

# Inspired formation of silica nanostructure

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Bio-mineralization processes are now fully recognized as inspiring systems for the design of new materials. In case of silica, the formation of diatom shell or sponge spicule has attracted much attention in the last decade. Mineral phase is thought to be formed by the controlled assembly of nano-particles generated from diluted precursor solutions, in the presence of bio-molecular templates. The production of nano-structured silica from inexpensive starting materials and under ambient conditions was inspired from the nature. It is well known that diatom algae produce highly ordered meso and macro-pores silica structure, using bio-analogous reaction conditions and reagents, such as water glass and Polyethylene Glycol (PEG), Polyethylene Imine (PEI) and Polyethylene Oxide (PEO) at room temperature. This was done in accordance with that found in the nature i.e. the active species in the formation of natural bio-silica in diatoms was polyamine called (sillafin). The produced silica was characterized using X-ray diffraction, particle size analyzer, fourier transform spectrophotometer and transmission electron microscope. The powder products were pressed in tablets and examined for biological activity. The obtained results revealed that the produced silica was in nano-sized particles; the morphology and the size of the silica produced found to be controlled by the polymer used. Also biological activity affected by the nature of the polymer.

عملية التكوين الحيوي للمعادن في الطبيعة أصبحت الآن مصدر من مصادر الإلهام لتصميم مواد جديدة. في حالة السيليكا يوجد كائنات دقيقة جذبت الانتباه بقدرتها على ترسيب السيليكا من الأوساط الطبيعية التي تعيش بها. بعض الطحالب البحرية لها هذه القدرة المذهلة على تحضير السيليكا في أحجام متناهية الصغر. في عملية مستوحاة من سلوك هذه الطحالب يتم تحضير السيليكا في أحجام متناهية الصغر من مواد بادئة رخيصة ومتوفرة وعند ظروف ميسرة للتفاعل. يتم في هذا التفاعل استخدام بعض البوليمرات كأوساط للتفاعل تساعد على تكوين السيليكا مثل (PolyEthylene Glycol (PEG), PolyEthylene Imine (PEI) and PolyEthylene Oxide (PEO). وهذا يتم بناء على الحقائق العلمية التي أثبتت أن العامل المسنول عن هذه العملية في الطحالب هو بوليمر سمي sillafin. السيليكا المحضرة تم توصيفها باستخدام X-ray diffraction, Particle Size Analyzer, Fourier Transform Spectrophotometer and Transmission Electron Microscope. أيضا تم دراسة مدى توافقها بيولوجيا للتطبيقات البيولوجية. أوضحت النتائج أن أشكال وأحجام السيليكا المحضرة يعتمد على نوع البوليمر المستخدم.

**Keywords:** Bio-mineralization, Bio-molecular templates, Silica Nanostructure, Biological Activity

## 1. Introduction

Biological processes in nature could be considered as sources of inspiration for material scientists. Water lilies, butterfly wings or marine organisms skeleton could be examples to construct water repellent materials [1], photonic structures [2] or optical fibers [3] respectively. Also, biomaterials that were found in nature are inspiring not only in their structures but also in the way they are made. Unicellular organisms in nature are

capable of forming nano-structure materials, a well known example is magnetite  $Fe_3O_4$  nanocrystals in magnetotactic bacteria [4] which exist in cytoplasmic membrane of the cell and allow its passive orientation in the magnetic field of the earth. Another example in mammals is the microstructure of tooth enamel in the rat, which is found to be composed of spaghetti-shaped crystals of carbonated apatite and the other example is a sponge with different size pores in the arm of the star *Ophiocoma wendtii* fig. 1-a, b [5]. A

striking example of natural nanostructure materials is the silica exoskeleton formed by unicellular algae named diatom. This exoskeleton consists of  $\text{SiO}_2$  nano particles in a porous network structure [6-9].

Silicon oxide minerals and silica based materials are not only considered as the main constituent of the earth crust but also have very wide applications in industries, technology and domestic purposes. Chemically produced silica in industry need sever conditions (high temperature, high pressure and /or strong acidic or alkaline medium) [10]. Diatoms are able to produce nano- structure silica from the eco-system where they live under ambient conditions. Studies on such behavior attract the scientists to clarify the biomenralization process and it was found that the agent controlling the preparation of silica in diatom is polyamine called sillafin. An important progress in studying the role of proteins in mediating the formation of silica in different morphologies by the use of different agents [11]. It was found that amine- containing silicification agents were used to produce helical silica nanotubes [12], meanwhile silica nanostructured thin film could be obtained by using tertiary amine – containing agents [13]. On the other hand p-lysin aggregates allowed the formation of micron-size silica hollow sphere [14] and mesoporous silica network could be obtained using arginine [15].

