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ARTICLE

Effect of incorporation of nano bioactive silica into commercial Glass Ionomer Cement (GIC)

M. Mabrouk ^{a,*}, M.M. Selim ^b, Hanan Beherei ^a, M.I. El-Gohary ^c

^a *Biomaterials Department, National Research Center, Cairo, Egypt*

^b *Surface Chemistry and Catalysis Laboratory, National Research Center, Cairo, Egypt*

^c *Physics Department, Faculty of Science, El Azhar University, Cairo, Egypt*

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Abstract Four types of bioactive nano-silica were prepared by different methods, and were used to improve commercial dental Glass Ionomer Cement (GIC) bioactivity. The prepared powder samples were characterized by X-ray diffraction (XRD) to identify the formed phase; particle size and morphology were assessed by transmission electron microscope (TEM). The bioactivity of the prepared powder samples and its dental cement blends were applied in simulated body fluid (SBF). The change in surface morphology and composition after soaking in SBF after week at 37 °C were determined by scanning electron microscopy with energy dispersive spectroscopy (SEM with EDS) and Fourier transform infrared analyses (FTIR). Our results confirmed that the prepared silica powder exists in nano-scale. Precipitations of carbonate-apatite on the silica surface were observed by FT-IR spectroscopy and scanning electron microscopy. Silica dissolution and re-precipitation phenomena were also observed from SEM. The relationship between both phenomena during the in vitro test is discussed mainly in terms of structural and microstructural

* Corresponding author. Address: Biomaterials Department, National Research Centre, Behoth Street, Dokki, Cairo, Egypt. Mobile: +20 10 7306227; fax: +20 2 3370931.
E-mail address: owka_05@yahoo.com (M. Mabrouk).



features of the silica. Combination of bioactive nano-silica with dental cement improves its bioactivity, which may be helpful to overcome marginal gap formation which is major disadvantage of the commercial dental cement.

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1. Introduction

Tooth decay is one of the most common diseases and accounts for almost half of all tooth extractions. Dental restorations do not last forever; over 60% of all restorative dentistry is for the replacement of restorations.

Secondary caries detection after placement of a restoration in a cavity may be due to one or more of many factors involving the material, the cavity, the patient and the operator or technique. In the absence of clearly defining factors involved in the occurrence or recurrence of the carious process, the presence of secondary caries is often interpreted as a function of the material properties if all other confounding factors are kept to minimum [1]. New restorative materials are often marketed and introduced into practice with limited evidence on their long-term clinical performance. Glass Ionomer Cement is commercial dental cement. The use of Glass Ionomer Cements has some limitations in very specific circumstances, like physical strengths, water sensitivity which leads to formation of marginal gap due to shrinkage of the dental cement.

The first Bioglass was invented by Hench [5]. Because of the good bioactivity, osteoconductivity and biodegradability [17,23], bioactive glasses have been used in clinic for more than 10 years, as bone repair materials [23,4]. In the last decade studies showed that the degradation products of bioactive glasses could stimulate the production of growth factors, cell proliferation and activate the gene expression of osteoblast [11,22]. Moreover, bioactive glass is the only one, which could bond to hard and soft tissue [6].

The *in vitro* behavior of bioactive silica-based materials obtained by sol-gel was mainly attributed to two factors. Li et al. [10] 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. Pereira et al. [18] reported that those silica-gel samples with high porosity volume and pore size > 2 nm are suitable for inducing *in vitro* HCA formation in simulated body solutions. Bioactive silica-gel samples evaluated by Li et al. [10] which induce HCA deposition also presented nano-size to a great extent. Furthermore, these samples also presented a particular interconnected microstructure with micrometric pores, but no comments were made about the influence of the sample microstructure on their *in vitro* response. The porous structure resulted from the hydrolysis and polycondensation of silicon alkoxides in the presence of an organic, non-reactive polymer. The presence of the polymer induces phase separation simultaneously with the polycondensation of alkoxide, and the resultant structure can be controlled from the synthesis parameters and initial composition of the reactive system [12].

