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Analytical control of wollastonite for biomedical applications by use of atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry

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Preliminary *in vitro* experiments revealed that wollastonite ($CaSiO_3$) is a potentially highly bioactive material that forms a hyroxyapatite (HA) surface layer on exposure to simulated body fluid with an ion concentration, pH and temperature virtually identical with those of human blood plasma. The formation of the HA layer is an essential requirement for an artificial material to be used as bioactive bone substitute. This finding opens up a wide field for biomedical applications of wollastonite. Biomaterials used as implants in the human body require strict control of trace elements and of the toxic species specified in American Society for Testing and Materials F-1185-88 (As, Cd, Hg and Pb) in ceramic hydroxyapatite for surgical implantation. In this work, two types of pseudowollastonite, the high temperature form of wollastonite, were analysed by using cold vapour atomic absorption spectrometry and hydride generation atomic absorption spectrometry, in order to determine the elements stated in the above-mentioned norm, and inductively coupled plasma atomic emission spectrometry to establish the SiO_2/CaO ratio of the two materials and analyse for all other impurities introduced by the raw materials and by the processes of synthesis, sintering and grinding. Barium and Mg were especially prominent in raw materials, and Zr, Y, Mg, W, Co and Ni come mainly from the processing.

Keywords: *Wollastonite; pseudowollastonite; biomaterials; impurities analysis; cold vapour atomic absorption spectrometry; hydride generation atomic absorption spectrometry; inductively coupled plasma atomic emission spectrometry*

One of the sequelae of World War II was the pressing need for developed countries to treat vast numbers of patients. In response, biomaterials science arose as a branch of the existing medical technology.

As a general rule, when an artificial material is implanted in the body, its is encapsulated by uncalcified fibrous tissue that isolates it from the surroundings. This is a normal reaction intended to protect the body from foreign substances. However, in the early 1970s, Hench *et al.*[1] found that a glass of the complex system Na_2O–CaO–SiO_2–P_2O_5 induced the formation of no fibrous tissue, but rather came into direct contact with the surrounding bone and formed a tight chemical bond with it.

Since then, other types of glasses and glass–ceramics have also been found to bind to living bone. These bone-binding materials are called bioactive materials.[2–4]

Hench and coworkers,[5–6] Gross and coworkers[7–8] and Kokubo[9] claim that the essential condition for a material to bind to living bone is the formation of an hydroxyapatite (HA) layer on its surface. Later, Ohura *et al.*[10] found that a CaO–SiO_2 glass

containing no P_2O_5 forms an HA surface layer when soaked in simulated body fluid (SBF), whereas a SiO_2-free CaO–P_2O_5 glass forms no such layer. This suggests that bioactive glasses and glass–ceramics can be more effectively obtained from the CaO–SiO_2 system than from CaO–P_2O_5.

The use of these materials as implants must be controlled in order to protect patients from undue risks associated with the implantation of foreign materials in the human body.[11]

With the aim of issuing analysis norms for materials to be used in biomedical applications in 1962 the ASTM (American Society for Testing and Materials) Technical Committee F-4 on Medical and Surgical Materials and Devises was formed. Since then, the Committee F-4 has released over 135 standards, specifications, practices and guidelines pertinent to medical materials and devices. These standards deal with the materials and devices used in the various medical disciplines including orthopaedics, cardiovascular, plastic and reconstructive surgery, and neurosurgery. Since 1976, the Food and Drug Adminstration (FDA)[12–14] has had the legal authority to regulate medical devices, especially with regard to their labelling, marketing, manufacture, processing, distribution and use.

The development and promulgation of international standards for medical devices is largely concentrated in two International Organisation for Standardisation (ISO) Technical Committees, *viz*. ISO TC-150 (Implants for Surgery) and ISO TC-194 (Biological Evaluation of Medical Devices). ISO standards describe the materials and devices they regulate, define their properties and performance requirements, and provide test methods for the evaluation and characterisation of materials intended for implantation in human bodies.

Recently, De Aza *et al.*[15–18] have shown in *in vitro* experiments for the first time, that polycrystalline pseudo-wollastonite (β-$CaSiO_3$) ceramic materials form an HA surface layer on exposure to SBF and are thus suitable for use as biomaterials. These materials have been tested in rats and dogs as potential implants[19] ever since the intitial results of De Aza *et al.*[15–18] became known.

