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Egypt. J. Chem. **55**, No. 4, pp. 399-411 (2012)

Preparation of Nano Bioactive Silica from Silica Gel

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BIOACTIVE silica was prepared by precipitation of the gel by acid from sodium silicate in the presence of ethylene glycol. The formed gel was homogeneously mixed with calcium carbonate powder. The produced solid was heated at 500^{o}C for 3 hr and the calcined material was treated with dilute hydrochloric acid to remove the carbonate. The investigation by X-ray showed that the produced solid before treatment contains amorphous silica with crystalline calcium carbonate phase. The treatment with HCl removed the crystalline calcium carbonate leaving pure amorphous silica in nano-sized particles as confirmed by SEM. The bioactivity of the obtained silica was performed by soaking it in simulative body fluid (SBF) for two weeks followed by investigation using SEM, IR and EDX.

Keywords: Bioactive silica, Calcium carbonate, Nano silica and Hydroxyapatite.

Silica gel was in existence as early as the 1640s as a scientific curiosity. It was used in World War I for the absorption of vapors and gases in gas mask canisters. In World War II, silica gel was indispensable in the war effort for keeping penicillin dry, protecting military equipment from moisture damage.

In chemistry, silica gel is used in chromatography as a stationary phase. In column chromatography, the stationary phase is most often composed of silica gel particles**.** The hydroxyl (OH) groups on the surface of silica can be functionalized to afford specialty silica gels that exhibit unique stationary phase parameters. These so-called functionalized silica gels are also used in organic synthesis and purification as insoluble reagents and scavengers.

The development of composites has been recognized as a promising strategy to fulfill the complex requirements of biomaterials. This new bioactive nanocoating enhances the effectiveness and improves the long term stability of orthopaedic dental implants. The work nanocoating, as it is referred to, is functionally graded from metal through oxides, silicates, silica, hydroxyl groups and hydroxyapatite (HA). The arrangement and integration of the layers enable the great bonding strength of the layer, and bioactivity is achieved by high silanol (hydroxyl) and HA content. The layer of bioactive silica is exceptionally hard and biologically active. Its hardness resists the formation of wear particles

and the high silanol and negative surface charge promote strong and rapid bonding to bone.

Bioactive glass and glass-ceramic have been developed for the tissue repairing from the discovery of bioglass and some of them are already clinically used. Since that, many attentions have been focused on designing of glass, glass-ceramics as well as organic/inorganic composites. It was well known that artificial materials have to form bonelike-apatite on their surfaces in the body environment, because the bioactive glass and glass-ceramics bond to livening bone through the bonelike apatite formed on their surface when implanted into the bone. As a bioactive component[(1)], it has been proposed that the Ca-SiO2 system can be good basic composition of bioactive glass-ceramics; since the dissolved Ca ion increases the degree of super saturation with respect to apatite and hydrated silica developed on them induce apatite formation when implanted into body environment.

Li *et al*.[(2)] proposed that the high concentration of SiOH groups on the sample surface could promote hydroxyl carbonated apatite nucleation. On the other hand, the surface microstructure of the silica-gel samples has also been related to in vitro bioactivity.

The aim of the work is to prepare nano bioactive silica using simple materials such as sodium silicate (commercial) and calcium carbonate (calcite). The product will be characterized by XRD, IR, SEM, EDX and soaking in SBF to follow up the formation of hydroxyapatite on the surface of the bioactive silica.

Experimental

Materials of Bioactive Silice

In this work sodium silicate solution 43% is used as starting material. Ethylene glycol, Hydrochloric acid and SBF were also used.

Preparation of bioactive silica

Silica gel was precipitated by using dilute hydrochloric acid solution in the presence of ethylene glycol (10%). The precipitate was filtered, washed, dried and calcined at 550^{o}C for 3hr. Another quantity of silica gel was also prepared in the same manner but the precipitate, after filtration, was mixed with 5% by weight calcium carbonate powder (1-5micron). The mixture was homogeneously mixed, dried and calcined at 550^{o}C for 3hr. The calcined solid with calcium carbonate was treated with dilute hydrochloric acid to remove the calcium carbonate and washed. Finally the solids were characterized.