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. Experimentally it was confirmed that pure silica gel prepared by hydrolysis and polycondensation of tetraethoxysilane in aqueous solution containing polyethylene glycol induces the formation of the apatite layer on its surface when the gel is soaked in a simulated body fluid (SBF). These results suggest that the silanol 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. proposed that; on the basis of molecular orbital calculation, only the silanol group forming trigonal siloxane can induce the apatite nucleation. However, this has not been proved experimentally [3,15].

In this work we aim to come over the disadvantage of GIC by incorporating nano-silica with little quantities prepared by different methods. The synthesis and *in vitro* evaluation of

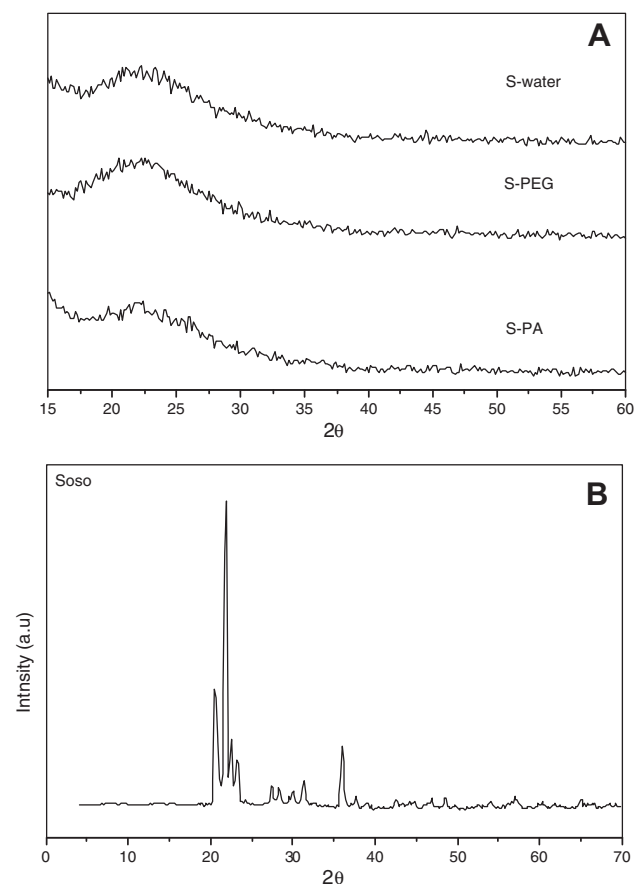
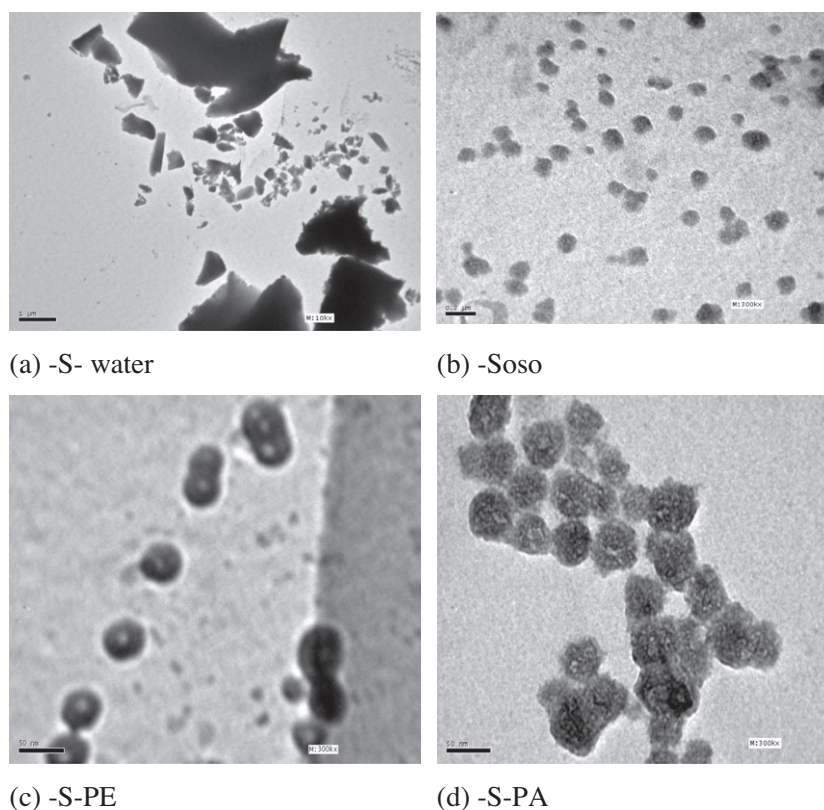


