DISSOLUTION BEHAVIOR OF BIOACTIVE GLASS CERAMICS WITH DIFFERENT CaO/MgO RATIOS

MUHAMMAD USMAN HASHMI, SAQLAIN ABBAS SHAH, SHAHZAD ALAM*, AHMAD SHAMIM

Physics Department, Government College University Lahore 54000, Pakistan
**PCSIR, Lahore 54600, Pakistan*

E-mail: usmanhashmi06@hotmail.com

Submitted Aug 30, 2009; accepted Feb 23, 2010

Keywords: Oxyacetylene Flame, Dissolution behavior, Atomic Absorption Spectroscopy, MgHydroxycarbonate apatite (Heneuite)

In this work, powders of three different compositions, each having 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF (% wt) and ratio of CaO/MgO varying from 11.5:1 to 1:11.5 were thoroughly mixed and melted under oxy-acetylene flame in a fire clay crucible that made the glass formation cheaper in time and cost. The melt of each composition was quenched in water to form three different glasses. Every glass was sintered at 950°C to form three glass ceramics named G1, G2 and G3 respectively. To study the dissolution behavior, each sample was immersed in a simulated body fluid (SBF) for 2, 5, 10, 20 and 25 days at room temperature. Thin film XRD analysis revealed that the samples with larger CaO/MgO ratio exhibited better bioactivity. pH of SBF increased efficiently in case of G1 whereas in case of G2 and G3, this increase was slower due to greater amount of MgO. The concentrations of Ca, P, Mg and Si ions were measured by Atomic Absorption Spectroscopy. EDS analysis showed the increase in P and Ca ions and presence of C in G1 after 5 days immersion and after 10 days, in case of G2 indicating the higher formation rate of hydroxycarbonate Apatite layer in G1 as compared to G2 due to greater CaO/MgO ratio whereas in G3 Mg-hydroxycarbonate apatite ($Ca(Mg)_5(CO_3)(PO_4)_3(OH)$) (heneuite) layer was recognized after 20 days showing the least bioactivity due to very large amount of Mg and the least CaO/MgO ratio.

INTRODUCTION

The materials that can withstand and perform their task properly within the living body without any harmful effects on surroundings are known as bio-materials [1]. It is vital to observe the effects of bio-material on surrounding tissues and the effects of surrounding tissues on the material [1]. Depending on these interactions between tissues and material, bio-materials are classified into two categories, first, in which the materials don't form bonds with surrounding tissues (bio-inert) and second, that can develop interfacial bonds with the host tissues (bio-active), the bio-active materials may be resorbable or non-resorbable [2]. It is notable that bio-activity is not only the property of material but it also depends on the solution in which material is to be immersed for *in-vitro* test [3] that is a very proficient technique to check the bio-activity *in-vivo* [4-6]. Jonasova et al [7] investigated a broad range of SBFs inclusive of the one having similar ionic composition as that of the human blood plasma containing even the same amounts of chloride and HCO^{3-} ions. But the experimental data [8-15] suggests that Kokubo's SBF-K9 (Table 1) is still the most favorable and widely used solution for the in-vitro examination owing to its efficient response while in interaction with the bioactive material. A basic feature of bio-active materials is the formation of bonelike hydroxycarbonate apatite layer (HCAp) on their surface in-vitro when the temperature, pH and ionic composition of the fluid are nearly equal to that of blood plasma [13]. The dissolution of bio-active glass in the *in-vitro* environment undergoes release of ions and the formation of silanol group on its surface that acts as a nucleating agent for HCAp layer [16,17]. It has been found that the glass-ceramics containing P_2O_5 exhibits quick formation of apatite layer on its surface as compared to glass-ceramics without P_2O_5 [18], however its presence lowers the mechanical strength [19]. In the later research, it has also been investigated that bio-activity of the glass in the *in-vitro* environment increases with increasing Na_2O/MgO ratio in the Na_2O–MgO–SiO_2 glasses [19].

Table 1. The comparison of ion concentrations of SBF and Human Blood Plasma (mmol/l).