Preparation of simulated body fluid (SBF)

The following reagents have to be stocked in a desiccator. Deionized water and distilled water were used for the preparation of SBF:

(1) Sodium chloride (NaCl),
(2) Sodium hydrogen carbonate ($NaHCO_3$),
(3) Potassium chloride (KCl),
(4) Di-potassium hydrogen phosphate trihydrate (K_2HPO_4 $3H_2O$).
(5) Magnesium chloride hexahydrate ($MgCl_2.6H_2O$),
(6) Calcium chloride ($CaCl_2$),
(7) Sodium sulfate (Na_2SO_4),
(8) Tris-hydroxymethyl aminomethane: ($(HOCH_2)_3CNH_2$) (Tris)
(9) 1M (mol/l) Hydrochloric Acid.
(10) pH standard solutions (pH 4, 7 and 9).

Ion concentrations of SBF

TABLE . 1. The ion concentrations of SBF are given in Table 1.

Ion	Ion concentrations (mM)	
	Blood plasma	SBF
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	103.0	147.8
HCO^-_3	27.0	4.2
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.2–7.4	7.40

Materials characterization

X-ray diffraction patterns were obtained at room temperature using A Philips X-ray diffractometer (Goniometer PW 1050/50) Co Ka radiation (λ= 1.78897 Å), employing cobalt radiation as the X-ray source. The x-ray tube was operated at 36KV and 16 mA, the samples were packed into a plastic holder, no adhesive or binder was necessary. Spectra were scanned at a rate 2^{o} min^{-1} in 4θ.

Scanning electron microscope (SEM) with EDX

The scanning electron microscope (SEM) and Energy dispersive X-rays (EDX) photographs were carried out using SEM Model Philips XL 30 attached with EDX unit، with accelerating voltage 30 k.V. , magnification 10x up to 400.000x and resolution for W. (3.5 nm). The samples were coated with carbon for both EDX and SEM assessment.

Infrared spectroscopy (IR)

The infrared spectroscopy is a very sensitive technique which can be used effectively to identify the bioactivity of the prepared silica samples by detection of the functional groups. The infrared spectroscopy was carried out using Nexus 670 IR Reflectance and Transmittance spectrometer –Company: Nicolet –Country: USA –Range: 4000-400 cm^{-1} -Resolution:4 cm^{-1}.

Results and Discussion

Figures 1-4 show XRD-analyses of the pure calcium carbonate, calcined pure silica, silica-calcium carbonate and silica calcium carbonate after removing of calcium carbonate. From these figures . it can be observed that the prepared silica and silica after removing calcium carbonate were amorphous. The silica-calcium carbonate solid showed two phases, a crystalline calcium carbonate phase and amorphous silica phase. This means that the combination of amorphous silica with crystalline calcium carbonate gave a composite with two different phases. The removal of the crystalline calcium carbonate from the calcined sample leaves pure amorphous silica.

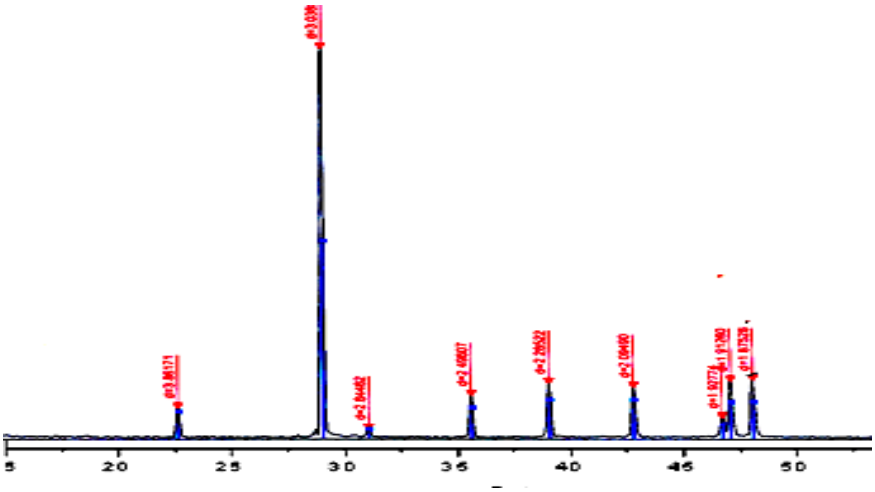

Fig. 1. XRD- of pure calcium carbonate.