Figure 1 Represents X-ray diffraction patterns of (a): three kinds of pure silica gels S-water, S-P amide, and S-PE prepared by hydrolysis and polycondensation of TEOS in different media precursors and heated at 600 °C for 2 h and (b): shows X-ray diffraction pattern of pure silica powder prepared by hydrolysis and polycondensation of sodium silicate precursor and heated at 900 °C for 2 h sample Soso.



(a) -S- water

(b) -Soso

(c) -S-PE

(d) -S-PA

Figure 2 TEM images of (a) S-water, (b) Soso, (c) S-PE, and (d) S-PA illustrating the morphological features of the sintered samples after aging the wet gel for 6 days.

series of silica samples is described. The in vitro behavior of the silica samples and nano silica dental cement blends were evaluated, taking into account the effect of their microstructure and stability in SBF.

2. Preparation methods

2.1. Preparation of silica via sol-gel

Three solutions of a, b, and c were prepared as follows:

- Deionized water.
- Deionized water with certain amount of polyacrylamide (company: BHD).
- Deionized water with certain amount of polyethylene glycol (company: Fluka).

A few drops of HNO_3 55 wt.% (Egyptian company for chemicals and pharmaceuticals) were added to each of the above solutions. A certain amount of TEOS (Merck, Germany) was added to each of the above mixtures with vigorous stirring for 5 min. The solutions were transferred to plastic Petri dishes with tightly sealed tops which were kept at 40°C for 18 h. The obtained wet gels were immersed in molar solution of HNO_3 for 6 h. HNO_3 was renewed every 2 h. The obtained gels were washed for several times and dried at 40°C for 6 days. Then the obtained gels were heated and the temperature was gradually increased up to 600°C with a rate of $100^\circ\text{C}/\text{h}$ and kept at 600°C for 2 h. The samples were named (S-water), (S-PAA) and (S-PEG), respectively.

2.2. Preparation of silica by treatment of sodium silicate

A certain amount of sodium silicate (Commercial 43% $\text{Na}_2\text{Si}_2\text{O}_5$ solution) was hydrolyzed in 400 ml distilled water, the mixture was treated by diluted acetic acid ($\text{CH}_3\text{CO}_2\text{H}$) (El Nasr Pharmaceutical Chemicals Company) till pH 6.8 with continuous stirring. After filtration and washing for several times the resulted gel was collected. One gram of calcium carbonate (CaCO_3) (VWR International Ltd.) and 1 g of Commercial Baking powder were added to the collected gel with continues stirring for 2 min at room temperature.

The above mixture was transferred into plastic dish and dried at 180°C for 3.5 h. The obtained powder was added to 50 ml of distilled water at pH 8.8. Few drops of molar solution of HCl (imported by El-Ghonemy Group, Egypt) were added to the above mixture till pH 6.8. After filtration and washing for several times the obtained precipitate was dried at 60°C for 3 days. The dried solid was calcined at 900°C with a rate of 2.5/min. the sample is named (Soso).