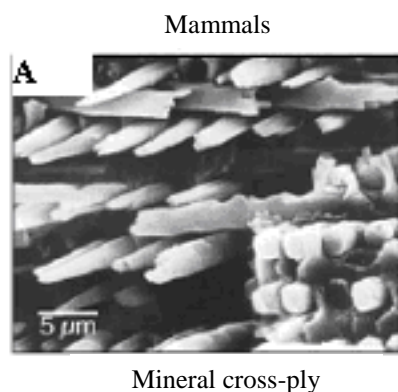
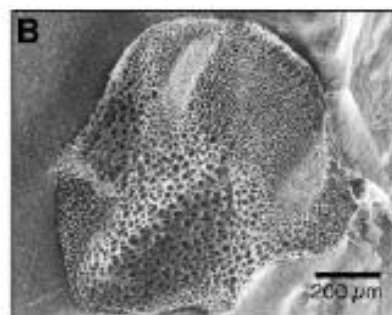


Fig. 1-a. Example from mammals is observed in the incisor of a rat, which generates crossed elongated crystals of carbonated apatite.



Spongy single crystal

Fig. 1-b. Sponge with different size pores in the arm of the star *Ophiocoma wendti*.

Inorganic materials, although diverse in composition, lack the structural and morphological variety that is one of the characteristics of polymeric, supramolecular and self-assembled structures [16-20]. A great deal of research effort has been devoted towards the development of efficient and innovative fabrication methods to obtain inorganic materials with well-defined morphologies, because of their potential applications in e.g. catalysis, chromatography, absorbance, drug-delivery, etc. To develop novel inorganic materials closely resembling these organic architectures, supramolecular structures such as organogels based on low molecular mass amines, and amphiphilic mesophases have been transcribed into inorganic materials [21, 22].

This work focus on the preparation, characterization and biological evaluation of nano-structured silica mediated by different polymers such as polyethylene glycol, polyethylene imine and polyethylene oxide at room temperature.

## 2. Materials and method

### 2.1. Materials

Sodium silicate.Nona hydrate extra pure  $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$  (water glass) was purchased from Marine Chemicals. PolyEthylene Glycol (PEG) with molecular weight 6000, PolyEthylene Imine (PEI) molecular weight 60000, 50% wt aqueous solution and PolyEthylene Oxide (PEO) powder with molecular weight 100000 were purchased

from across organics. Stimulant body fluid, SBF was prepared using analytical grade reagents listed in table 1.

## 2.2. Method

The aim of this work is to mimic diatom biosilica formation by using dilute sodium silicate solution as cheap and readily available source of silica. PEG, PEI and PEO were used as mediating polymers.

A dilute sodium silicate solution 10% in distilled water was stirred at room temperature for 3 hours. Few drops of 10 % polymer solution was added drop wisely in a weight ratio 10:1 silicate: polymer. A gelatinous precipitate of silica was formed. The precipitate was filtered, washed and dried at 120 °C over night. The dry products were characterized using X-Ray Diffraction Study, Particle Size Analyzer, Fourier Transform spectrophotometer and Transmission Electron Microscope. The powder products were pressed in disks and examined for biological activity.

## 2.3. Biological activity study

The obtained silica were pressed in form of disks and immersed in stimulant body fluid SBF in a way that a disk of one gram of silica in 100 ml of SBF. After one week of immersion the disk were dried and examined by EDX technique to identify the precipitated materials to evaluate the biological activity and the influence of polymer used in preparation of silica. The chemical modification and the elemental composition of the prepared silica due to the biological treatment were studied by Fourier transform infrared spectrophotometry and SEM- EDX analysis technique.

