{F}$; PDF 15-876) in the strontium-free glass-ceramic (GSr-0) after heat treatment at 775°C (Figure 2A) or 800°C/1h. Both fluorapatite and åkermanite ($\text{Ca}_2\text{MgSi}_2\text{O}_7$; PDF 35-592) were present after heat treatment at 850 or 900°C/1h (Figure 2B). For clarity, only XRD patterns after heat treatment at either 775°C or 900°C for 1h are displayed in Figure 2 while crystallographic lattice cell parameters are summarized in Table 3 for all temperatures. Sr-substituted fluorapatite was present in all Sr-containing glass-ceramics after heat treatment

at 775 or 800°C (Figure 2A). Meanwhile, both Sr-substituted fluorapatite and Sr-substituted åkermanite were present after heat treatment at 850 or 900°C (Figure 2B).

Effect of heat treatment temperature on lattice parameters

Rietveld refinement analyses are summarized in Table 3. There was a decrease in the fluorapatite cell parameter a with increasing heat treatment temperature (Figure 3A), while the c parameter remained unchanged. The åkermanite cell parameter a increased from 850 to 900°C while the c parameter remained unchanged (Figure 3B). A decrease in the Sr-fluorapatite cell parameter a was also observed with increasing heat treatment temperature (Figure 3A). Concurrently, lattice parameter c decreased for composition GSr-12 and increased for GSr-18 and GSr-24, with increasing heat treatment temperature. There was no significant change in lattice parameters for åkermanite between 850 and 900°C for these three compositions (Table 3).

Effect of strontium additions on lattice parameters

The lattice parameter a for fluorapatite increased linearly ($R^2>0.99$) with the amount of strontium in the composition regardless of heat treatment temperature (Figure 3A). Similarly, the lattice parameter a for åkermanite increased linearly ($R^2=0.99$) with the amount of strontium in the composition (Figure 3B).

The linear increase in the lattice parameter a for fluorapatite and åkermanite as a function of strontium content indicates a progressive replacement of strontium for calcium in both crystal structures. When both phases forming a solid solution present the same crystalline structure, the extent of strontium for calcium substitution can be estimated by applying Vegard's law⁴⁷ and plotting cell volume from published data^{48–50} as a function of strontium content. As shown in Figure 3C, it can be estimated that the extent of strontium substitution in the fluorapatite lattice increased from about 25% in composition GSr-12 to about 48% in GSr-18 and up to 62% in GSr-24. In the case of the åkermanite cell, calculations showed that the degree of substitution of strontium for calcium was 38, 53 and 65 % in compositions GSr-12, GSr-18, and GSr-24, respectively (Figure 3D).

Microstructural characterization

Characteristic micrographs for each glass-ceramic composition after heat treatment at 800°C for 1h are shown in Figure 4(A through D). Submicrometer flower-shaped spherulitic fluorapatite crystals dominated the microstructure for all compositions. A progressive increase in the number of crystallization sites, associated with a decrease in mean crystal diameter was observed as the amount of strontium in the glass composition increased (Table 4). A very different microstructure, best described as a coast-to-island microstructure, developed after heat treatment at 900°C for 1h (Figure 5A through D). Large islands in darker contrast (2–5 µm in length), together with small spherical islands (<1 µm in diameter) in brighter contrast can be seen, with similar, shape, size and distribution across compositions, regardless of strontium content. A consistent increase in the number of the smaller islands per surface area from GSr-0 to GSr-24 may confirm that strontium acts as a nucleating agent for fluorapatite nucleation.

Chemical solubility and ion release

The chemical solubility, as expressed by the weight loss, increased with strontium content, reaching a maximum for composition GSr-24 of 2.6 ± 1.2 wt. % in TRIS-HCl buffer (pH 7.4) and 10.4 ± 0.6 wt.% at pH 3 in citric acid buffer (Figure 6A).

The amount of strontium released in solution during and after incubation in either TRIS-HCl buffer or citric acid buffer measured by atomic absorption spectroscopy is displayed in Figure 6B. There was a linear increase ($R^2=0.99$) with strontium content, regardless of buffer type, reaching a maximum of 547 ± 80 ppm in TRIS-HCl and 1252 ± 290 ppm in citric acid buffer for glass GSr-24.

The amount of strontium released from the sintered glass-ceramics (SG) in Tris-HCl buffer solution as a function of time is displayed in Figure 6C. The amount released was lowest for SGSr-12, and increased linearly with the amount of strontium in the composition.

Importantly, the amount released continued to increase between 70 and 120 h, indicating a sustained release rather than a burst release.