2.3. Silica disks preparation

Silica samples of $5\text{ mm} \times 5\text{ mm} \times 3\text{ mm}$ were prepared by molding and comprising of silica powder.

2.4. Cement bioactive silica blend preparation

Cylinders of 4 mm in diameter and 6 mm in long were made from GIC for bioactivity test. The control GIC was prepared with self curing Glass Ionomer Cement (Medicem-liquid batch

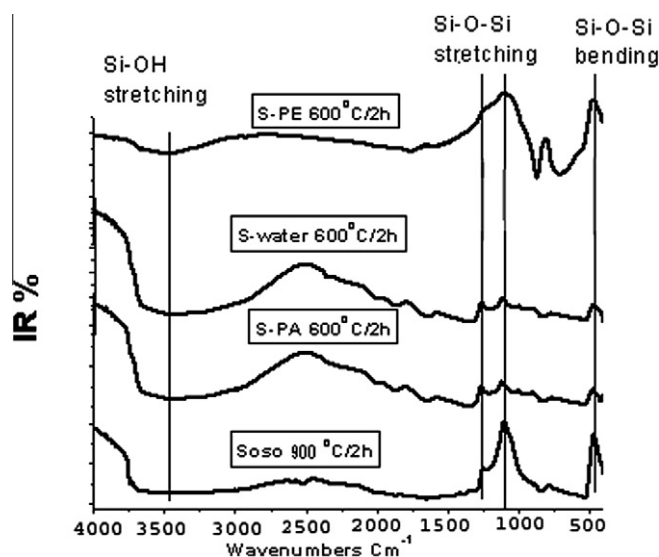


Figure 3 Shows the results of IR spectra of the under investigated silica powder prepared by the above mentioned methods before immersion in SBF.

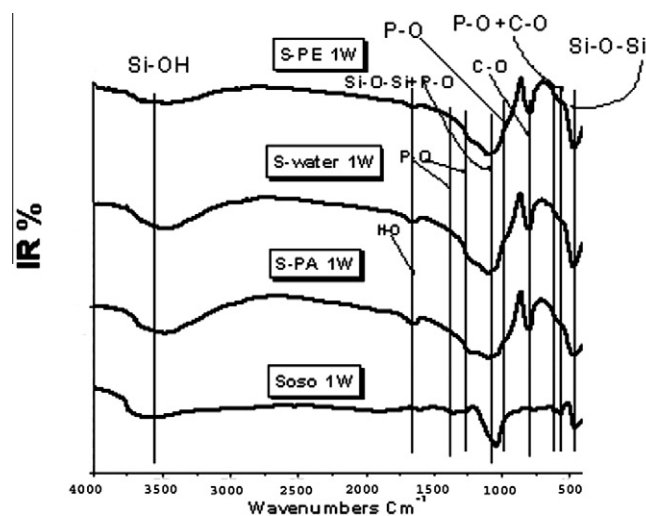


Figure 4 Shows the results of IR spectra of the under investigated silica powder prepared by the above mentioned methods after immersion in SBF for 1 week.

No. 461137 and powder – Neumuenster/Germany). The GIC containing bioactive silica was prepared from the same batch with (0.01, 0.02 and 0.04) wt.% with the prepared bioactive silica prepared samples. The bioactive silica and GIC powder were manually mixed and the required powder liquid ratios were prepared as recommended by manufacturer.

2.5. Soaking of the prepared samples in simulated body fluid

Samples were soaked in SBF that resembles the ionic composition of human plasma. It was confirmed that this fluid can fairly precisely reproduces apatite formation on the surfaces of various kinds of bioactive glasses and glass-ceramics in vitro [15]. After soaking the samples in simulated body fluid for 7 days, they were removed from the fluid and gently washed with deionized water. The specimens were dried at room temperature.

