A new route to sol-gel crystalline wollastonite bioceramic

Luqman A. Adams*, Enobong Reginald Essien and Elsie Effah Kaufmann

*Department of Chemistry, University of Lagos, Lagos, Nigeria; †Department of Chemical and Food Sciences, Bells University of Technology, Ota, Nigeria; ‡Department of Biomedical Engineering, School of Engineering Sciences, University of Ghana, Legon, Ghana

ABSTRACT
Artificial bone graft materials formed from wollastonite have been extensively used in bone repair because of their high degree of bioactivity and biocompatibility, thereby justifying the development of a protocol for large-scale production. This work reports a novel route for preparing wollastonite via the sol-gel process using bentonite clay as a cheap silica source. The obtained wollastonite was characterized for morphology, elemental composition, phase composition and bioactivity using scanning electron microscopy, energy dispersive X-ray analysis, X-ray diffraction and Fourier transform infrared spectroscopy. Results obtained revealed that wollastonite phase was successfully formed in the material and it showed ability to induce formation of apatite within 0.5 day in biological fluid, an indicator for bone-bonding capability. Overall, the wollastonite prepared from the bentonite clay exhibited properties comparable to that synthesized from commercially obtained sodium metasilicate. Hence, our synthetic route may be useful for commercial-scale preparation of wollastonite.

1. Introduction
Over the past two decades, artificial bone implant materials formed from wollastonite (CaSiO$_3$) have been extensively applied in the field of orthopedics as graft substitutes to treat bone defects [1–5]. Interest in CaSiO$_3$ ceramics stems from studies showing that they promote osteoblasts attachment, proliferation and differentiation [2,6–8]. Furthermore, CaSiO$_3$-based glasses induced the formation of apatite when immersed in simulated body fluid (SBF), at a rate higher than other bioglass and glass-ceramics [9,10].

Formation of apatite is considered essential for synthetic grafts to bond to their hosts in vivo [11,12]. The high degree of bioactivity of silicate-based glasses, generally, has been attributed to the release of critical concentrations of soluble silica and calcium ions when degrading in biological fluids [13]. Bioactivity is determined not only by the composition but also by other factors, such as the size and shape of the material, as well as the preparation method.

The sol-gel processing technique permits the preparation of glasses with high-specific surface area and pore volume compared with glasses produced by conventional melting process. The sol-gel route involves controlled hydrolysis and condensation of silicon alkoxides or metal salts to form a suspension of colloidal particles (sol), which further undergoes polycondensation to form a gel, which is an interconnected network structure. A high silica-rich surface enhances biocompatibility and bioactivity by increasing the rate of hydroxyapatite formation [5].

The synthetic route to sol-gel-derived wollastonite often involves the use of the conventional silica precursors: tetraethyl orthosilicate (TEOS) and sodium silicate (Na$_2$SiO$_3$). Lakshmi and Sasikumar, 2015 [13] studied the bioactivity of needle-like wollastonite prepared from TEOS. Xie et al. [14] prepared CaSiO$_3$ composite coated with graphene plates to improve the mechanical properties of the CaSiO$_3$ synthesized from TEOS as silica source. Lin et al. [15] fabricated and characterized 45S5/CaSiO$_3$ macroporous bioceramics by preparing the CaSiO$_3$ from Na$_2$SiO$_3$. Papynov et al. [16] synthesized highly porous wollastonite ceramic materials with immobilized Au-NPs by employing sodium metasilicate (Na$_2$SiO$_3$·5H$_2$O) as silica source.

The current study is devoted to developing a new commercial-scale route for synthesizing high-quality wollastonite. Specifically, this work aims to study the possibility of preparing wollastonite from bentonite clay as a cheap and novel starting material. Thereafter, the properties of the wollastonite will be compared against results obtained from using sodium metasilicate as a conventional precursor.

2. Materials and methods

2.1. Materials
The material used for synthesizing the wollastonite were as follows: bentonite clay (obtained locally from
Nigeria); sodium hydroxide (NaOH), sodium metasilicate (Na$_2$SiO$_3$.9H$_2$O) (Sigma-Aldrich); and calcium nitrate tetrahydrate Ca(NO)$_3$.4H$_2$O (LOBA Chemie).