	Na^+	Ca^+	K^+	Mg^{2+}	Cl^-	HCO_3^{2-}	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	2.5	5.0	1.5	147.8	4.2	1.0	0.5
Blood Plasma	142.0	2.5	5.0	1.5	103.8	27.0	1.0	0.5

Some researchers show the excellent properties of glasses having Mg [20-23]. Mg is one of the major substitutes for Ca in biological apatites and Mg-hydroxyapatite is expected to have outstanding biocompatibility and biological properties because 0.72 and 1.23 (wt %) of Mg is present in bone and dentin respectively [24]. It has also been reported that Mg is closely linked with mineralization of calcified tissues [24] and ultimately influences mineral metabolism [25]. The function of Mg is not fully understandable and has been suggested that Mg directly stimulates osteoblast production. Mg deficiency has been found to have harmful effects on skeletal metabolism [26], causing termination of bone development, lower bone quality, strength and thickness and enhance bone fergility [27]. Synthesis of Glasses with low silica amount (25-29 mol %) and high Mg contents (31-36 mol %) have also been reported, suggesting that more improvements at the surfaces and in the glasses can produce potentially attractive bioactive materials [28]. It is noteworthy that replacement of CaO by MgO in the composition of SiO_2 glasses would alter their stability and enhance the mechanical properties of the glass [29].

It is a known fact that CaO and MgO are network modifiers but in some reports the role of Mg has been documented with possibility of their role as network former [30-31]. Thus, it is very essential to illuminate the function of Mg in the glassy structures and in-vitro environment with replacement of CaO by MgO and the present investigation is an attempt to study the dissolution behavior of three glass ceramics samples each having 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF (% wt) and ratio of CaO/MgO varying from 11.5:1 to 1:11.5. Structural characterization of the leached surface of *in-vitro* test glass samples is carried out by thin film X-Ray Diffraction. The pH of the SBF is measured before and after immersion of glass samples for various time periods. Atomic absorption spectroscopy is used to study the concentrations of different ions in the SBF, before and after immersion of glass samples for various time periods. Compositional analysis of the leached surface was performed by EDS.

EXPERIMENTAL

Preparation of Samples

Powders of three different compositions, 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF- 46 CaO-4 MgO, 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF-25 CaO- 25 MgO and 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF-4 CaO-46 MgO (% wt) were thoroughly mixed and melted under oxy-acetylene flame in a fire clay crucible. The melt of each composition was quenched in water to form three different glasses. Every glass was powdered using agate mortar and pestle for several hours. 5 wt.% Polyvinyl Alcohol (PVA) was used as organic binder for compaction and powder was compacted under a pressure of 10 tons/cm^2 in a hydraulic press. The glass compacts were sintered at 950 °C for three hours to form glass ceramics, in a muffle furnace operating at the ramp rate of 5°C/min. The samples were maintained at 700°C for 1 hour for the creation of nucleation sites before sintering. The furnace was switched off at the end of each sintering process till the room temperature was achieved and samples were taken out.

The samples were named as G1, G2 and G3 respectively according to CaO/MgO ratios.

Preparation of SBF K-9

To study the dissolution behavior of these samples in the *in-vitro* environment, Kokubo's SBF- K9 [32] was selected on account of its characteristics of rapid response on interaction with bioactive materials and capacity to maintain its properties for long period of time.

For preparation of solution, NaCl, $NaHCO_3$, KCl, K_2HPO_4. $3H_2O$, $MgCl_2$. $6H_2O$, $CaCl_2$, Na_2SO_4 and $(CH_2OH)_3CNH_2$ respectively, were added in ultra pure water (in beaker) one by one by keeping it on a magnetic stirrer with heater at 37°C. In order to avoid increase in pH of solution, $(CH_2OH)_3CNH_2$ was added in the solution little by little. By keeping the electrodes of pH meter in the solution, pH was adjusted at 7.4 by HCl.

All the samples were put in the solution in different bottles and taken out after respective time periods and cleaned with distilled water and dried at 100°C for two and half hours.

Characterization

X- Ray diffraction patterns were obtained to identify the phase composition of G1, G2 and G3 before immersion in SBF using Panalytical X'pert PRO MPD θ-θ X-Ray Diffractometer.

The *in-vitro* test of bio-activity was carried out by immersing the glass ceramics samples in SBF (mass/vol = 0.002 gm/cm^3 at ambient temperature) for different time intervals.