Table 1  
SBF reagents amount

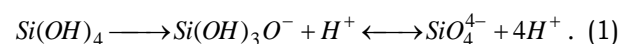
Amount	Reagent	Order
8.035 g	NaCl	1
0.355 g	NaHCO <sub>3</sub>	2
0.225 g	KCl	3
0.231 g	K <sub>2</sub> HPO <sub>4</sub> .3H <sub>2</sub> O	4
0.311 g	MgCl <sub>2</sub> .6H <sub>2</sub> O	5
39 ml	1.0M-HCl	6
0.292 g	CaCl <sub>2</sub>	7
0.072 g	Na <sub>2</sub> SO <sub>4</sub>	8
6.118 g	Tris	9
0-5 ml	1.0M-HCl	10

## 2.4. Stimulant body fluid preparation

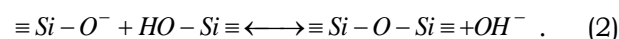
The SBF solution was prepared by dissolving reagent chemicals of NaCl (8.035g), NaHCO<sub>3</sub> (0.355 g), KCl (0.225 g), K<sub>2</sub>HPO<sub>4</sub>.3H<sub>2</sub>O (0.231 g), MgCl<sub>2</sub>.6H<sub>2</sub>O (0.311 g), CaCl<sub>2</sub> (0.292 g) and Na<sub>2</sub>SO<sub>4</sub> (0.072 g) into less than 1000 ml deionized water. The fluid was buffered at physiological pH 7.40 at 37°C with tri- (hydroxymethyl) aminomethane (6.118 g) and hydrochloric acid, and the solution was made up to 1000 ml with additional water. The ion concentrations of SBF here are very close to those of human blood plasma.

## 3. Results and discussion

Silica formation involves two steps; the first step is the polymerization of molecular precursor [23].



The second step is further condensation



(≡ Represents bonds with -OH, -O- and/or -O-Si groups).

In case of induced precipitation of silica using a polymer as salicifying agent, the polymer used plays three roles:

1. It serves as flocculation agent in the formation of silica sols.
2. The interaction between silica and polymer steer polymerization
3. Polymer induces phase separation in which there are polymer rich and silica rich phases [24, 25].

### 3.1. X-Ray diffraction study

X-ray diffraction analysis for samples was carried out using *X-ray Diffractometer* of (Schimadzu-7000, U.S.A.). X-Ray Diffraction (XRD) analysis was performed. To evaluate the phase composition, XRD spectra were obtained with a 30 kW Rigaku rotating anode diffractometer fitted with a copper target. XRD spectra were obtained between 20° and 80° (2θ) in continuous scan with 4 degree/ min.

using the standard  $\theta - 2\theta$  geometry. The spectra were compared to standard spectra from the Joint Committee on Powder Diffraction Standards (JCPDS) database. X-ray diffractogramme fig. 2 show the significant pattern of silica produced from PEG, PEI and PEO at  $2\theta$  23.43. The crystallite size calculated from scherr equation [26] and found to be 1.7 nm, 1.854 nm and 1.85 nm for PEG, PEI and PEO respectively. The slight decrease in the crystallite size could be due to the less polymer chain length in case of PEG.

Scherr equation

$$\beta = 0.89\lambda / L \cos \theta \quad (3)$$

Where  $\beta$  is the full width at half maximum peak (FWHM) of diffraction peak,  $\lambda$  is the wavelength of X-ray employed to do this measurement,  $L$  is the thickness of film, and  $\theta$  is the peak position of Bragg peak.

### 3.2. Particle size analyzer

Particle Size Analyzer (PSA), Beckman Coulter-USA, data giving the mean particle size of the produced silica by using PEG, PEI and PEO to be 353 nm, 289 nm and 408 nm respectively. The lower mean particle size of silica produced by using PEI could be explained by the assumption cited in previous literature that the basic medium decrease the aggregation of the particles. In basic medium some of the silanol groups Si-OH deprotonated

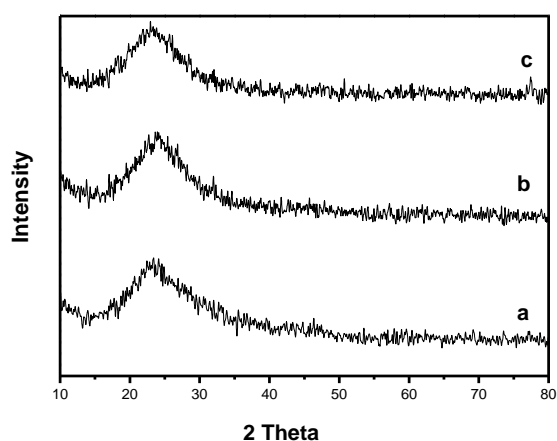


Fig. 2. XRD diffraction Pattern of Silica mediated by PEG, PEI and PEO denoted as a, b and c respectively.

so the particles bear negative charges, these negative charges hinder the aggregation due to Coulombic repulsive forces [27].

