

Synthesis and *In Vitro* Investigation of Sol-Gel Derived Bioglass-58S Nanopowders

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The aim of this research is the synthesis of bioglass-58S nanopowders by sol-gel method. Also, the effect of aging time of parent sols on the morphology, structure and particle size was investigated. Bioglass-58S powders were analyzed by X-ray diffraction patterns (XRD), Fourier transform infrared spectroscopy (FTIR), zetasizer instrument, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The XRD results showed that the powder is amorphous and glassy. According to FTIR spectroscopy, silicate bonds were formed in all powders. Zetasizer curves proved that the particle sizes of the powders and agglomerates have increased with aging time. The SEM images confirmed these results, too. Additionally, the TEM observations revealed that the increase of aging time caused the growth of grains with the size between 50-200 nm. The *in vitro* biological behavior of bioglass-58S powders were investigated by immersing the bioglass discs (made from the powders) in the simulated body fluid (SBF). The XRD patterns and SEM images confirmed the formation of the hydroxyapatite (HA) phase.

Keywords: *bioglass, sol-gel, aging, SBF, hydroxyapatite*

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1. Introduction

In 1969, Hench et al. discovered that rat bones can bond chemically to certain silicate-based glass compositions [1]. This group of glasses was later termed “bioactive glasses”. This kind of glasses has shown appropriate *in vitro* and *in vivo* behaviors. For example, Sepulveda et al. found that the bioglass after *in vivo* implantation has no local or systemic toxicity, no inflammation and no foreign-body response [2]. Also, Hench et al. showed that there is a genetic control of the cellular response of osteoblast to bioactive glasses [3]. Bioactive glasses can be attached to soft and hard tissues with a strong chemical bond. The bioglass with the chemical composition of $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$ can bond to soft and hard tissue without formation of intermediate fibrous layer.

Bioglass-58S is a type of biomaterial for bone regeneration and scaffolds of bone tissue engineering because of its high bioactivity, osteoconductivity and biodegradability [4–6].

Also, bioglass is used for more than ten years in clinical applications [7, 8]. Recent studies have shown that the degradation products of bioactive glasses can stimulate the production of growth factors, cell proliferation of osteoblasts and activate the gene expression [9, 10]. Vallet-Regi et al. showed that increasing of specific surface area and pore volume of bioglass can accelerate the deposition process of hydroxyapatite [6]. Furthermore, the biomaterials in nanometric scale can stimulate the reaction of biomaterials and cells [11, 12]. So, it can be said that the nano bioglass (NBG) can enhance the osteoconductivity property.

A lot of new biomaterials can be produced by sol-gel method. Sol-gel is a proper method for studying and controlling biochemical reactions. In this method, the process temperature is low and can produce the glasses with high porosity and large specific surface area. The high bioactivity of the sol-gel derived bioglass is caused by residual hydroxyl ions, micro pores and high specific surface area [13]. Indeed, the synthesis of bioglass by sol-gel method is a prospective procedure

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Table 1. Ion concentrations of the simulated body fluid (SBF) and human blood plasma (mmol/L) [13].

	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Cl ⁻	HCO ₃ ⁻	HPO ₄ ²⁻	SO ₄ ²⁻	pH
SBF	142	5.0	1.5	2.5	147.8	4.2	1.0	0.5	7.40
Human Plasma	142	5.0	1.5	2.5	103.0	27	1.0	0.5	7.2–7.4

for commercial applications. Simulated body fluid (SBF) is usually used as the medium in which the *in vitro* biological behavior of biomaterials is evaluated [14].

In this research, bioglass-58S nanopowder synthesized by sol-gel method was studied. The effect of aging time (an important factor for commercial sol-gel method) on the morphology, structure and particle size was investigated by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), zetasizer instrument, scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Also, *in vitro* investigation of bioglass powder was carried out, by immersing the powder in the SBF.

2. Experimental

2.1. Materials

The selected precursors for synthesis of bioglass-58S were tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP), ethanol, calcium nitrate tetrahydrate, nitric acid and ammonia. All materials were purchased from Merck Company.