Structural characterization of the leached surface of in-vitro test glass ceramics samples was performed by thin film X-Ray Diffraction (Panalytical X'pert PRO MPD θ-θ X-Ray Diffractometer) using Cu Kα1 radiation(λ=1.54056° A) by fixing the sample at an angle of 1° to the X-Ray beam.

The pH of the SBF was measured before and after immersion of glass samples for various time periods using the pH meter (PCSIR manufactured).

In order to study the dissolution behavior of the samples in detail, concentrations of different ions in the SBF, before and after immersion of glass samples for various time periods, were measured using Atomic Absorption Spectroscopy (180-80, Hitachi).

Compositional analysis of the leached surface was performed by EDS (S-3700N, Hitachi, Japan).

RESULTS AND DISCUSSION

XRD Analysis

Figure 1 shows the XRD patterns of G1, G2 and G3 before immersion in SBF showing their phase compositions. Figures 2, 3 and 4 show the thin film XRD patterns of surfaces of G1, G2 and G3 respectively after immersion in SBF for 2, 5, 10, 20 and 25 days. Figure 2 shows that HCAp phase appears on G1 after 5 days followed by increase in its intensity and appearance of its more phases in upcoming days indicating that G1 starts bond formation after 2 days as indicated by other characterization techniques. Figure 3 shows the appearance of HCAp after 10 days due to less CaO/MgO ratio whereas in G3 (Figure 4), Mg-hydroxycarbonate apatite (heneuite) phase appears after 20 days instead of HCAp due to a large amount of MgO in G3 and the least CaO/MgO ratio indicating that bio-activity of the glass is decreased due to greater amount of MgO in the composition that causes the resistance in calcium phosphate layer formation owing to higher Mg^{2+} ions [33].

pH Measurements

The variations in pH values are measured to understand the dissolution behavior of all the samples in the *in-vitro* environment. It is observed (Figure 5) that pH of SBF increases to a large extent (7.4 to 7.92) in case of G1 due to greater concentration of Ca^{2+} ions in G1 and its large exchange with H^+ or H_3O^+ ions in SBF [34-35] that results in the formation of HCAp after 2 days. Whereas in case of G2 and G3, pH of SBF increases but not so quickly that may be due to greater amount of Mg in these samples because pH drop depends on the amount of Mg.

The greater the amount of Mg, the lower the pH drop [36].

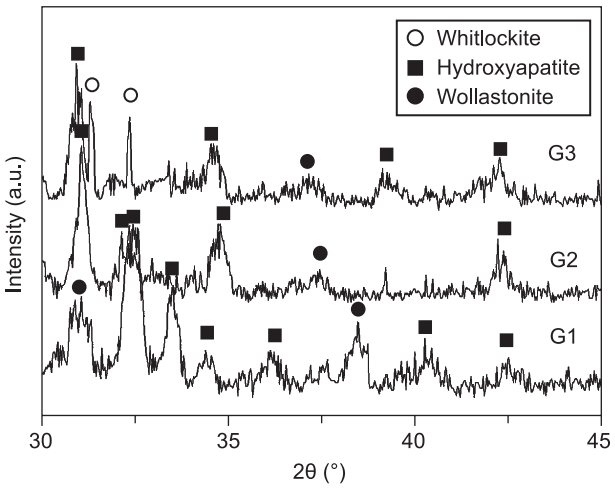

Figure 1. XRD pattern of G1, G2 and G3 before immersion in SBF.

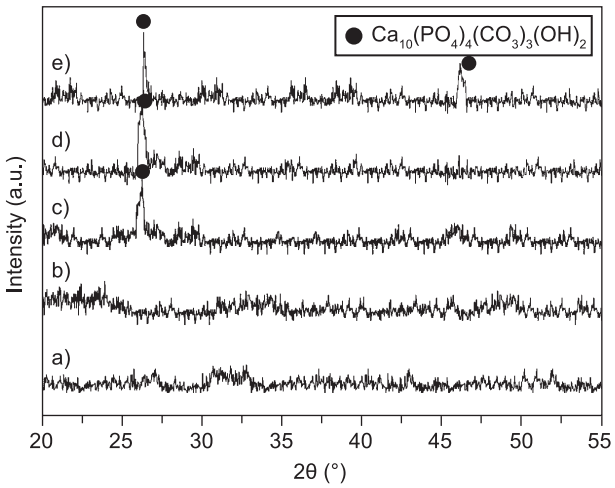

Figure 3. Thin Film pattern XRD of surface of G2 after different immersion periods: a) 2 days, b) 5 days, c) 10 days, d) 20 days, e) 25 days.