### 3.3. Transmission Electron Microscopy (TEM)

The morphology of the synthesized powders was studied by Transmission Electron Microscope (TEM) Jeol JEM 1230 with max. mag. 600kx, resolution 0.2 nm which is presented in fig. 3. The samples were prepared by sonication for 30 minutes. The samples are denoted a,b and c for silica prepared using PEG, PEI and PEO respectively. It was found that the morphology and the size of the produced silica differ with the used polymer. The silica mediated by polyethylene glycol has the rod shape with 150-200 nm in length and 30-50nm in diameter and silica produced from polyethylene imine has a shape of small rods with about 50 nm lengths and 10 nm diameters. Meanwhile silica mediated by polyethylene oxide has the rod shape with elongated length and diameter 30-50 nm. This difference could be attributed to the change of molecular weight of the polymer that is indicating the polymer chain length as well as the nature of the polymer as mentioned before that PEI produced negative charge hindered the aggregation of silica particles [25].

### 3.4. Chemical modification and elemental composition study

#### 3.4.1. Fourier transform Infrared spectroscopy FTIR

FTIR analysis was performed using Fourier transform infra red spectrophotometer FTIR-8400 S Shimadzu- Japan. FTIR spectra showed the characteristic pattern of Silica mediated with PEG, PEI and PEO denoted as G, I and O respectively and presented in fig. 4. The SiO<sub>2</sub> stretching vibration is recorded to be at 1140 cm<sup>-1</sup>. The peak observed at 1638 cm<sup>-1</sup> indicates the presence of physically adsorbed water in the silica samples. Also the broad peak at 3000 cm<sup>-1</sup> is attributed to adsorbed H<sub>2</sub>O molecules within the crystal structure of Silica. The FTIR pattern after biological treatment show no significant changes and this could be explained by the presence of the

stretching vibration of phosphate group at  $1092\text{ cm}^{-1}$  and  $1097\text{ cm}^{-1}$  which mean that they exist in the same region of the vibration corresponding to  $\text{SiO}_2$  vibration. Meanwhile the area under the peaks of the biologically treated samples increased which may indicate the interference of the peaks corresponding to both  $\text{SiO}_2$  and phosphate group precipitated from the SBF media.

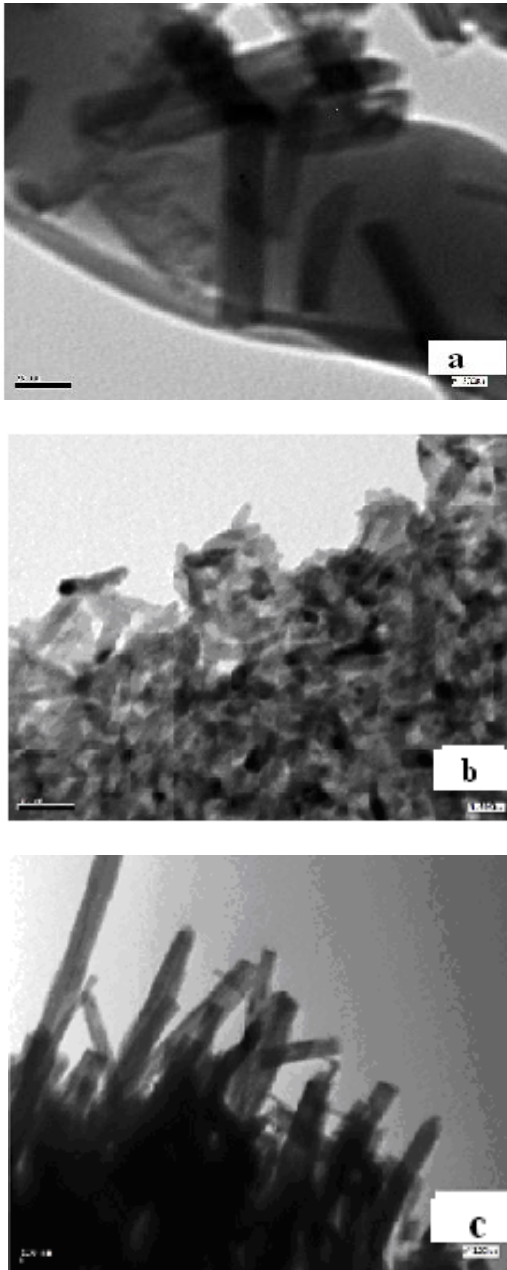


Fig. 3. TEM images of Silica mediated by PEG, PEI and PEO denoted as a, b and c respectively.