2.2. Methods

An alkali medium was used for sol-gel synthesis of bioglass-58S. The composition was chosen with the weight ratio of SiO₂: CaO: P₂O₅ = 58:23:9. First, TEOS and distilled water were dissolved in a 2 M nitric acid – ethanol solution to produce acidic sol, which was then stirred for 30 minutes in the room temperature. Afterward, calcium nitrate tetrahydrate was added to the acidic sol. In a separate container, 2 M ammonia – ethanol solution was made and added dropwise to this solution. In order to study the effect of time on gelation behavior, the bioglass sol was aged for

1 hour, 1, 2, 3 and 4 days. The resultant gel was dried at 80 °C and heat treated at 600 °C for 2 hours. After grinding and sieving, the powders were compacted into discs (10 mm in diameter and 3 mm in thickness) for *in vitro* studies in the SBF.

The standard SBF was prepared following the specification described in literature [13] by dissolving reagent chemicals of NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂, and Na₂SO₄ in deionized water. The ionic concentrations of the SBF were close to human blood plasma, as shown in Table 1. The pH of the system was customized by Tris-HCl. The solution was transferred to a chemical flask. The *in vitro* tests were undertaken immersing the discs of bioactive glass in a container filled with the SBF. Every disc, before the immersion in the SBF, was washed in pure acetone, rinsed with distilled water and air-dried. The discs were immersed in the SBF solution for 7, 14 and 21 days and remained in an incubator at 37 °C. The ratio of disc surface area to solution volume of the SBF was 0.1 cm⁻¹. After each time period, the samples were removed from the solution, gently rinsed with distilled water and dried at room temperature.

2.3. Characterization Instruments

In order to determine the structural changes in the bioglass powders, Fourier transform infrared spectroscopy (FTIR, Bruker, Vector 33, Germany) using KBr pellets technique in the range of 4000–600 cm⁻¹ was applied. The particle size distribution of the powders was measured by zetasizer instrument (Malvern Co, HS C1330-3000, England). Scanning electron microscopy (SEM, Streoscan360) and transmission electron microscopy (TEM, Philips EM208) were used to investigate the microstructure and

morphology of the bioglass powders. Preparation of the bioglass samples was done by ultrasound instrument. The bioglass powders were stirred in ethanol three times for ten minutes to avoid the agglomeration of the powders.

Before and after soaking in the SBF, the surface morphology of the specimens was examined by the scanning electron microscopy (SEM, XL30, Philips, Holland). Inductive Coupled Plasma (ICP, ICP-AES3410 ARL, Switzerland) was acquired to measure ionic concentration in the SBF solution. X-ray diffraction (XRD, D500, Siemens, Germany) measurements were carried out with a $\text{Cu}_{K\alpha}$ radiation ($\lambda = 1.5418 \text{ \AA}$). In addition, the pH of SBF solution was also monitored by using a pH meter.

3. Results and discussion

Fig. 1 shows the FTIR spectra of the bioglass-58S powders obtained from the sols aged for different time periods. The peak around 3470 cm^{-1} is related to the presence of physical water. The shoulder at 1250 cm^{-1} is assigned to the Si-O-Si bending mode. Also, the small shoulder at 958 cm^{-1} shows the Si-O-Ca vibration mode [15].

The peak at $1200\text{--}900 \text{ cm}^{-1}$ is related to P-O or Si-O. The Si-O can be grouped as Si-O 'bridging' and 'non-bridging'. In fact, some of the oxygen atoms are not directly connected to another Si atom in the glass network and the presence of cations increases the formation of Si-O groups. The non-bridging oxygens are also related to the Si-OH groups that break in the silica network. Therefore, the presence of non-bridging oxygens leads to the shift of the Si-O pick around $1040\text{--}940 \text{ cm}^{-1}$ [13].

Interestingly, the intensity of the Si-O-Si, Si-O-Ca and P-O peaks of the powders obtained from sols aged for different time periods did not show any significant differences; this proves that the structure of bioglass-58S has been formed in the first one hour. The number of carbonate bonds decreased with rising of the aging time. It seems, on the basis of the C-O bands, that the amount of carbonates decreased with increasing the aging time.