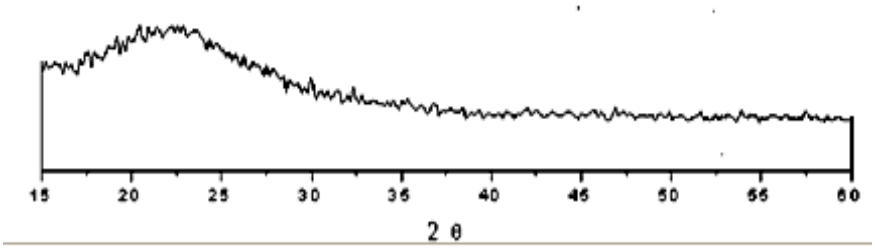

Fig. 2. XRD- of pure silica gel.

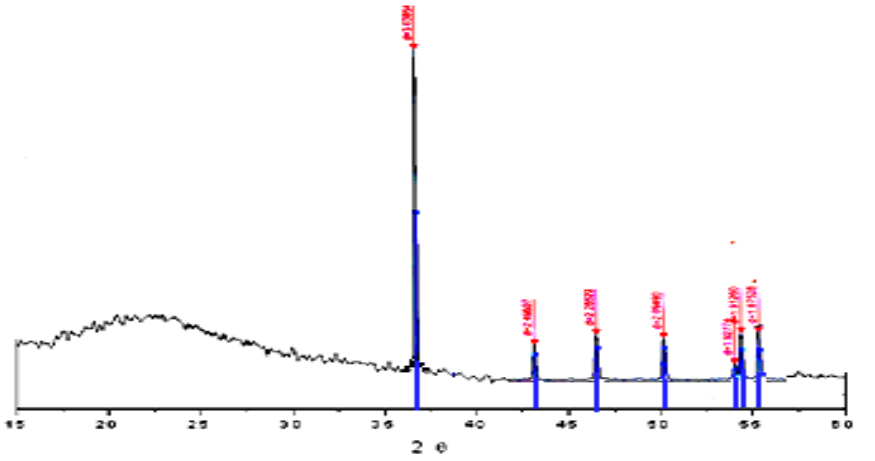

Fig. 3. XRD- of composite of silica gel with calcium carbonate .

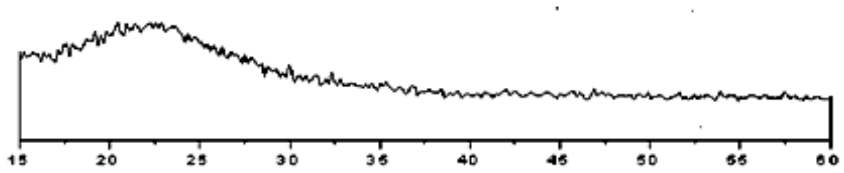

Fig. 4. XRD- of composite of silica gel after removal of calcium carbonate.

SEM-results

Figure 5 shows that the pure silica is particles with smooth surface. The treatment with calcium carbonate gave roughness to the silica surface (see Fig. 6).