Actually biological tests are under development. As a complementary study, in the present work, a method for the analytical control of impurities in pseudowollastonite is developed. This is the first reported analytical method for impurities in pseudowollastonites intended for use as biomaterials.

The chemical analysis of a potential bioactive pseudowollastonite material should include the following determinations:

(*a*) Every element considered detrimental to health above given levels by international regulations. In this respect, ASTM F 1185–88,[20] a widely accepted standard for the analysis of hydroxyapatite ceramic for surgical implants, requires analysing for As, Cd, Hg and Pb, the concentrations of which in the biomaterial should not exceed 1, 2, 5 and 10 ppm, respectively.

(*b*) Other impurities present in the raw materials or introduced during the synthesis and sintering processes or the subsequent grinding of the materials.

(*c*) The SiO_2/CaO ratio, which is essential with a view to establishing the crystal phases present in pseudowollastonite materials and also to predict the potential presence of a vitreous phase.

These analyses cannot be effectively implemented by using any available instrumental technique. In fact, no single technique is sensitive enough to determine As at concentrations below 1 µg g^{-1} and simultaneously quantify CaO and SiO_2 in contents above 40% with adequate precision (*viz.* a relative standard deviation less than 1%).

In this work, cold vapour atomic absorption spectrometry (CVAAS), hydride generation atomic absorption spectrometry (HGAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) were used in combination to perform the above-mentioned analyses. Thus, CVAAS was used to determine Hg and HGAAS to determine As. On the other hand, ICP-AES was used to analyse for all other impurities, in addition to CaO and SiO_2 (the last two were determined by using an internal standard).

Experimental

Materials and characterization

Two different pseudowollastonites were studied, namely: (*a*) sintered pseudowollastonite (psW-S) obtained in the laboratory by solid-state reaction; and (*b*) electrofused pseudowollastonite (psW-M), industrially obtained. Scheme 1 shows the processing routes used in the synthesis and subsequent treatments of both pseudowollastonites.

Sintered pseudowollastonite (psW-S) consisted of nearly equiaxial grains of average diameter ≈ 21.3 µm with round and closed pores of average size ≈ 7.8 µm. It contained very little vitreous phase that was mainly located at triple junctions between grains, its SiO_2/CaO molar ratio being about 0.73.
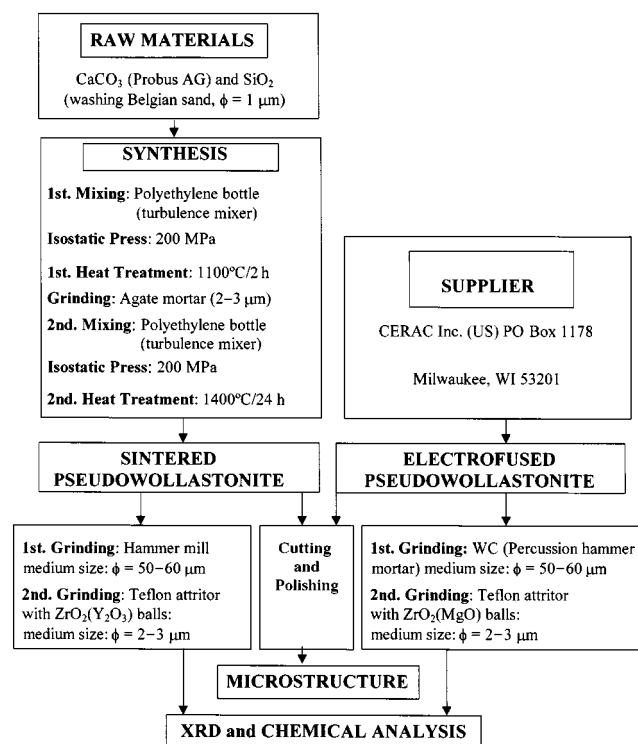
On the other hand, psW-M was composed of columnar grains with an average length-to-width ratio of $\approx 10:1$ with no pores. It also contained a silica-rich vitreous phase among elongated psW crystals, with a SiO_2/CaO molar ratio about 2.69 as determined by X-ray wavelength dispersive spectroscopic microanalysis (WDS).

Mineralogically, the two samples consisted of well-crystallized pseudowollastonite (see Fig. 1). In addition, psW-M contained small amounts of highly distorted α-cristobalite due to partial devitrification of the silica rich vitreous phase.