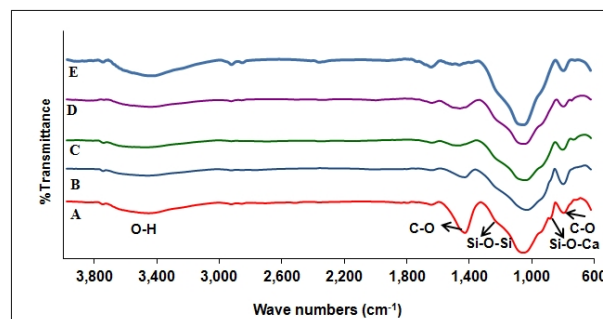


Fig. 1. FTIR images of the bioglass-58S powders obtained from the sols aged for different time periods; A) 1 hour, B) 1 day, C) 2 days, D) 3 days and E) 4 days.

Fig. 2 shows the scanning electron microscopy (SEM) images of the bioglass-58S powders obtained from the sols aged for different time periods. These powders have the amorphous random structure without any crystals and agglomerates. As can be seen in these images, all of powders include the nano-grains and the enhanced tendency to agglomeration as the gelation time increases. In fact, the powders had enough time for aggregation.

Fig. 3 shows the transmission electron microscopy (TEM) images of the powders obtained from the sols aged for different time periods with the resolutions between $\times 20000$ – $\times 50000$. The grains of the powders have a spherical shape. According to these images, the particle sizes have been growing up with increasing the aging time and, in the powders which had enough time for agglomeration, the particles aggregated beside each other randomly without any recognizable shape. Presumably, it can be said the crystalline structure can be formed by increasing the time and the heating temperature because the powders have enough time for the formation of regular crystalline structure as a result of diffusion “(nucleation and growth) mechanisms”.

Fig. 4 (A-C) shows the zetasizer curves of the bioglass-58S powders obtained from the sols aged for 1, 3 and 4 days. As can be observed in these images, the particle sizes grow up with increasing the aging time. The average particle sizes of