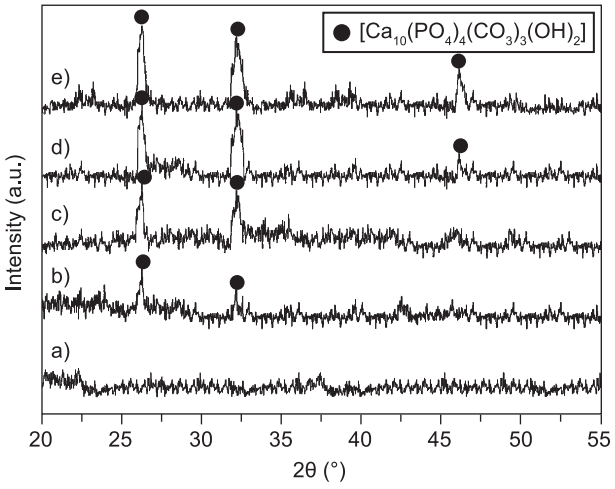

Figure 2. Thin Film XRD pattern of surface of G1 after different immersion periods: a) 2 days, b) 5 days, c) 10 days, d) 20 days, e) 25 days.

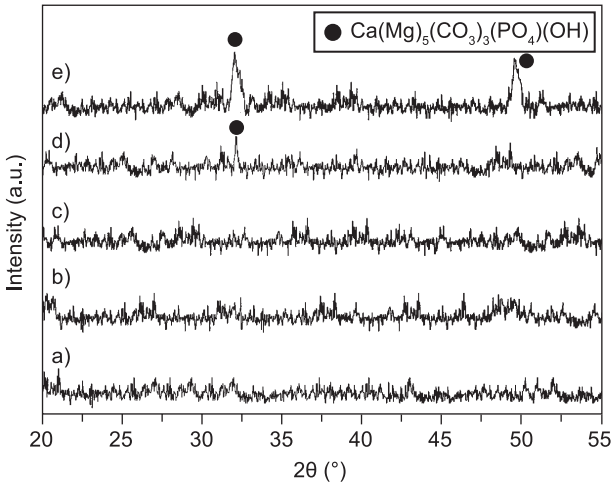

Figure 4. Thin Film XRD pattern of surface of G3 after different immersion periods: a) 2 days, b) 5 days, c) 10 days, d) 20 days, e) 25 days.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy was used to measure the concentrations of Ca, P, Si and Mg ions in SBF to study the dissolution behavior of samples in more detail.

It is found that in G1, concentration of Ca and Mg ions increases in SBF in first 2 days (Figure 6) that is attributed to exchange of Ca or Mg ions from G1 with H^+ or H_3O^+ ions from SBF and as a result, silanol group (Si__OH) is formed on the glass surface due to reaction of Si with OH^- ions that act as nucleation agent for HCAp formation. After 2 days, Concentration of Ca and P ions decrease continuously in SBF that may be attributed to transfer of these ions from SBF to the immersed sample showing the formation of HCAp layer on G1 as conformed by XRD. Concentration of Si greatly increases in SBF showing the high dissolution rate of part of Si from Si__OH group that helps in formation of calcium phosphate layer.

In case of G2, Ca and P ion concentration increases in SBF up to 5 days (Figure 7) and after that it starts decreasing gradually showing the formation of HCAp. This delay in HCAp formation may be attributed to greater amount of Mg in G2. Concentration of Si increases in G2 but not so quickly due to less CaO/MgO ratio.

In case of G3, the rate of exchange of ions between sample and solution is very slow (Figure 8) due to very large amount of Mg that resists the exchange of ions and thus the bone bonding mechanism.