Apparatus and operating conditions

ICP-AES

A Jobin-Yvon JY-38 VHR sequential spectrometer equipped with an ICP source was used for ICP-AES determinations. The instrument was furnished with a monochromator of 3600 grooves mm^{-1} Its reciprocal linear dispersion was 0.26 nm mm^{-1} and its resolution 0.0026 nm. The specifications for the ICP source were frequency, 56 MHz; induced power, 1600 W; reflected power, < 5 W. The operating conditions were as follows: plasma gas (argon) flow-rate, 16 l min^{-1}; aerosol carrier gas (argon) flow-rate, 0.3 l min^{-1}; coil cooling water flow-rate, 0.13 l min^{-1}; sample introduction, Meinhard (Santa Ana, CA, USA) high-salt concentric glass TR-C2-30 nebulizer with a humidifier at the argon aerosol carrier inlet; sample delivery, PS Analytical Ltd. peristaltic pump (2.1 ml min^{-1}); measurement time, 3 integrations of 400 ms step^{-1}; observation height, 16 mm.

CVAAS and HGAAS

A Thermo Jarrel Ash model Smith-Hiefje 21 atomic absorption spectrophotometer was used for AAS measurements. The instrument included a single-beam light system and monochromator mounted on a diffraction grating of the Ebert-Fostie type that afforded a maximum resolution of 0.09 nm. It was equipped with a model AVA 440 system, also from Thermo Jarrel Ash, for generating mercury cold vapour or hydrides. A Smith-Hiefje background correction device was also included, use of which for Hg and As was unnecessary because virtually no matix effects were present.

Reagents

Lithium tetraborate (Merck, Poole, Dorset, UK; RA grade); sodium hydroxide on pellet from Merck (RA grade); HF, HCl and HNO$_3$ (Merck Suprapur grade); H$_2$SO$_4$ (Merck RA grade); sodium borohydride pellets (Merck Spectrosol); 1 g l^{-1} highly pure stock standard solutions (Riedel-de-Haën, Hannover, Germany); an yttrium internal standard prepared by dissolving

Scheme 1 Processing of the sintered and electrofused pseudowollastonites.
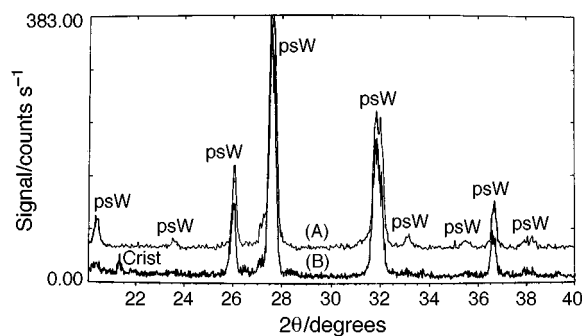
Fig. 1 X-ray diffraction of the pseudowollastonite samples obtained by solid state reaction (A) and by electrofusion (B). (psW = pseudowollastonite; Crist = α-cristobalite.)

1.00 g of Y_2O_3 (Johnson Mathey, Royston, Herts, UK; 99.995 pure) in 50 ml of HCl 1 + 1 and diluting to 1000 ml; and de-ionized water of resistivity less than 18 MΩ were used.

Decomposition of samples

Arsenic (HGAAS)

Triplicate samples of 0.5000 g were digested with 10 ml of *aqua regia*; the mixture was initially allowed to stand at room temperature for 1 h and then heated in an open beaker at 90 °C for 2 h. The digest was filtered and washed with de-ionized water in order to remove the silica. The resulting solution was diluted to 100 ml with de-ionized water. Finally, a 25 ml aliquot of this soluton was poured into the reaction vessel. As^{5+} was then reduced to its hydride by using 5 ml of a 10% sodium borohydride solution in 2.5% NaOH, evolved gases being swept into a heated quartz tube cell for atomic absorption measurement.

Mercury (CVAAS)

Triplicate samples of 0.1000 g were digested with 5 ml of a H_2SO_4/HNO_3 1 + 1 mixture in a closed Teflon pump at 180 °C for 90 min. Once cold, the reaction mixture was transferred to a polypropylene bottle and its volume made to exactly 10 ml. The mixture was then transferred to the reaction vessel and Hg^{2+} reduced to metal mercury by using 10 ml of a 3% sodium borohydride solution in 1% NaOH. The resulting mercury vapour was swept to a quartz tube for subsequent analysis by AAS.