2.2. Preparation of wollastonite from sodium metasilicate (WoNS)

The wollastonite (CaO.SiO$_2$) was prepared by first dissolving 5.00 g of the Na$_2$SiO$_3$.9H$_2$O in deionized water under magnetic stirring condition at room temperature. Thereafter, 4.17 g of Ca(NO)$_3$.4H$_2$O was added gradually to the mixture over 45 min with stirring. Then, the mixture was allowed a reaction time of 2 h after completing the addition. The gel formed was washed five times in 400 ml deionized water to get rid of NaNO$_3$, the by-product of the hydrolysis process before filtering by suction. Afterward, it was dried at 100°C for 42 h and heated at 700°C for 2 h. The equation for the reaction is shown in Equation 1.

$$\text{Na}_2\text{SiO}_3(\text{aq})+\text{Ca(NO}_3)_2.4\text{H}_2\text{O(s)} \rightarrow \text{CaSiO}_3(s)+2\text{NaNO}_3(\text{aq})+4\text{H}_2\text{O(l)}$$

(1)

2.3. Preparation of wollastonite from bentonite clay (WoBC)

SiO$_2$ was extracted from the bentonite clay to form Na$_2$SiO$_3$ by boiling with 1 M NaOH (270 ml) at 120°C for 2 h, and for the reaction equation, see ref [17]. After allowing to cool, the mixture was filtered, and then Ca(NO)$_3$.4H$_2$O was added to the filtrate gradually with stirring at room temperature for 2 h. The gel obtained was washed, dried and calcined in a similar procedure to that reported in Section 2.2. A summary of the process is depicted in the flowchart shown in Figure 1.

2.4. Characterization

The microstructure of the samples was evaluated in a scanning electron microscope (SEM; JEOL JSM 6390LV, Tokyo, Japan), while elemental analyses were performed by energy dispersive X-ray (EDX) from an SEM machine (SEM: Phenom ProX, The Netherlands) having a back-scattered electron detector operated at 15 kV.

The diffraction patterns of the samples before and after immersion experiment in SBF were obtained at a step size of 0.1° and a scan time of 1 s per step with a Cu Ka radiation operating source of wavelength ($\lambda$) = 0.154060 nm at 45 kV, 40 mA in the 2$\theta$ range from 5° to 90° from an X-ray diffractometer (XRD; PANalytical EMPYREAN Series 2, Almelo, The Netherlands). The strongest diffraction peak (2$\theta = 30^\circ$) was used to calculate the crystallite size of the main phase in the crystalline sample (wollastonite – CaSiO$_3$) according to the Scherrer equation:

$$\varsigma = \left(\frac{k \cdot \lambda}{\beta \cdot \cos \theta}\right)$$

(2)

where $\varsigma$ is the crystallite size, $k$ the Scherrer constant, equal to 0.89, $\lambda$ the wavelength of the Cu Ka X-ray (1.5406 x 10$^{-10}$ m) and $\beta$ the full width at half maximum (FWHM) of the strongest diffraction peak.

Assessment of the nature of bonds present in the glass network and confirmation of apatite formation on the surface of the samples were performed using

![Figure 1. Flowchart for preparing the wollastonite bioceramic from bentonite clay.](image-url)
Fourier transform infrared spectroscopy (FTIR) with attenuated total reflectance (Bruker-Alpha, Platinum ATR) operating in the wavenumber range of 4000–500 cm\(^{-1}\).

2.5. In vitro bioactivity determination in simulated body fluid (SBF)

Bioactivity tests to form apatite on the materials were conducted by soaking the samples in SBF (pH 7.4) at 36.5°C according to the standard in vitro procedure proposed by Kokubo and Takadama [18] using the following analytical grade reagents: NaCl, NaHCO\(_3\), KCl, K\(_2\)HPO\(_4\)·3H\(_2\)O, MgCl\(_2\)·6H\(_2\)O, CaCl\(_2\), trishydroxymethyl aminomethane [Tris-buffer, (CH\(_2\)OH):3CNH\(_2\)] and 1 M HCl to obtain a similar ionic concentration compared with human blood plasma [19]. Samples were immersed in SBF solution in clean sterilized plastic bottles at a concentration of 0.0022 g/ml and placed in an incubator for a maximum of 7 days. After removal of the samples from the SBF, they were rinsed with deionized water, and then further washed in 100% ethanol and left to dry at ambient temperature for 1 day.