EDS Analysis

Tables 2, 3 and 4 show the intensities of EDS spectrum for materials components of G1, G2 and G3 respectively, before and after immersion in SBF for different days. Table 2 shows that after immersion of G1 in SBF for 2 days, EDS illustrates the gradual decrease

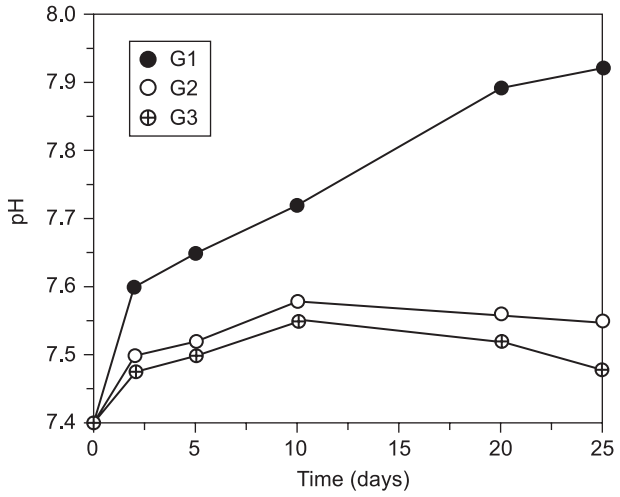

Figure 5. pH changes in SBF with different soaking times.

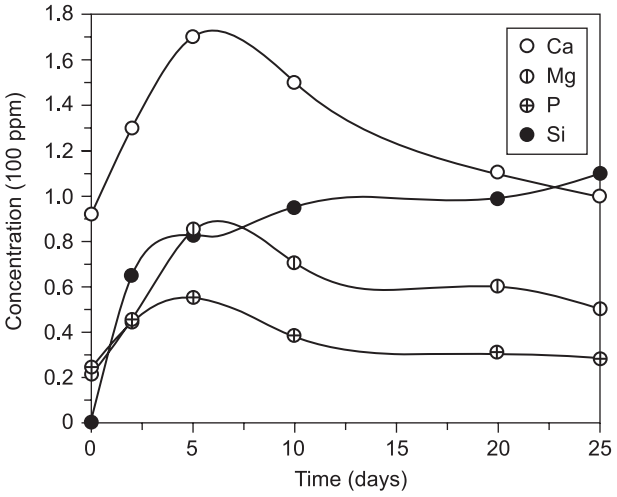

Figure 7. Variation of Ions concentration in SBF, in case of G2, with different soaking times.

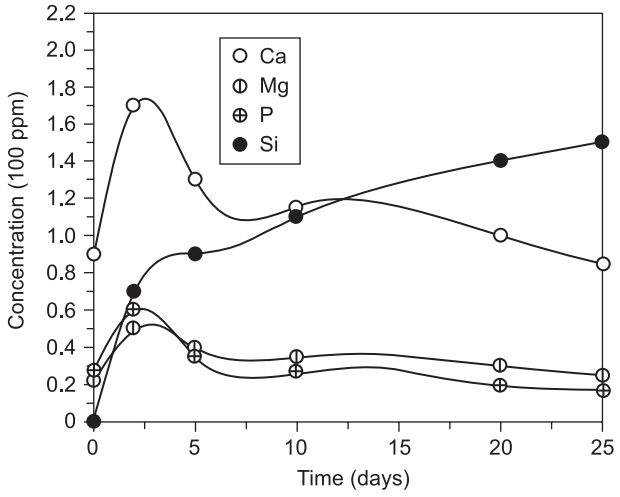

Figure 6. Variation of Ions concentration in SBF, in case of G1, with different soaking times.

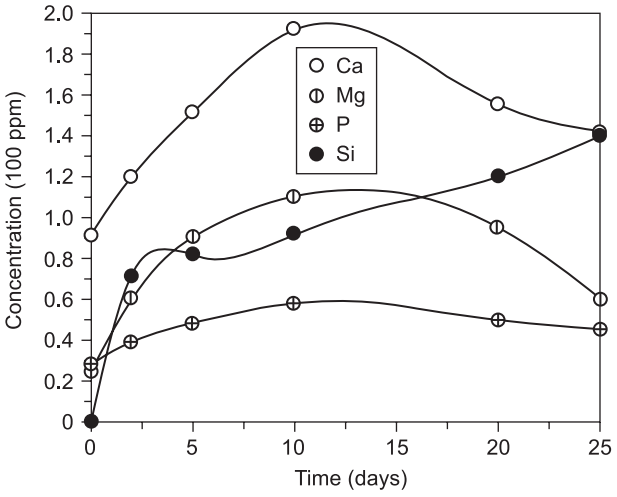

Figure 8. Variation of Ions concentration in SBF, in case of G3, with different soaking times.

in Ca and P ions from G1 due to their exchange with H^+ or H_3O^+ ions from SBF and after that, intensities of EDS spectrums show the continuous increase in P and Ca ions concentration and presence of C in G1 indicating the formation of HCAp layer as conformed by XRD and AAS analysis.