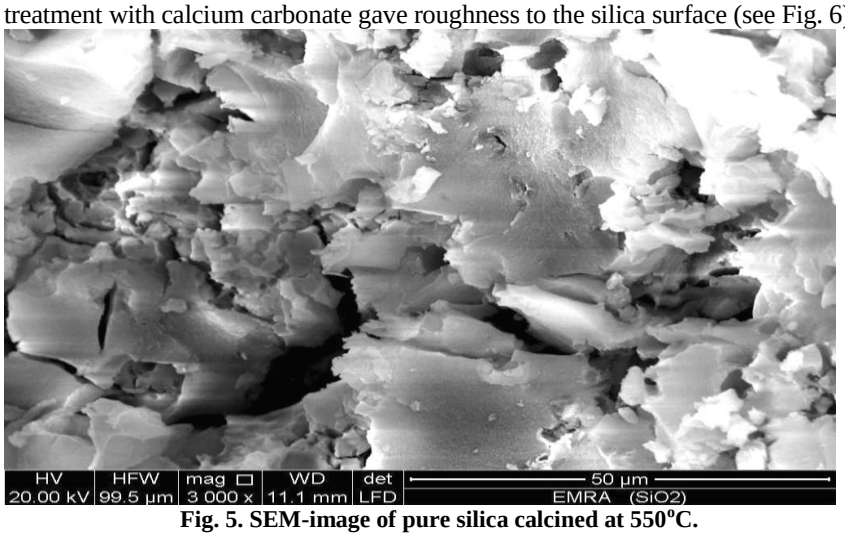

Fig. 5. SEM-image of pure silica calcined at 550°C.

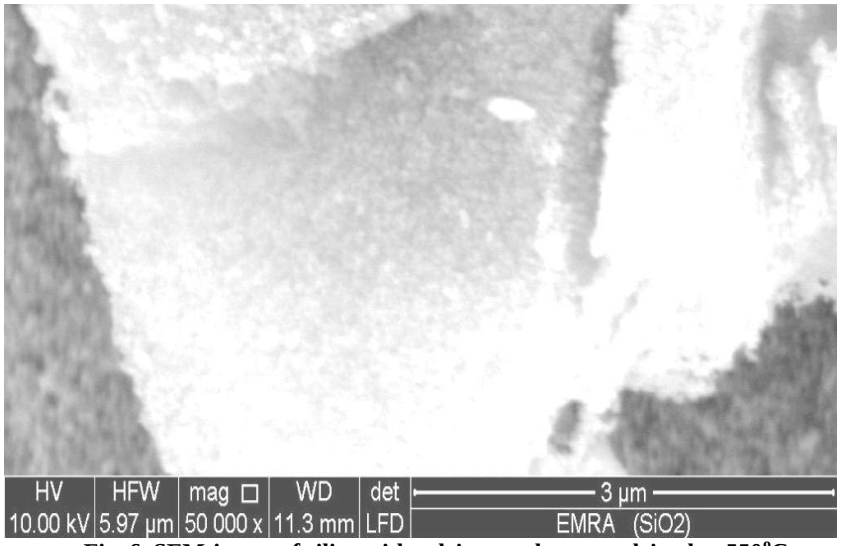

Fig. 6. SEM-image of silica with calcium carbonate calcined at 550°C.

After the treatment with HCl, spherical particles with diameter ranging from 45-150 nm appeared in the SEM- image(Fig.7). These spheres were formed as a result of the dissolution of calcium carbonate from the composite.