3. Results and discussion

3.1. Morphology

The surface morphology and EDX spectra of WoNS and WoBC before immersion in SBF are depicted in Figure 2. The microstructures of both samples are rough and porous, but the surface of WoBC (Figure 2(b)) appears fractured when compared to the more compact WoNS (Figure 2(b)). It is possible that the fracture was caused by particle deformation during the heat treatment process. Surface roughness is considered necessary in a biomaterial serving as an artificial graft to enhance cell and protein adsorption, while porous structure is useful for cell infiltration, nutrient delivery and waste removal [19]. The EDX spectra confirm the elemental composition of wollastonite in both samples and also validate the possibility of forming wollastonite from bentonite clay. Furthermore, it is worthy to note that Na was absent in both spectra, which is attributable to the efficiency of the gel-washing procedure to remove the NaNO\(_3\) by-product (Equation (1)).

The SEM/EDX micrographs of the samples after immersion in SBF for 0.5, 1, 3, 5 and 7 days are shown in Figure 3. As observed, after immersion for 0.5 day only few apatite balls emerged on the surface of WoNS, Figure 3(a). In addition, apatite concentration increased slightly after 1 day (Figure 3(b)) and continued to increase as immersion duration increased to 3, 5 and 7 days in SBF, Figure 3(c–e), respectively. However, the apatite-forming pattern of WoBC after immersion for the same period (Figure 3(f–j)) is slightly different. After the initial 0.5-day immersion, the surface appears to be dominated by agglomerated apatite crystallites, which also increased with immersion duration, Figure (g–j). Apatite formation is supported by the appearance of P and increase in Ca intensity in the EDX spectra of both samples as incubation period in SBF increased. At the same time, Si decreases in intensity in the spectra of both samples, signifying increase in apatite density on the surface of the glass samples or degradation of the samples in SBF, thus leading to low detection of Si by EDX.

3.2. Diffraction patterns

The XRD patterns of the sintered samples are presented in Figure 4. The only peaks identified in both samples matched wollastonite [20] when indexed using the standard PDF (JCPDS #00–043-1460) in angular positions and reflection indices. It is important to point out that no intense peaks were observed beyond 2\(\theta\) 42°, even the prominent peak usually present around 2\(\theta\) 50° in the diffractogram of wollastonite, appeared diffusive. This could be attributed to the low sintering condition of 700°C for 2 h, which was adopted in order to prevent crystallization of the glass. Under this condition, the material could only attain a short-range ordering of the silicate in its structure, thus resulting in peaks with very low

Figure 2. SEM and EDX spectra of (a) WoNS and (b) WoBC wollastonite samples before immersion in SBF.
intensities, especially at high diffraction angles. This speculation can further be advanced by considering the result of a previous synthesis of wollastonite \[ \text{CaSiO}_3 \], where a peak comparable in intensity to the one near \( 2\theta \, 28^\circ \) (202) \( \text{(Figure 4)} \) was obtained around \( 2\theta \, 50^\circ \) when the sample was heated at 900°C for 5 h.

According to Equation (2), the average size of the \( \text{CaSiO}_3 \) crystallites in WoNS was 53.30 nm, while that
in WoBC was 24.60 nm. The larger crystallite size of WoNS implies that it experienced a higher degree of crystallization than WoBC as evident in the XRD diffractograms, which shows that WoNS exhibited sharper diffraction peaks than WoBC. Nonetheless, both samples presented a similar diffraction pattern.

Figure 5 shows the diffraction patterns of WoNS and WoBC after immersion in SBF for 0.5, 1, 3, 5 and 7 days. The angular location and the reflection indices of the new peaks in both samples correspond to apatite phase according to standard reference files ([21] and JCPDS PDF#9–0432). As the period of immersion of both samples in SBF increased, the apatite peaks increased, whereas the wollastonite peaks decreased in intensity due to increase in concentration of apatite on their surface and degradation of the silicate peaks, respectively. These observations agree with the results of the SEM and EDX (Figure 3). However, the number of apatite peaks in WoNS (Figure 5(a)) slightly outnumbered those in WoBC (Figure 5(b)). Overall, it is gratifying to observe that the growth of apatite on the surface of the bentonite clay-derived wollastonite exhibited a similar pattern to those from commercial sodium metasilicate. Apatite formation on the surface of a material is considered crucial for bone-bonding reactions to occur [11,12,22]. Furthermore, degradability of a bioactive glass in a biological fluid is an important requirement for a material to serve as a temporary scaffold for bone regeneration [5].