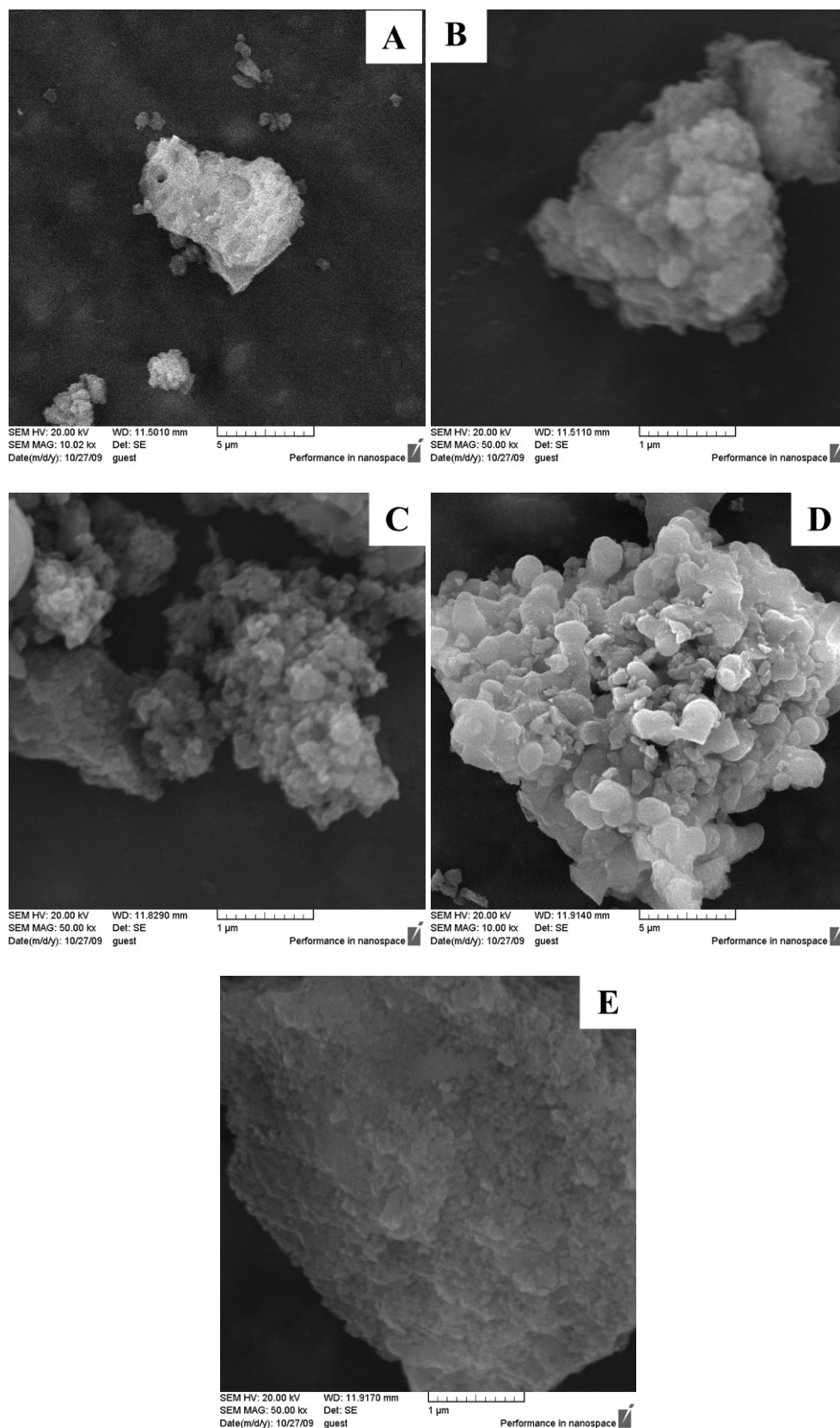


Fig. 2. SEM images of the bioglass-58S powders obtained from the sols aged for different time periods; A) 1 hour, B) 1 day, C) 2 days, D) 3 days and E) 4 days.