Other impurities (ICP-AES)

Quintuplicate samples of 1.000 g were digested in a Teflon beaker with 5 ml of concentrated HNO_3 and 10 ml of concentrated HF. The mixture was evaporated to dryness on a hot plate at 190 °C in order to remove tetrafluorosilicate and residual HF. Then, 5 ml of concentrated HF and 5 ml of de-ionized water were added to the residue, which was heated at 100 °C until complete dissolution. The resulting solution was finally diluted to 100 ml with de-ionized water.

Silicon and calcium (ICP-AES)

Quintuplicate samples of 0.2000 g were mixed with 2.00 g of lithium tetraborate in a small porcelain crucible and then quantitatively transferred to a high-purity graphite crucible. The mixture was fused in a muffle furnace at 1000 °C for 20 min and carefully, quickly poured into a 400 ml glass beaker containing 100 ml of 5% v/v HCl for dissolution with the aid of a Teflon-coated magnetic bar. The solution was subsequently filtered to remove graphite particles and diluted to 200 ml with de-ionized water. Prior to analysis, 5 ml of this solution + 1 ml of the 1000 µg ml^{-1} Y solution as internal standard was diluted to 100 ml with de-ionized water.

Preparation of standards and calibration

Calibration graphs for the impurities quantified by ICP-AES, CVASS and HGAAS were constructed from a blank solution and a standard of appropriate concentration. Both solutions and standards used to determine the detection limits were matrix-matched to the decomposition reagents in the final solutions of sample and calcium macro constitutents.

The calibration graphs for Ca and Si were constructed from two-elemental standards containing a high (40 µg ml^{-1}) or a low concentration (20 µg ml^{-1}) of both elements. The standards also included 10 µg ml^{-1} of Y as internal standard and the same amounts of reagents used to attack the sample following 1 + 19 dilution.

Results and discussion

Analytical lines

Wavelength profiles in the vicinity of the analytical ICP-AES lines for 62 elements were used to determine the qualitative composition of impurities in the samples. These are potential impurities taking into account the nature of the raw materials used in the synthesis of both pseudowollastonites as well as those introduced by the method of synthesis (solid state synthesis or electrofusion) and processing routes (grinding media). Those established by the ASTM-1185-88 for hydroxy-apatite implants have been also taken into account. The lines for the elements present were also used for their quantifications because they were free of spectral interferences from the other impurities. Table 1 gives their wavelengths, in addition to those for the AAS signals for As and Hg.

Limit of determination (C_D)

The C_D values[21] for the elements studied were taken to be five times their respective limits of detection (C_L). These in turn were considered to be twice the standard deviation σ for the background signal produced by the pure main element (Ca) in the pure solvent (matrix).

Table 1 Analytical lines used in the ICP-AES and AAS determinations

Element	Wavelength/nm	Element	Wavelength/nm	Element	Wavelength/nm
Ag^{I}	328.068	Cr^{II}	267.716	Ni^{II}	231.604
Al^{I}	237.324	Cu^{I}	324.754	P^{I}	214.914
As^{I*}	193.696	Fe^{I}	271.441	Pb^{II†}	220.353
B^{I}	249.678	Hg^{I*}	253.652	Sn^{I}	270.651
Ba^{II}	493.409	K^{I}	766.491	Ti^{II}	336,121
Cd^{I†}	228.802	La^{II}	379.478	W^{II}	207.911
Ce^{II}	413.765	Mg^{II}	279.553	Y^{II}	371.030
Co^{II}	228.616	Mn^{II}	294.920	Zr^{II}	343.823

* Measured by CVAAS and HGAAS. † Not detected, but included in ASTM F-1185-88.

Table 2 Smallest determinable concentrations of the elements studied, both in solution and in the solid

Element	Solution/ng ml^{-1}	Solid (ppm)	Element	Solution/ng ml^{-1}	Solid (ppm)	Element	Solution/ng ml^{-1}	Solid (ppm)
Ag	40	4	Cr	50	5	Ni	50	5
Al	10	1	Cu	50	5	P	900	90
As	5	1	Fe	20	2	Pb	50	5
B	30	3	Hg	100	2	Sn	100	10
Ba	10	1	K	1000	100	Ti	50	5
Cd	5	0.5	La	10	1	W	50	5
Ce	20	2	Mg	2	0.2	Y	10	1
Co	25	2.5	Mn	50	5	Zt	30	3

The background noise produced by Ca and an adverse effect on the C_L for all the impurities was determined by ICP-AES. This was not the case with AAS, where the preliminary separation of arsenic as a hydride and of mercury as cold vapour considerably decreased background noise.