3.3. FTIR confirmation of phase and bioactivity

The FTIR result of WoNS at 0 day (Figure 6(a)) shows peaks located at, 1010, 931, 682 and 644 cm\(^{-1}\). The peak at 1010 cm\(^{-1}\) is considered as the asymmetric stretching vibration of Si–O–Si bonds [23], while the peak at 931 cm\(^{-1}\) is attributed to its symmetric stretching mode. The peaks at 682 and 644 cm\(^{-1}\) are due to the vibration of Si–O bonds in silicate tetrahedra, all of which are sharp, suggesting crystalline nature. The spectrum of WoBC (Figure 6(b)) reveals a prominent peak at 1407 cm\(^{-1}\), associated with the presence of CO\(_3^2-\). This is supported by a C–O deformation mode, which appears as a sharp peak near 873 cm\(^{-1}\). The presence of carbonate group in the sample could be due to absorption of atmospheric CO\(_2\) by NaOH [24] during the extraction of SiO\(_2\) from the bentonite clay. The presence of silicate in WoBC sample is confirmed by the peaks at 970 cm\(^{-1}\) (asymmetric

![Figure 5. XRD patterns of (a) WoNS and (b) WoBC after immersion in SBF for 0.5, 1, 3, 5 and 7 days showing the formation of apatite.](image-url)
stretching vibration of Si–O–Si) and 712 cm$^{-1}$ (silicate tetrahedron) (Figure 6(b)). The silicate peaks in WoBC are less intense than in WoNS, implying that WoBC was more amorphous than WoNS, as observed earlier in the XRD result (Figure 4).

After incubation of WoNS for 0.5 days in SBF (Figure 7(a)), no significant changes in vibrational mode are observed, except the emergence of a small band around 1630 cm$^{-1}$ considered to be due to OH group of absorbed water by the sample. After 1–3 days in SBF, a new peak developed at 565 cm$^{-1}$, which is characteristic of P–O bending mode in PO$_4^{3-}$ [25]. A similar trend is observed in WoBC (Figure 7(b)), but after 3 days in SBF, the PO$_4^{3-}$ group is observed at 563 and 602 cm$^{-1}$. These features confirm the formation of hydroxyapatite on the surface of the samples as identified earlier using SEM/EDX and XRD. With extension in incubation period in SBF to 5 and 7 days, the phosphate peaks in both samples become more intense due to increase in apatite concentration on their surface, while the silicate peaks broaden and become less intense due to degradation of the materials in SBF.

4. Conclusion

Bioactive wollastonite glass samples were prepared from commercial sodium metasilicate and bentonite clay. The purpose was to develop a protocol for low-cost commercial production of wollastonite. Consequently, the performance of the wollastonite
prepared from bentonite clay was compared with that formed from the commercial sodium metasilicate. Analyses showed that wollastonite phase was successfully formed on both samples, and apatite began to grow on their surface within 0.5 day of immersion in SBF. In general, the wollastonite obtained from bentonite clay exhibited properties comparable to the one prepared from commercial sodium metasilicate, judging from its composition, morphology, diffraction patterns and, most importantly, bone-bonding ability. Hence, bentonite clay, which is cheap and widely available, has the potential for use as a convenient source of silica for preparing wollastonite ceramics for application in bone repair.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Enobong Reginald Essien https://orcid.org/0000-0003-2379-3640

References

[1] Idaszek J, Zinn M, Obarazanek-Fojt M, et al. Tailored degradation of biocompatible poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/calcium silicate/poly(lactic-co-glycolide) ternary composites: an in vitro study. Mater Sci Eng C Mater Biol Appl. 2013;33(7):4352–4360.

[2] Fei L, Wang C, Xue Y, et al. Osteogenic differentiation of osteoblasts induced by calcium silicate and calcium silicate/β-tricalcium phosphate composite bioceramics. J Biomed Mater Res B Appl Biomater. 2012;100(5):1237–1244.