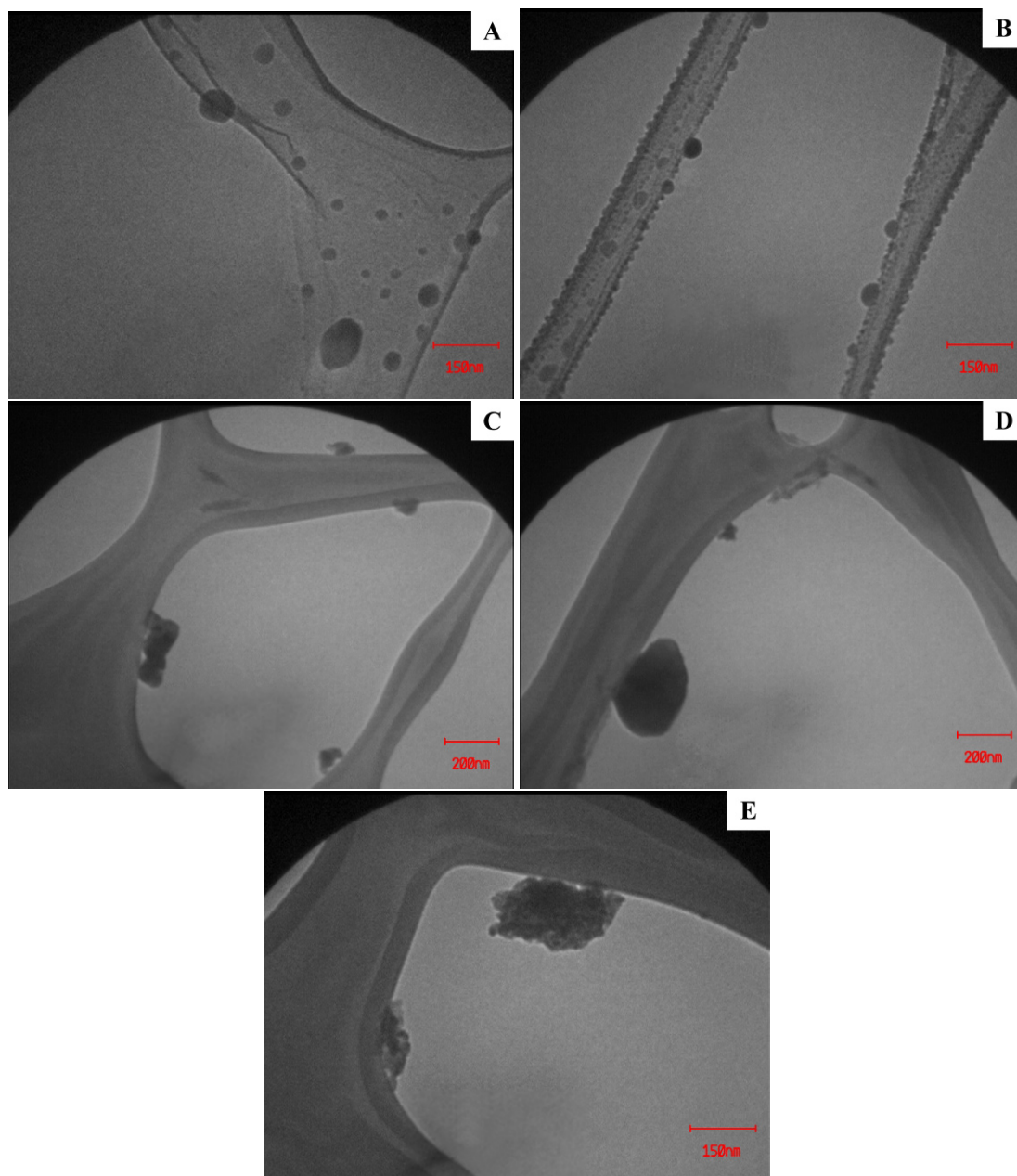


Fig. 3. TEM images of the bioglass-58S powders obtained from the sols aged for different time periods; A) 1 hour, B) 1 day, C) 2 days, D) 3 days and E) 4 days.

powders aged for 1, 3 and 4 days were 182, 1054 and 1661 nm, respectively. The powders obtained from the sols aged for longer times include the larger particle sizes in micrometer scales. It means that the powders grains can grow up with the increasing of aging time.

The XRD patterns of the bioglass powders (obtained from the sol aged for 1 day) before and after the soaking in the SBF for different time periods are presented in Fig. 5. The sample before immersion (A) have broad diffraction bands, confirming its amorphous and glassy nature. Hydroxyapatite (HA) phase has been formed

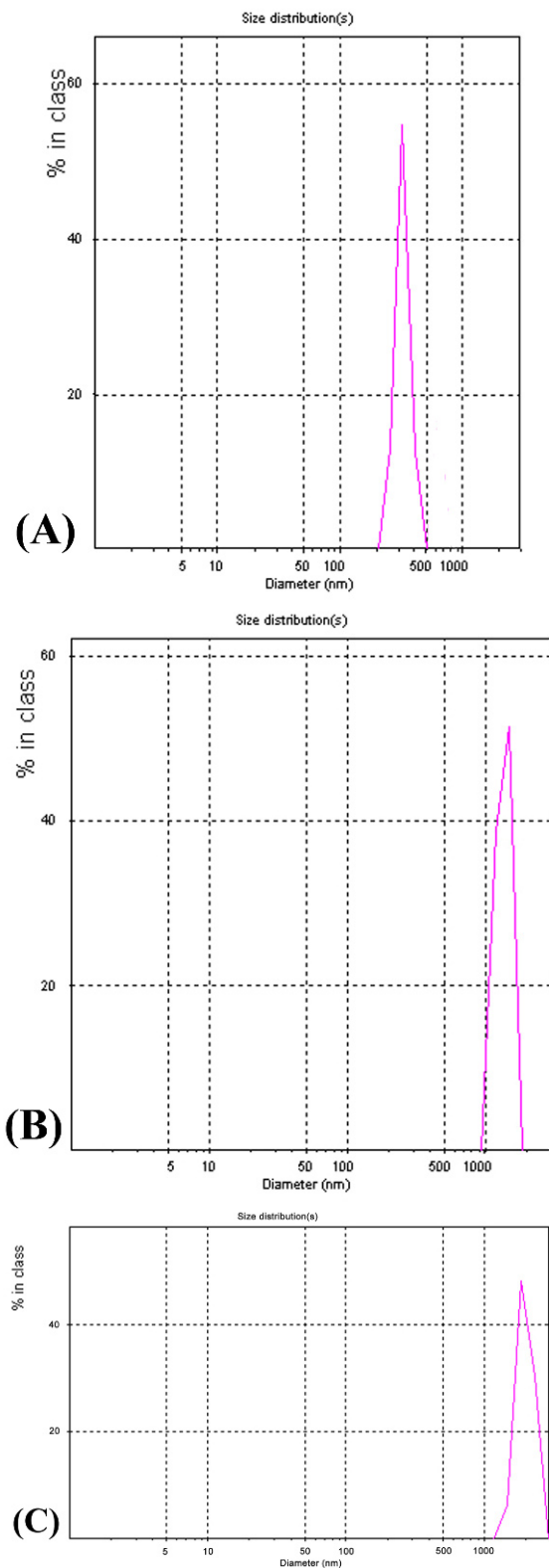


Fig. 4. Zetasizer images of the bioglass-58S powders obtained from the sols aged for: A) 1 day, B) 3 days, and C) 4 days.