Discussion

The mean density of the glasses increased linearly with the amount of strontium in the glass composition, this was expected due to the higher atomic mass of strontium (87.62) compared to calcium (40.08). The incorporation of strontium in the glassy matrix of silicate glasses is also expected to increase radio-opacity, which is desirable in both dental applications and bone tissue engineering for enhanced x-ray imaging⁵. Differential thermal analyses revealed the presence of two crystallization exotherms attributed respectively to fluorapatite and åkermanite from x-ray diffraction analyses. Strontium additions lowered the crystallization temperature of Sr-fluorapatite compared to the strontium-free glass. A study by Hill *et al.*, on a glass-ceramic in the $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-P}_2\text{O}_5\text{-CaO-CaF}_2$ system showed that, at low levels, strontium remained in the glassy matrix and promoted surface nucleation of fluorapatite⁵. Our results also showed that strontium acted as a nucleating agent, as indicated by the increase in the number of nucleation sites for Sr-fluorapatite (Table 4), resulting in a very dense microstructure (Figure 4D). In addition, Rietveld refinement analyses demonstrated that strontium was successfully substituted for calcium in both crystalline structures. This progressive substitution led to a linear increase in crystallographic lattice cell volumes, due to the larger ionic radius of strontium (132 pm), compared to calcium (114 pm)^{5, 51}. Substitutions of strontium for calcium in the hydroxyapatite structure have been the focus of extensive research work due to the potential biomedical applications of strontium-substituted hydroxyapatites as bone fillers, or incorporated into bone cement compositions^{23, 27, 28, 30, 31, 52}. The presence of strontium in cell and calvarial cultures has been shown to enhance the replication of pre-osteoblastic cells and stimulate bone formation while blocking osteoclastic activity^{15, 16, 53}. Meanwhile fluorapatite has also received interest as a host structure to sequester radionuclides such as $^{90}\text{Sr}^{29}$. Fluorapatite crystallizes in the hexagonal system, space group $P6_3/m$ ^{54, 55}. The distribution of Sr^{2+} cations between the $6h$ or $4f$ sites in the fluorapatite unit cell was refined by Michie *et al.*⁴⁸, who proposed the use of atomic scale local density calculations to predict energies and lattice parameters in mixed calcium-strontium fluorapatite solid solutions. It was concluded that because calcium

sites are equally accessible, strontium can substitute for calcium in either cationic sites, with a preference for *6h* sites at low concentrations. Our results confirmed a linear increase in fluorapatite cell volume with increasing strontium content in the glass composition.

Meanwhile, at higher temperatures, from 850°C and above, strontium also substituted in the åkermanite crystalline structure. Åkermanite crystallizes in the tetragonal system, space group *P-421m*, with a layered structure composed of MgO₄ and SiO₄ tetrahedra connected by Ca in eightfold coordination between layers^{56, 57}. This type of configuration allows strontium for calcium substitutions in the åkermanite structure, within the Ca-Sr åkermanite solid solution domain^{49, 51}. Calculations from our lattice parameters measurements indicated that the extent of strontium for calcium substitution was comparable between fluorapatite and åkermanite, reaching 62 and 65%, respectively for GSr-24. The microstructure observed after heat treatment at 900°C for 1h is tentatively interpreted as large åkermanite islands in darker contrast and smaller fluorapatite islands in brighter contrast. To our best knowledge, this is the first report of a glass-ceramic system containing both substituted phases. The presence of both Sr-fluorapatite and Sr-åkermanite may be beneficial to sustained release of strontium due to their markedly different degradation rates at physiological pH^{32, 58}.

Our results showed that the solubility of the glasses and glass-ceramics in Tris-HCl and citric acid buffers increased with strontium content in the glass composition. Additionally, the increase in solubility as a function of strontium content was accompanied by a linear increase in the amount of strontium released in solution. While these results are consistent with published data on bioactive strontium-containing silicate or phosphate-based glasses^{1, 25, 26, 59, 60}, work by Goel *et al.* reported that strontium additions led to a decrease in the chemical degradation of CaO-MgO-SrO-SiO₂-P₂O₅-CaF₂ glasses³⁹. This highlights the extreme complexity of the effect of chemical composition on the physical properties and dissolution behavior of bioactive glasses. Strontium amounts considered in the present work (up to 24 mol.%) were substantially higher than those explored in the study by Goel *et al.* (up to 10 mol.%)³⁹. A recent study by Bruckner *et al.*⁶¹ pointed out that while the mixed alkali effect is known to reduce ion release from conventional silicate glasses, compared to compositions based on one alkali oxide only, substitution of smaller alkali ions with cations of larger ionic radius expands the silicate network and increases dissolution rates. This is the case for example with Sr²⁺ replacing Ca²⁺ cations, a privileged mechanism in the present work. The total amount of strontium released in solution after incubation for 5 days in TRIS-HCl is within therapeutic range (0–547 ppm)^{9, 62, 63} and was similar between glasses and glass-ceramics subjected to sintered cycle (Figure 6B). The linear increase in strontium released in solution with the amount of strontium present in the glass composition may offer a simple approach to *in situ* delivery of controlled strontium doses, with different levels of release tailored by composition and degree of crystallization. Moreover, the release of strontium in TRIS-HCl buffer continued to increase at 72 and 92 hours and up to 5 days, for all strontium-containing compositions. This demonstrates that a sustained release of strontium can be obtained in this compositional range.