3. Results and discussion

3.1. X-ray diffraction (XRD) results

Fig. 1(a) shows the x-rays diffraction patterns of different silica prepared by different methods in amorphous phase and (b) shows the crystalline phase of silica due to high temperature of heat treatment.

3.2. Transmission electron microscope (TEM) images

Distinct morphologies for the prepared silica samples were a result of the different sintering temperatures and different dispersion medium. Fig. 2 illustrates the morphological development in the sintered samples with different methods; three kinds of grain shapes were recognized. Firstly large

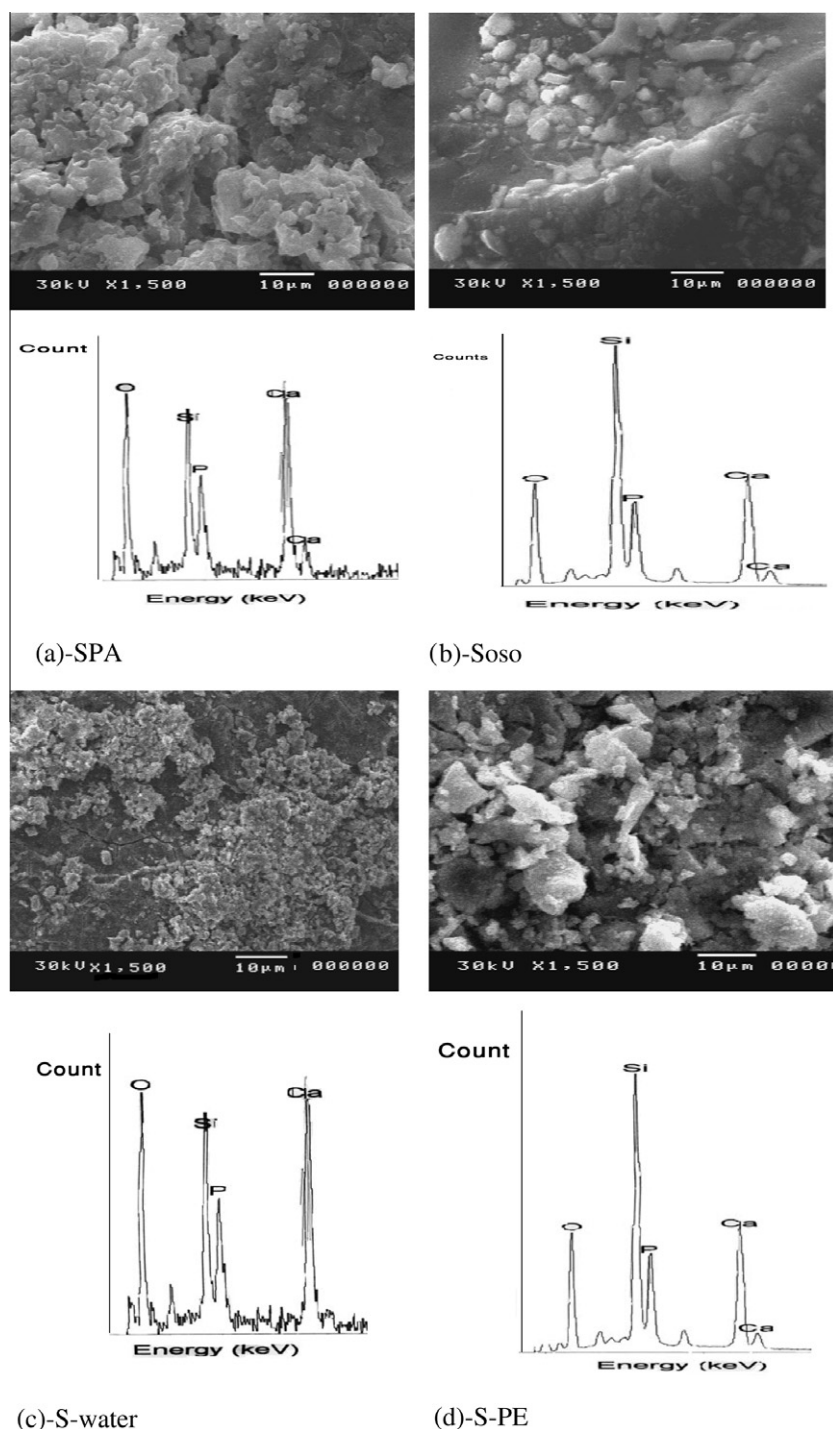


Figure 5 SEM micrographs and EDS of (a) S-PA, (b) Soso, (c) S-water and (d) S-PE after soaking in SBF for 1 week.

fragment-like grains with particle size ranging from 100 to 200 nm could be detected in Fig. 2a. This one is prepared using TEOS as a dispersed particle in the distilled water. Second finely dark sphere-like grains with