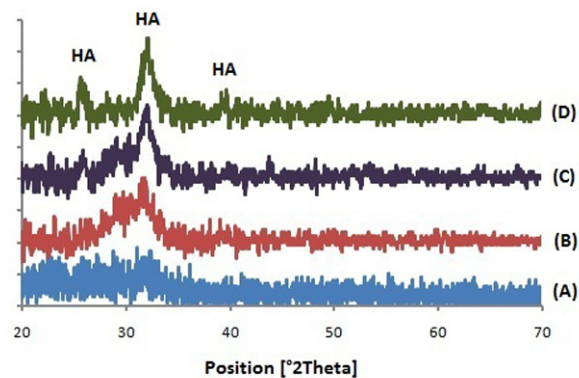


Fig. 5. The XRD patterns of the surface of bioglass disc before (A) and after soaking in the SBF for 7 days (B), 14 days (C) and 21 days (D).

after immersion of the bioglass discs in the SBF for 7 days (B). The intensity of the HA phase has developed significantly with the soaking time (C;D) and the bands of the HA phase became sharper which was confirmed by further transformation of bioglass to hydroxyapatite.

Fig. 6 shows the SEM images of the bioglass discs surfaces (obtained for the sol aged for 1 day) before and after soaking in the SBF. After immersion of the bioglass samples in the SBF, a heterogeneous surface with the aggregates of spherically shaped hydroxyapatite particles (sizes ranging from nano size to micron size) was created. The formation of the hydroxyapatite particles on the glass surfaces after immersion in the SBF for 7 to 21 days was confirmed by the XRD analysis. As can be observed, the spherical particles are completely covering the sample surface after 21 day immersion.

The concentration of the P, Ca and Si ions was measured in the SBF solution on the 7th, 14th and 21st day by the ICP experiment (Fig. 7). As can be observed, after 2 weeks, the concentrations of Ca and Si ions increased from 89.1 ppm to 229 ppm and from 0 to 39.4 ppm, respectively, whereas the concentration of P ion in the solution decreased. The decreasing of P ion concentration in the SBF solution shows that the amount of P ion released from glass cannot adjust its consumption caused by apatite deposition. Furthermore, pH