Conclusion

Strontium substitution for calcium in this fluorapatite-based silicate glass-ceramic system led to the crystallization of strontium-substituted fluorapatite with further crystallization of strontium-substituted akermanite after heat treatment at 850°C and higher. Strontium had a marked effect on the microstructure by reducing crystal size and increasing crystal number density.

Meanwhile, strontium additions led to an increase in solubility for both glasses and glass-ceramics in this system, associated with a sustained release of strontium in solution at levels proportional to the amount present in the glass composition.

This glass-ceramic system could provide a practical approach for the production of strontium-releasing glass or glass-ceramic scaffolds for bone tissue engineering with sustained release capabilities.

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Figure 1.
Differential thermal analysis thermograms for the various glasses.

Figure 2.
X-ray diffraction patterns for the various glass-ceramics after heat treatment at 775°C for 1h (A) or 900°C for 1h (B).

Figure 3.

Variation of lattice parameter a as a function of strontium content for fluorapatite (A) and akermanite (B). Estimates of strontium content in fluorapatite (C) and akermanite (D) from unit cell volume.

Figure 4. Scanning electron micrographs after heat treatment at 800°C for 1h. A: GSr-0; B: GSr-12; C: GSr-18; D: GSr-24.

Figure 5.
Scanning electron micrographs after heat treatment at 900°C for 1h. A: GSR-0; B: GSR-12;
C: GSR-18; D: GSR-24.

Figure 6.

Weight loss (A) and strontium release (B) as a function of strontium content after incubation for 120 h. Strontium release as a function of time in TRIS-HCl buffer for the various glass-ceramics (C).

Table 1

Chemical composition of the glasses prepared (mol. %).

	GSr-0	GSr-12	GSr-18	GSr-24
SiO ₂	31.5	31.5	31.5	31.5
MgO	21.2	21.2	21.2	21.2
Nb ₂ O ₅	0.2	0.2	0.2	0.2
CaO	24.0	12.0	6.0	0.0
SrO	0.0	12.0	18.0	24.0
Na ₂ O	2.3	2.3	2.3	2.3
K ₂ O	3.8	3.8	3.8	3.8
Al ₂ O ₃	0.8	0.8	0.8	0.8
CaF ₂	11.2	11.2	11.2	11.2
P ₂ O ₅	5.0	5.0	5.0	5.0

Table 2

Density, glass transition and crystallization temperatures for the various glass compositions.

Glass (mol.% SrO)	Density (g/cm ³)	T _g (°C)	Exotherm 1 T _{El} (°C)	Exotherm 2 T _{E2} (°C)	T _(E2-El) (°C)
GSr-0	2.927 ± 0.008	654 ± 3	880 ± 1	900 ± 2	20
GSr-12	3.188 ± 0.005	627 ± 7	833 ± 3	875 ± 15	42
GSr-18	3.262 ± 0.021	637 ± 0	834 ± 1	883 ± 5	49
GSr-24	3.353 ± 0.021	643 ± 3	836 ± 8	909 ± 3	73

Rietveld refinement results for lattice parameters of fluorapatite, åkermanite and Sr-containing matching phases (NP: Not Present)

Glass	Heat treatment temperature (°C)	Fluorapatite – Sr-fluorapatite			Akermanite – Sr-akermanite		
		Lattice parameter a (Å)	Lattice parameter c (Å)	Cell volume V (Å ³)	Lattice parameter a (Å)	Lattice parameter c (Å)	Cell volume V (Å ³)
GSr-0	775	9.4313	6.8986	531.4		NP	
	800	9.4108	6.8994	529.2		NP	
	850	9.3967	6.8967	527.4	7.8185	5.0103	306.3
	900	9.3828	6.8957	525.7	7.8205	5.0103	306.4
GSr-12	775	9.5397	7.0402	554.9		NP	
	800	9.5344	7.0344	553.8		NP	
	850	9.5205	7.0040	549.8	7.8776	5.0687	314.5
	900	9.5054	6.9907	547.0	7.8782	5.0712	314.7
GSr-18	775	9.6059	7.1093	568.1		NP	
	800	9.5980	7.1160	567.7		NP	
	850	9.5906	7.1459	569.2	7.9033	5.0946	318.2
	900	9.5882	7.1555	569.8	7.9019	5.0989	318.4
GSr-24	775	9.6589	7.1893	580.9		NP	
	800	9.6498	7.1893	579.8		NP	
	850	9.6484	7.1985	580.3	7.9203	5.1226	321.3
	900	9.6386	7.1949	578.9	7.9198	5.1277	320.6

Table 4

Microstructural characteristics of the various glass-ceramics after heat treatment at 800°C for 1h.

Glass composition	Crystal number density (per μm^2)	Crystal size (μm)
GSr-0	0.299 $\pm$ 0.018	1.66 $\pm$ 0.55
GSr-12	1.083 $\pm$ 0.088	0.93 $\pm$ 0.02
GSr-18	2.587 $\pm$ 0.342	0.55 $\pm$ 0.02
GSr-24	6.191 $\pm$ 0.203	0.36 $\pm$ 0.01