The intensities of EDS spectrums of G2 and G3 before and after immersion in SBF for 2, 5, 10, 20 and 25 days are shown in Tables 2 and 3 respectively. These tables show that in case of G2, concentration of Ca and P decreases gradually up to 5 days and after that their concentration start increasing that is attributed to formation of HCAp layer whereas in case of G3, concentration of Ca and P decreases continuously up to 10 days and after that their concentration starts increasing and small intensities of Mg also appears due to large amount of Mg in G3 that is attributed to formation of Mg-hydroxycarbonate apatite layer as conformed by XRD results.

CONCLUSION

- Glasses with three different compositions, each having 34 SiO_2-14.5 P_2O_5-1 CaF_2-0.5 MgF (% wt) and ratio of CaO/MgO varying from 11.5:1 to 1:11.5 were prepared by melt-quench method using oxy-acetylene flame in a fire clay crucible that made the glass formation cheaper in time and cost.
- Each glass was sintered at 950°C to prepare three glass ceramics of different compositions and in-vitro dissolution behavior was studied by soaking the samples in SBF for different time periods.
- EDS analysis of soaked samples, thin film XRD of their surfaces and variation in pH and ions concentrations of SBF with different soaking times revealed the dependence of surface reactivity of glass ceramics on the composition and its direct proportional relationship with CaO/MgO ratio.
- The formation of calcium phosphate layer is the fastest in G1 as compared to G2 and G3 due to the large amount of CaO/MgO ratio and the most powerful reaction.

Table 2. The EDS spectrum intensities of materials components for the sample G1 (a.u.).

	Ca	P	Si	Ca	O	C	Mg
Before immersion	645	707	578	495	335	0	0
After 2 days	599	635	674	463	394	0	0
After 5 days	638	700	622	495	392	87	0
After 10 days	663	732	590	516	396	105	0
After 20 days	665	753	580	523	390	114	0
After 25 days	674	769	555	527	364	155	0

Table 3. The EDS spectrum intensities of materials components for the sample G2 (a.u.).

	Ca	P	Si	Ca	O	C	Mg
Before immersion	592	693	580	424	348	0	0
After 2 days	555	612	610	390	374	0	0
After 5 days	502	486	638	323	399	0	0
After 10 days	537	534	624	346	381	52	0
After 20 days	596	626	578	385	355	93	0
After 25 days	619	684	555	415	337	105	0

Table 4. The EDS spectrum intensities of materials components for the sample G3 (a.u.).

	Ca	P	Si	Ca	O	C	Mg
Before immersion	502	587	590	413	351	0	82
After 2 days	440	486	605	420	362	0	70
After 5 days	376	385	624	236	387	0	61
After 10 days	316	261	645	178	399	0	70
After 20 days	413	401	605	231	369	54	80
After 25 days	500	537	576	371	346	77	96

Acknowledgement

Authors acknowledge the cooperation of Dr. Riaz Ahmad, chairman of the Physics department, GC University Lahore and support of Higher Education Commission, Government of Pakistan. Authors are grateful to Dr. Muhammad Mujahid, Department of Chemical and Materials Engineering, National University of Science & Technology (NUST) and Dr. Abdul Majeed, Brooklyn Hospital Centre, Brooklyn NY U.S.A, for their technical assistance.