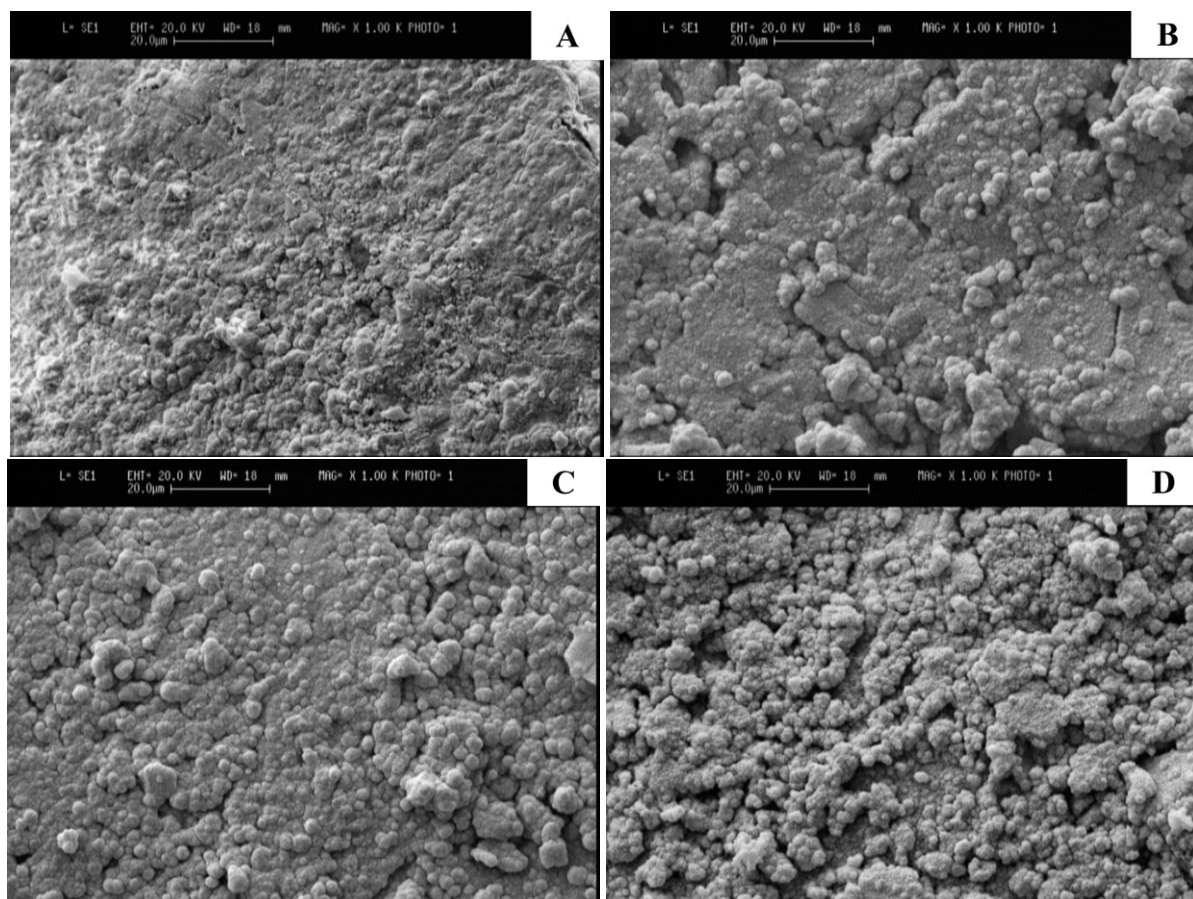


Fig. 6. SEM morphology of the surface of bioglass disc before (A) and after soaking in the SBF for 7 days (B), 14 days (C) and 21 days (D).

variation with time increased up to 8 during the first 7 days of immersion and then no significant change took place. It is clear from the results that an interaction between the SBF and the glass samples has occurred.

Zhou et al. considered two opposite processes: the release of Ca ions from the glass, and the consumption of Ca ions due to the formation of the apatite layer. Therefore, when the release rate of Ca ions is higher than the consumption rate, the pH will increase, or in contrast, the pH will decrease [16]. The pH value did not change significantly after 14 days and simultaneously the amount of Ca ion remained nearly invariable.

4. Conclusions

In this research, the bioglass-58S nanopowders were successfully synthesized by sol-gel method and the effect of aging time of the parent sols on the structure, particle size and morphology of the powders was investigated. The formation of glassy structure was confirmed by XRD pattern. FTIR results proved the existence of silicate bonds. The presence of carbonates decreased with increasing the aging time. SEM and zetasizer results showed that the tendency to agglomeration intensified with increasing the aging time. According to TEM images, the grain sizes grew up from 50 nm to 200 nm with increasing the aging time.

The XRD patterns and SEM images confirmed that hydroxyapatite (HA) phase had been formed