Table 2 shows the C_D values obtained, referring to solution and solid samples.

Determination of impurities

The analytical chemical results obtained for the two pseudo-wollastonites are shown in Table 3. Samples were analysed in quintuplicate by ICP-AES and in triplicate by CVAAS and HGAAS. The results are the averages of each replicate series.

As can be seen from Table 3, neither material contained As, Cd, Hg or Pb concentrations exceeding the levels established in ASTM F-1185-88 for ceramic hydroxyapatite for surgical implants. Also, the electrofused material contained much larger amounts of impurities than the sintered material.

On the other hand, the results also inform of the potential origin of the impurities. Thus, while both materials were ground in Teflon ball-mill attritors (see Scheme 1), the electrofused material was harder than the sintered material as zirconia–magnesia balls were softer than zirconia–yttria balls. Both conditions are consistent with a higher degree of Zr contamination of the electrofused material.

The presence of Y in the sintered material arose from the fact that it was ground with zirconia–yttria balls, the Y_2O_3 content in which typically ranges from 3.5 to 13.5%. The presence of W in the electrofused material was the obvious result of one of the grinding steps involving the use of a WC percussion hammer mortar. Cobalt Ni and Cr are constituents of W alloys in WC mortars, so they were logically present in the electrofused pseudowollastonite. Boron and Ag originated from the graphite electrodes of the arc use in the electrofusion process.

Finally, all other impurities encountered are not related to the processing and grinding operations and originated from the raw materials used. In this respect the following findings warrant special comment:

(*a*) The $CaCO_3$ and SiO_2 used to obtain the electrofused pseudowollastonite were less pure than those employed in its sintered counterpart.

(*b*) Barium was an exception to the previous rule because it occurred at a higher concentration in the sintered material as the likely result of the $CaCO_3$ being obtained by precipitation, where Ba is a commonplace impurity.
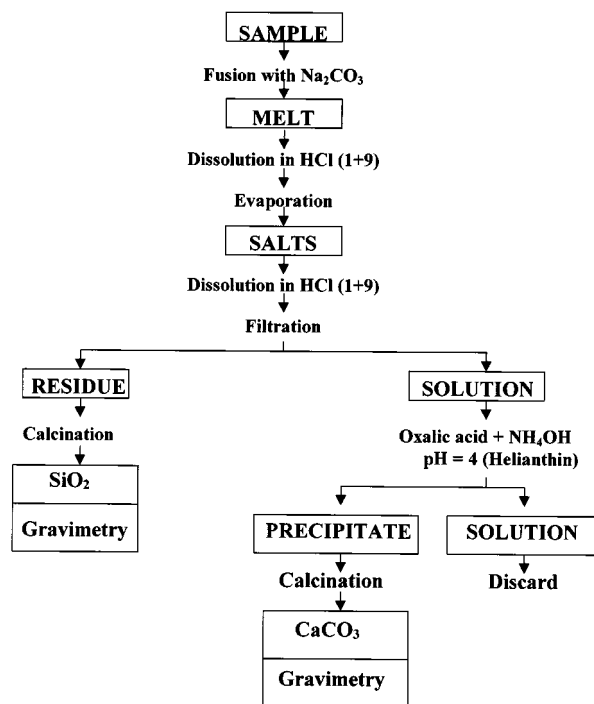
(*c*) The Mg content in the electrofused material, too high to be exclusively ascribed to contamination through grinding with zirconia–magnesia balls, suggests that dolomite (Mg, Ca) CO_3 must have been present as an impurity of $CaCO_3$ raw material.

(*d*) The Al, Fe, Ti, Mn, K and P contents obtained are consistent with those usually found in the two raw materials studied.

Determination of CaO and SiO₂

The selected ICP-AES lines for the analytes and internal standard were as follows: Ca^{II}, 317.933 nm; Si^I 251.611 nm; Y^{II}, 371.030 nm. All three were interference free.