References

1. Nicolodi L., Sjolander E., Olsson K.: *Biocompatible Ceramics- An Overview of Applications and Novel Materials*, KTH, 4, 2004.
2. Boccaccini A.R., Gough J.E.: *Tissue Engineering using Ceramics and Polymers,* CRC press New York, Washington, WP Ltd. Cambridge England, 4, 2007.
3. Peitl O., Zanatto E.D., Hench L. L.: J. Non-Cryst. Solids *292*, 115 (2001).
4. Li G., Zhou D., Xue M., Yang W., Long Q., Cao B., Feng D.: App. Surf. Sci. *235*, 559 (2008).
5. Kokubo T., Takadama H.: Biomaterials *27*, 2907 (2006).
6. Ohtsuki C., Kokubo T., Yamamura T.: J. Non-Crys. Solids *143*, 84 (1992).
7. Jonasova L., Helebrant A..: Ceramics-Silikaty *46*, 9 (2002).
8. M. R. Filqueras, G. La Torre and L. L. Hench: J. Biomed. Mater. Res. 27, 1485 (1993).
9. Nakamura O.K., Yamamuro T., Ebisawa Y., Kokubo T., Kokubo Y., Oka M.: J. Mater. Sci. Mater. Med. *3*, 95 (1992).
10. Hench L.L., La Torre G. in: Yamamuro T.Y., Kokubo T., Nakamura T. (Edns): *Bioceramics*, Japan, vol. 5, 67, 1992.
11. L. L. Hench, O. H. Inderson and G. La Torre, in: W. Bonfield, G. W. Hasting and K. E. Turner (Edns), *Bioceramics*, UK, vol. 4, 155, 1991.
12. Hench L.L. in: Hulbert J.E., Hulbert S.F. (Edns), *Bioceramics*, U.S.A, vol. 3, 43 1991.
13. Kokubo T., Kushitani H., Sakka S.: J. Biomed. Mater. Res. *24*, 721 (1990).
14. Peitl O., La Torre G., Hench L.L.: J. Biomed. Mater. Res. *30*, 509 (1996).
15. Li P., Zhang F., Kokubo T.: J. Mater. Sci. Mater. Med. *3*, 452 (1992).
16. Hench L.L.: J. Am. Ceram. Soc. *81*, 1705 (1998).
17. Saboori A., Rabiee M., Moztarzadeh F., Sheikhi M., Tahriri M., Karimi M.: Mater. Sci. & Eng. C *29*, 335 (2009).
18. Padilla S., Roman J.: Biomaterials *26*, 475 (2005).
19. Roy D.: J. Phys. Chem. Solids *68*, 2321 (2007).
20. Hauret G., Vaills Y., Luspin Y., Gervais F., Cote B.: J. Non-Cryst. Solids *170*, 175 (1994).
21. Cervinka L., Berghova J., Trojan M.: J. Non-Cryst. Solids *121*, 192 (1995).
22. Hoppe W., Walter G., Knanold R., Stacheland D., Baiz A.: J. Non-Cryst. Solids *28*, 192 (1995).
23. Hoppe W.: J. Non-Cryst. Solids *195*, 138 (1996).
24. Gutowska I., Machoy Z., Machaliski B.: J. Biomed. Mat. Res. *75*, 788 (2005).
25. Althoff J., Quint P., Krefting B. R., Hohling H. J.: Histochemistry *74*, 541 (1982).
26. Liu C.C., Yeh J.K., Alola J.F.: J. Bone Miner. Res. *3*, S104 (1988).
27. Li X., Ito A., Wang X., LeGeros L.Z.: Acta Biomaterelia *5*, 508 (2009).
28. Periera D. et. al: J. Eur. Ceram. Soc. *24*, 3693 (2004).
29. Vallet-Regi M., Salinos A.J., Roman J., Gil M.: J. Mater. Chem. *9*, 515 (1999).
30. Merzbacher C., White W.B.: J. Non-Cryst. Solids 130, 18 (1991).
31. F. Gervais, A. Blin and D. Massiot, J. Non-Cryst. Solids *89*, 384 (1997).
32. Ohtsuki C.: *How to prepare Simulted Body Fluid*, http://mswebs.naist.jp/LABs/tanihara/ohtsuki/SBF/index.html
33. Driessens F.C.M., Yerweck R.M.H.: *Biominerals*, CRC press, Bocaraton, FL 1990.
34. Hench L.L., Wilson J.: Surf. Active Biomater. Sci. *226*, 630 (1984).
35. Barrare F., Bayrolle P., Van Blitterswijk C.A., Groot K.D.: Bone *25*, 107S (1999).
36. Kibalezyc W., Chritoffersen J., Christoffersen R. M., Zielenkiewics A., Zielenkiewics W.: J. Cryst. Growth *106*, 355 (1990).