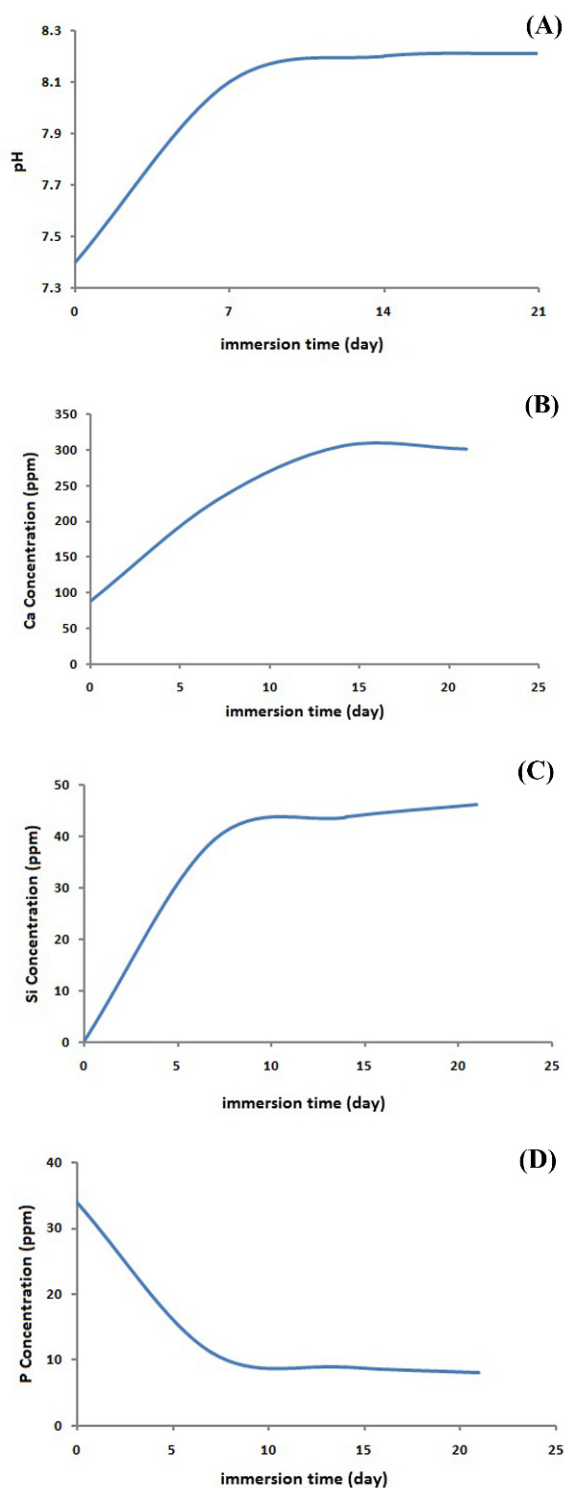


Fig. 7. Variation of pH, Ca, Si and P concentrations in the SBF with immersion time.

after immersion of the bioglass discs in the SBF solution. Furthermore, the pH value increased with time up to 8 during the first 7 days of immersion and then no significant change were observed. The synthesized nanopowders can affect positively the acceleration of the reaction of tissue and other materials and finally improve the osteoconductivity and bioactivity process.

References

- [1] HENCH L.L., SPLINTER R.J., ALLEN W.C, GREENLEE T.K., *J. Biomed. Mater. Res.*, 2 (1971), 117.
- [2] SEPULVEDA P., JONES J.R., HENCH L.L., *J. Biomed. Mater. Res.*, 59 (2002), 340.
- [3] HENCH L.L., POLAK J.M., *Science* 295 (2002), 1014.
- [4] HENCH L.L., *Curr Orthopaed* 14 (2000), 7.
- [5] WILSON J., LOW S.B., *J. Appl. Biomater.*, 3 (1992), 123.
- [6] VALLET-REGI M., RAGEL C.V., SALINAS A.J., *Eur. J. Inorg. Chem.*, 6 (2003), 1029.
- [7] HENCH., XYNOS I.D, POLAK J.M., *J. Biomat. Sci-Polym. E.*, 15 (2004), 543.
- [8] HENCH L.L., *J. Amer. Ceram. Soc.*, 74 (1991), 1487.
- [9] LOSSDORFER S. et al., *Biomater.*, 25 (2004), 2547.
- [10] VALERIO P., PEREIRA M.M., GOES A.M., LEITE M.F., *Biomed Mater.*, 4 (2009), 045011.
- [11] WEBSTER T.J., ERGUN C., DOREMUS R.H.L., SIEGEL R.W., BIZIOS R., *J. Biomed. Mater. Res.*, 51 (2000), 475.
- [12] WEBSTER T.J., SIEGEL R.W., BIZIOS R., *Biomater.*, 20 (1999), 1221.
- [13] KOCUBO T., TAKADAMA H., *Biomater*, 27 (2006), 2907.
- [14] BALAMURUGAN A. et al., *Mater. Lett*, 60 (2006), 3752.
- [15] PEREIRA M.M., CLARK A.E., HENCH L.L., *J. Biomed. Mater. Res.*, 28 (1994), 693.
- [16] ZHOU Z., RUAN J., ZOU J., ZHOU Z., *J. Uni. Sci. Tech. Beijing.*, 15 (2008), 290.

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