The results obtained for the two samples were compared with those provided by the usual laboratory procedure (see Scheme 2). Table 4 gives both sets of results and their precision as relative standards deviations (RSD) for five replicates of each sample. The high consistency between the results and also the high precision of the method validate it for the determination of CaO and SiO_2 by ICP-AES.

Table 3 Impurity contents (ppm) in the two types of pseudowollastonite. (Concentration ranges correspond to the standard deviation for the five sample replicates)

Element	psW-S	psW-M	Element	psW-S	psW-M
Ag	<4	8 ± 4	K	210 ± 50	300 ± 50
Al	1200 ± 100	1600 ± 100	Lu	<1	5 ± 2
As	<1	<1	Mg	175 ± 21	3500 ± 200
B	6 ± 2	80 ± 9	Mn	70 ± 5	160 ± 11
Ba	164 ± 12	18 ± 3	Ni	<5	51 ± 4
Cd	<0.5	<0.5	P	110 ± 8	104 ± 6
Ce	5 ± 2	15 ± 3	Pb	<5	<5
Co	<2.5	8 ± 2	Sn	<10	15 ± 4
Cr	<5	28 ± 6	Ti	36 ± 6	55 ± 6
Cu	7 ± 2	38 ± 3	W	<5	80 ± 5
Fe	125 ± 20	650 ± 50	Y	30 ± 5	<1
Hg	<2	<2	Zr	250 ± 15	540 ± 40

Scheme 2 Procedure used for the conventional determination of SiO_2 and CaO in the pseudowollastonties.

Table 4 Analytical results for macroconstituents in the two types of pseudowollastonite

	psW-S		psW-M	
	Gravimetry	ICP-OES	Gravimetry	ICP-OES
Material (oxide)	m (%) ± RSD	m (%) ± RSD	m (%) ± RSD	m (%) ± RSD
SiO_2	51.30 ± 0.13	51.49 ± 0.27	56.26 ± 0.14	56.20 ± 0.29
CaO	47.80 ± 0.20	47.70 ± 0.40	42.43 ± 0.23	42.21 ± 0.41
SiO_2/CaO (theoretical = 1.071)	1.073	1.079	1.326	1.335

Conclusion

The results obtained allow the following conclusions to be drawn:

(*a*) The electrofused pseudowollastonite contains more impurities than its sintered counterpart (0.73% *vs*. 0.24%).

(*b*) The presence of Zr, Y, W, Co, Ni, Cr and Mg can largely be ascribed to the prolonged grinding required to ensure adequate grain fineness in the material. On the other hand, Zr, Y and Mg come from the attrition mills, where the grinding bodies were zirconia balls partly stabilized with yttria and magnesia respectively. Also, W came from the WC mortar, which include Co, Ni and Cr in their alloys.

(*c*) The proposed ICP-AES methodology allows one to analyse for Pb and Cd at the concentration levels established by ASTM F-1185-88 (5 ppm for Cd and 30 ppm for Pb). This is not the case with Hg and As, the maximum allowed levels for which (5 and 3 ppm, respectively, are not afforded by this technique and require the use of CVAAS and HGAAS, respectively.

(*d*) Due to the fact that there were no electrofused or sintered pseudowollastonite standard samples, we had recourse to an attack process previously used in other silicate-based materials,[22] which does not produce any loss of volatile elements. This and the absence of spectral interferences in the selected lines in ICP-AES guarantee reliable results in the analysis of the impurities.

(*e*) The precision obtained in the ICP-AES determination of SiO_2 and CaO is comparable to that afforded by gravimetric procedures. The excellent result obtained in this respect can be ascribed to: (*i*) the flux ($Li_2B_4O_7$) and graphite crucible used to disaggregate samples, which ensured through solubilization of silica; and (*ii*) the use of a Y internal standard.

(*f*) The microstructures observed and the presence of very small amounts of highly distorted cristobalite in the psW-M sample are consistent with the SiO_2/CaO ratios obtained and the procedures used to produce the materials studied (electrofusion and sintering). In addition, the higher impurity content in the psW-M sample is consistent with its higher content in vitreous phase.

(*g*) Based on the results, both materials can be used for bone implants. In this respect, even that with the higher impurity content exhibited no adverse reaction to SBF, rather, the reactivity of both materials was similar.

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