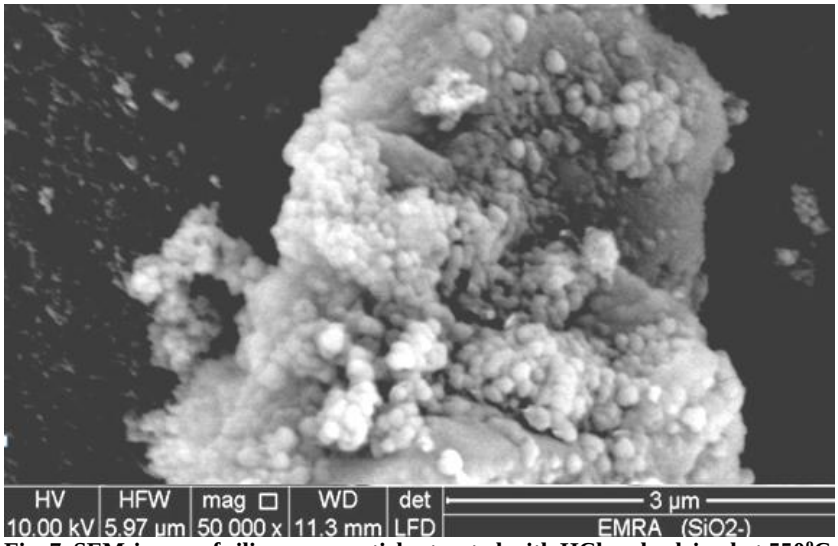

Fig. 7. SEM-image of silica nanoparticles treated with HCl and calcined at 550°C.

SEM images also show the morphology of Apatite (AP) layer formed on silica sample after two weeks of soaking in SBF (Fig. 8). The granules appear covered by a dense layer of closely packed particles or forming a thin layer composed of a Ca–P phase. These findings suggest the growth of preformed AP-precipitates during the soaking in SBF.

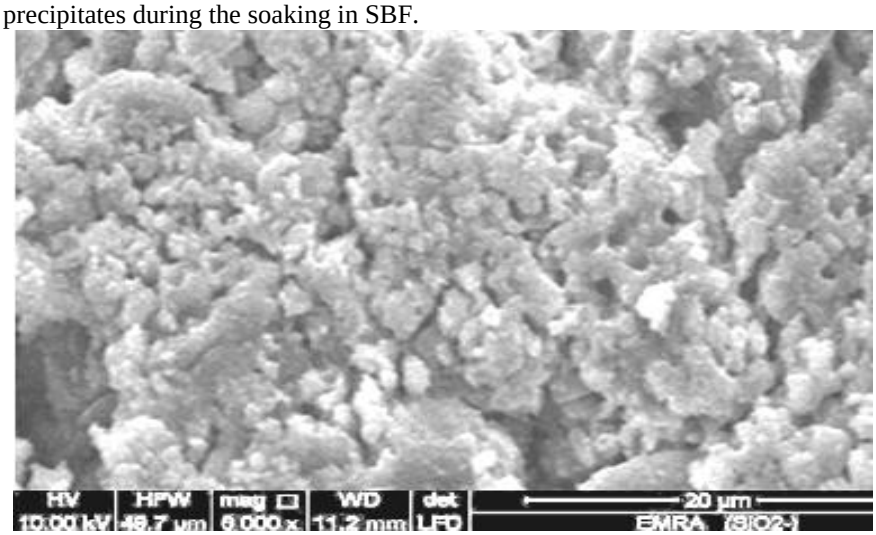

Fig. 8. SEM image shows the morphology of Apatite (AP) layer formed on silica sample after two weeks of soaking in SBF.

The IR spectra (Fig. 9a) shows well polymerized silica as indicated by the sharpness of the strong Si–O–Si bands, *i.e*. Si–O–Si bending at 465 cm^{-1} and Si–O–Si asymmetric stretching at 1088 and 1246 cm^{-1}[3]. Other silica network bands were located at 560,790, and 970 cm^{-1}. The 560 cm^{-1} bands are associated with Si–O–Si bending of three- or four member rings (cyclic tri- and tetrasiloxanes)[3-8]. The 790 and 970 cm^{-1} bands are usually assigned to symmetric Si–O–Si stretching and Si–OH stretching, respectively [10] . The broad band at 3400 cm^{-1} is associated with H-bonded Si-OH stretching vibrations and H-bonded water [9-12]. The band at 3600 cm^{-1} could be assigned to isolate Si-OH stretching [13-15] .

(Figure. 9b) shows the results of IR spectra of the under investigated silica powder prepared by the above mentioned method after soaking in SBF for 2weeks. The results of bioactivity are in agreement with the results in the literatures [16,17]. Also, the bioactivity results obtained showed that the bone like –apatite layer early formed on the surface of the silica. This layer is important to enhance bone forming *in vivo*.