particle size ranging from 20 to 70 nm were obtained in Fig. 2b. This dark sphere-like silica sample is prepared using sodium silicate in the distilled water. In Fig. 2c and d the silica is prepared using TEOS as a dispersed particle in the polyethylene glycol and polyacrylamide, respectively as a dispersion medium.

From the detailed TEM analysis on different samples, it can be shown that the particle size and morphologies of the prepared samples were found to be dependent on the preparation method, i.e. starting materials, and dispersion medium.

3.3. Infrared spectroscopy (IR) measurements

IR spectra in Fig. 3 which shows well polymerized silica as indicated by the sharpness of the strong Si–O–Si bands, i.e.

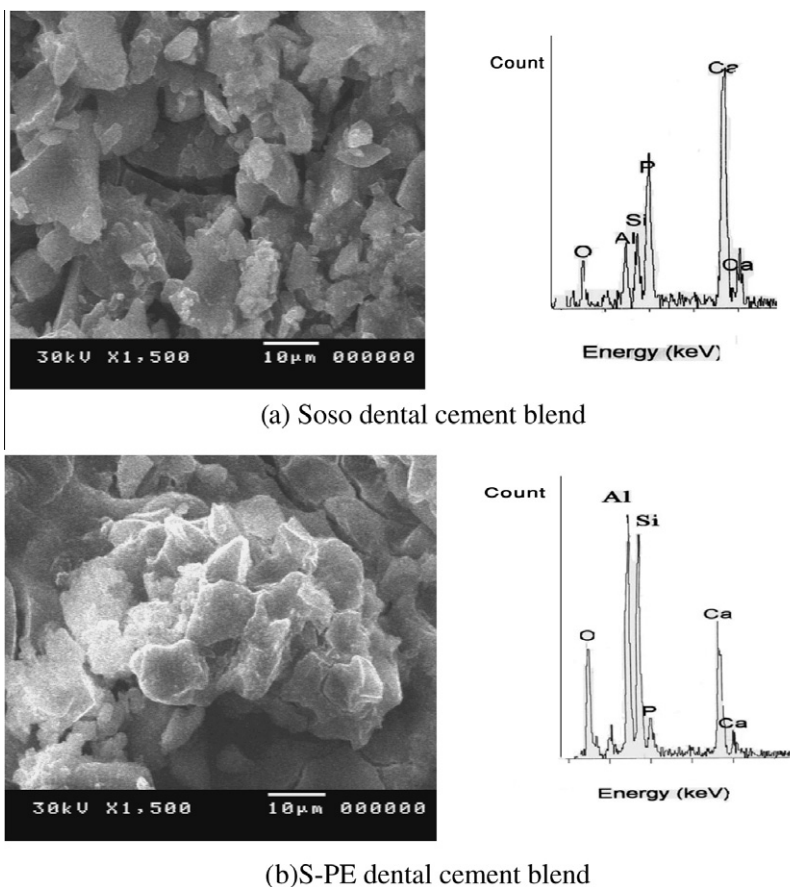


Figure 6 SEM image and EDs for dental cement blend containing 0.04 weight ratio of (a) Soso dental cement blend and (b) S-PE dental cement blend after soaking in SBF for 1 week shows a finely thin layer composed of a Ca-P phase.