[3] Ni S, Chang J, Chou L. A novel bioactive porous CaSiO₃ scaffold for bone tissue engineering. J Biomed Mater Res A. 2006;76(1):196–205.

[4] Rezqhat H, Ramasamy W, Wu C, et al. The incorporation of strontium and zinc into a calcium-silicon ceramic for bone tissue engineering. Biomaterials. 2010;31(12):3175–3184.

[5] Xynos ID, Edgar AJ, Butterly LD, et al. Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass 45S5 dissolution. J Biomed Mater Res. 2001;55(2):151–157.

[6] Ni S, Chang J, Chou L, et al. Comparison of osteoblast-like cell responses to calcium silicate and tricalcium phosphate ceramics in vitro. J Biomed Mater Res B Appl Biomater. 2007;80(1):174–183.

[7] Saravanan S, Vimalraj S, Vairamani M, et al. Role of mesoporous wollastonite (calcium silicate) in mesenchymal stem cell proliferation and osteoblast differentiation: a cellular and molecular study. J Biomed Nanotechnol. 2015;11(7):1124–1138.

[8] Hung CJ, Hsu HI, Lin CC, et al. The role of integrin αv in proliferation and differentiation of human dental pulp cell response to calcium silicate cement. J Endod. 2014;40(11):1802–1809.

[9] De Aza PN, Guitian F, De Aza S. Bioactivity of wollastonite ceramics: in vitro evaluation. Scr Metall Mater. 1994;31(8):1001–1005.

[10] De Aza PN, Luklinska Z, Anseau MR, et al. Morphological studies of pseudowollastonite for biomedical application. J Microsc. 1996;182(1):24–31.

[11] Balas F, Kawashita M, Nakamura T, et al. Formation of bone-like apatite on organic polymers treated with a silane-coupling agent and a titania solution. Biomaterials. 2006;27(9):1704–1710.

[12] Hench LL. The story of bioglass. J Mater Sci Mater Med. 2006;17(11):967–978.

[13] Lakshmi R, Sasikumar S. Influence of needle-like morphology on the bioactivity of nanocrystalline wollastonite – an in vitro study. Int J Nanomedicine. 2015;10(Suppl 1):129–136.

[14] Xie Y, Li H, Ding C, et al. Effects of graphene plates’ adoption on the microstructure, mechanical properties, and in vivo biocompatibility of calcium silicate coating. Int J Nanomedicine. 2015;10:3855–3863.

[15] Lin K, Chang J, Liu Z, et al. Fabrication and characterization of 45S5 bioglass reinforced macroporous calcium silicate bioceramics. J Eur Ceram Soc. 2009;29(14):2937–2943.

[16] Papynov EK, Shichalin OO, Mayourov YV, et al. Sol-gel and SPS combined synthesis of highly porous wollastonite ceramic materials with immobilized Au-NPs. Ceram Int. 2017;43(11):8509–8516.

[17] Essien ER, Olaniji OA, Adams LA, et al. Sol-gel-derived porous silica: economic synthesis and characterization. J Minerals Mater Characterization Eng. 2012;11(10):976–981.

[18] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials. 2006;27(15):2907–2915.

[19] Hench LL, Polak JM. Third-generation biomedical materials. Science. 2002;295(5557):1014–1017.

[20] Kanazaki M, Stebbins JF, Xue X. Characterization of 45S5 bioglass reinforced macroporous calcium silicate bioceramics. J Eur Ceram Soc. 2015;35(24):6382–6388.

[21] Ulian G, Valdrè G, Corno M, et al. The vibrational spectroscopy of TEOS to silica gel and glass by vibrational spectroscopy. Geophys Res Lett. 1992;19(4):752–759.

[22] Hughes JM. The many facets of apatite. Am Mineral. 2015;100(3–6):1033–1039.

[23] Matos MC, Ilharco LM, Almeida RM. The evolution of TEOS to silica gel and glass by vibrational spectroscopy. J Non-Cryst Solids. 1992;147:232–237.

[24] Cerrutti M, Morterra C. Carbonation formation on bioactive glasses. Langmuir. 2004;20(15):6382–6388.

[25] Oliveira JM, Correia RN, Fernandes MH. Effects of Si speciation on the in vitro bioactivity of glasses. Biomaterials. 2002;23(2):371–379.