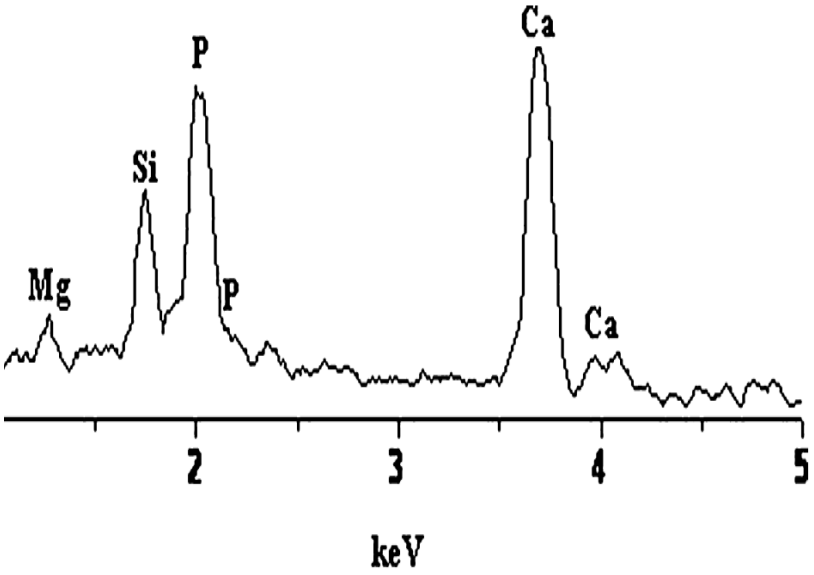

Fig. 9. EDX-spectrum of SiO_2 nanoparticles after 14 days soaking in SBF.

Kokubo[3] suggested that for the bioactive silica forming surface silanols upon hydration, a silica layer is produced by exchange of ions in the SBF, and that this layer acts as a nucleation agent for apatite crystal formation. Once the apatite crystals are formed، they grow by consuming calcium and phosphate ions from the SBF. In addition, the filler dissolution rate is important, since the faster the dissolution rate is the faster the ions come into contact with the SBF solution, promoting the surface formation of apatite. In order for bioactive silica to bond to bone, a series of reactions must take place that includes dissolution, precipitation and ion exchange.

Si-O-Si

The energy dispersive X-ray analysis (EDX) results for bioactive silica surface after in- vitro show the changes in Ca, P and Si at different immersion periods (Fig. 10). The ratio of calcium to phosphorus shows a decreasing trend as a function of exposure time. The EDX data from selected areas of silica also showed different values of the Ca/P ratio (Fig .10).

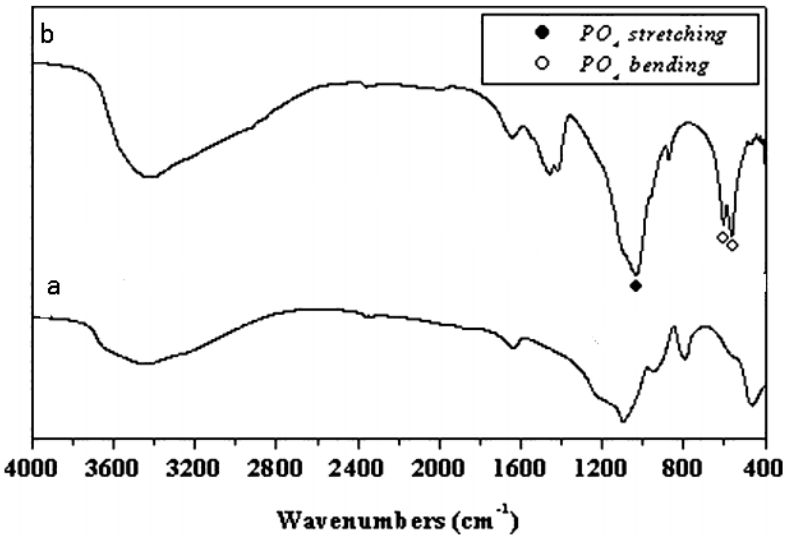

Fig. 10. IR spectra of SiO_2 (a) as-prepared; (b) after 14 days soaking in SBF.