Si–O–Si bending at 465 cm^{-1} and Si–O–Si asymmetric stretching at 1088 and 1246 cm^{-1} [20]. Other silica network bands were located at $560, 790,$ and 970 cm^{-1} . The 560 cm^{-1} band is associated with Si–O–Si bending of three- or four member rings (cyclic tri- and tetrasiloxanes) [20,21,9,13]. The 790 and 970 cm^{-1} bands are usually assigned to symmetric Si–O–Si stretching and Si–OH stretching, respectively [13]. The broad band at 3400 cm^{-1} is associated with H-bonded Si–OH stretching vibrations and H-bonded water [13,8,7,14]. The band at 3600 cm^{-1} could be assigned to isolate Si–OH stretching [19,17,2].

Fig. 4 shows the evolution of the surface, which points to a formation of an apatite-like layer indicating that the intensity of the silicate absorption bands at $1085, 606$ and 462 cm^{-1} decrease as a function of soaking time in SBF, while new bands that can be assigned to the phosphate group at $1043, 963, 603, 566$ and 469 cm^{-1} and to the carbonate group at $1490, 1423$ and 874 cm^{-1} appear after the immersion of the silica in SBF for a week.

3.4. Scanning electron microscope (SEM) with (EDs) results

SEM images in Fig. 5 shows the morphology of apatite (AP) layer formed on chosen silica samples after a week of soaking in simulated body fluid (SBF). The granules appear covered by a dense layer of closely packed particles or forming

a thin layer composed of a CaP phase. These finding suggest growth of preformed AP-precipitates during the immersion in SBF. We can note that in sample (Soso) the intensity of carbonated Ca–P layer precipitated on its surface is greater than on the surfaces of the other three samples (S-PE, S-PA and S-water).

EDs: shows the element concentrations, the formed layer consisted of Ca, P and Si which indicates that the formed layer is apatite (AP) layer formed on the chosen silica samples after a week of soaking in SBF. The granule surface is uniformly covered with fine precipitates composed of a CaP phase.

Results of SEM–EDS analyses (Fig. 5) show a detectable change in the surface morphology and composition (elemental distribution). The appearance of Ca and P concentrations indicate the formation of an apatite-like material. Determined amount of Ca and determined amount of P make the ratio between them is equal to (Ca/P ratio) 1.65 observed for the apatite-like layer is approximately the same value as that reported for bone apatite [4,11].

3.5. Scanning electron microscope (SEM) with (EDs) results of dental cement blend

Two silica cement blends samples were chosen to make SEM and EDs for them due to their smaller particle size. Fig. 6: SEM images and EDs spectrums for dental cement blends

contain 0.04 weight ratios of Soso and S-PE samples after soaking in SBF for 1 week.

For dental cement blend (Soso sample) SEM and EDs spectrum shows finely thin layer composed of a Ca-P phase and for dental cement blend (S-PE sample) shows that the granules appear covered by a dense layer of closely packed particles.

4. Conclusion

From the above results it can be concluded that:

The FT-IR showed that the prepared silica have variable bioactivities depending on the starting material, method of preparation, and dispersion medium. TEM images showed that the prepared bioactive silica existed in nanoscale. The prepared silica in nanoscale shows bioactivity more than those in greater scale as confirmed by SEM with EDs for both silica samples (Soso and S-PE) and their blends. The surface of the prepared silica samples and their blends after week of immersion in SBF were uniformly covered with fine precipitates composed of a calcium phosphate phases, incorporation of bioactive nano-silica with GIC enhances its bioactivity properties leading to preventing of marginal gap formation.

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