Bioactivity

HCA layer formation on the surface of the bioactive materials has shown that reactions occur on the material side in five stages. These stages are the fastest for the highest level of bioactivity. Surface reaction stages I-V on a bioactive glass in aqueous solution are summarized below.

Stage (I): Rapid exchange of Na^+ or K^+ with H^+ or H_3O^+ from solution.

$$Si\text{-}O\text{-}Na^+ + H^+ + OH^- \rightarrow Si\text{-}OH + Na^+_{(solution)} + OH^-$$

Stage (II): Loss of soluble silica in the form of Si $(OH)_4$ to the solution resulting from breakage of Si-O-Si bonds and formation of Si-OH (silanols) at the glass solution interface :

$$2\ (Si\text{-}O\text{-}Si) + (H_2O) \rightarrow 2Si\text{-}OH + 2OH\text{-}Si$$

Stage (III): Condensation and repolymerization of a SiO_2 rich layer on the surface that is depleted in alkalis and alkaline earth cations.

$$2\ (Si\text{-}OH) + 2\ (OH\text{-}Si) \rightarrow Si\text{-}O\text{-}Si\text{-}O\text{-}Si\text{-}O + H_2O$$

Stage (IV) : Migration of Ca^{2+} and PO^{3-}_4 groups from the SBF to the surface through the SiO_2-rich layer forming a $CaO-P_2O_5$-rich film on top of the SiO_2-rich layer, followed by growth of an amorphous $CaO-P_2O_5$-rich film by incorporation of soluble calcium and phosphate from solution.

Stage (V): Crystallization of the amorphous $CaO-P_2O_5$ film by incorporation of OH, CO^{2-}_3, or F anions from solution to form a mixed HCA layer.

Concerning the mechanism of the apatite formation on the surfaces of bioactive glasses and glass-ceramics, it has been proposed that hydrated silica developed on their surfaces in the body induces nucleation of the apatite.

These results suggest that the silanols group formed on the surface of the silica gel in the SBF could be responsible for the apatite nucleation. On the other side, recently West *et al.*[8] proposed that on the bases of molecular orbital calculation, only the silanol group forming trigonal siloxane can induce the apatite nucleation. However, this has been proved experimentally[3].

Conclusions

1- Silica gel was prepared by acidification of a solution of commercial sodium silicate.
2- The prepared silica gel was homogeneously mixed with calcium carbonate and heated at 550 ^oC.
3- The calcium carbonate in the produced composite was leached by hydrochloric acid.
4- The formed silica was examined by soaking it in simulated body fluid for two weeks and it showed good bioactivity as confirmed by EDX and IR.
5- The obtained bioactivity of silica can be attributed to the presence of some ions of calcium in the silica as cited in literature.

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Received 11/11/2012;
accepted 28/11/2012)

تحضير السليكا النا نومترية النشطة بيولوجيا من سليكات الصوديوم

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تم تحضير السيليكا النشطة بيولوجيا من من سيليكات الصوديوم في وجود الإثيلين جلايكول. وخلطت مع مسحوق كربونات الكالسيوم . تم حرق الصلب الناتج عند500درجة مئوية لمدة 3 ساعات وتمت معالجة المادة المكلسة مع حمض الهيدروكلوريك المخفف لإزالة الكربونات. وأظهر التحليل بواسطة الأشعة السينية أن المادة الصلبة الناتجة قبل العلاج بالكربونات تحتوي على سليكا فير متبلورة والسيلكا مع الكربونات تعطى سيليكا غير متبلورة مع كربونات الكالسيوم البلورية. إزالة الكربونات بحمض الهيدروكلوريك وكربونات الكالسيوم البلورى تعطى السيليكا النقية غير المتبلورة تاركة سليكا علي شكل جزيئات فى حجم النانو كما أكد الميكروسكوب النافذ. تم إجراء تجارب النشاط الحيوي للسيليكا التي تم الحصول عليها عن طريق النقع في SBF لمدة أسبوعين تليها التحليل باستخدام SEM وEDX.