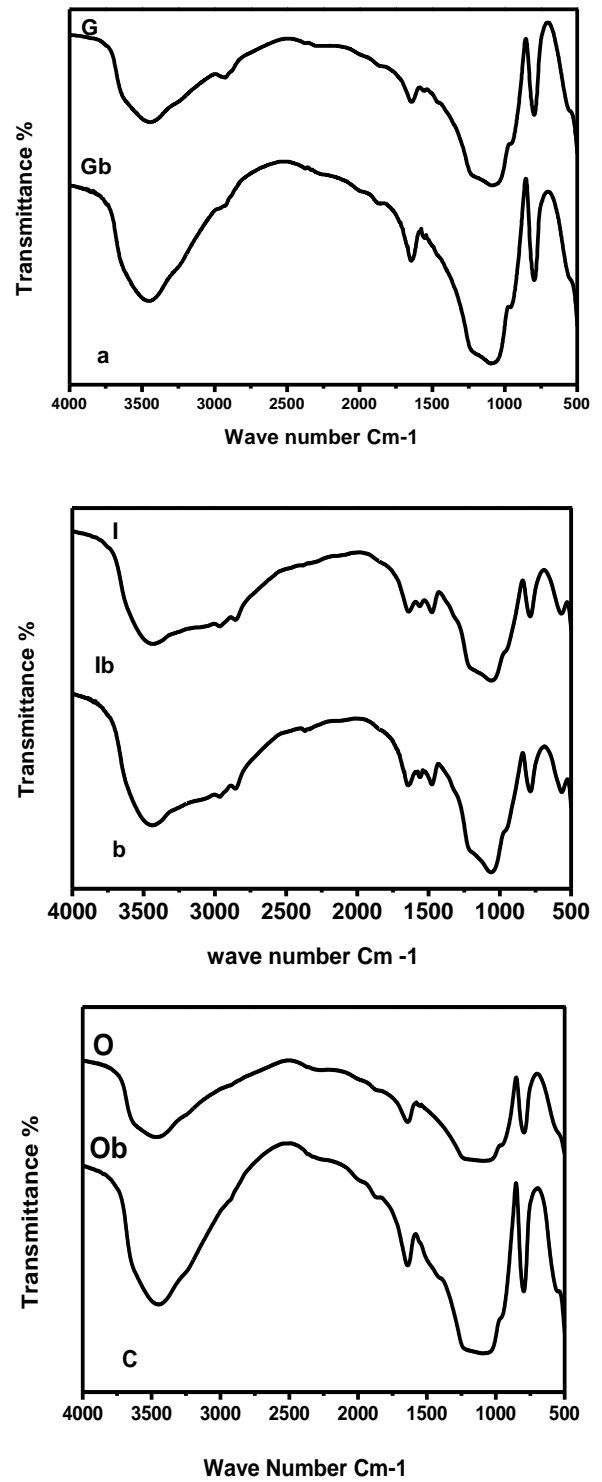
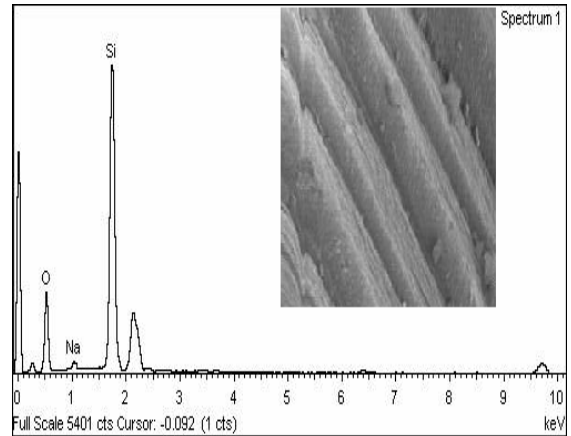


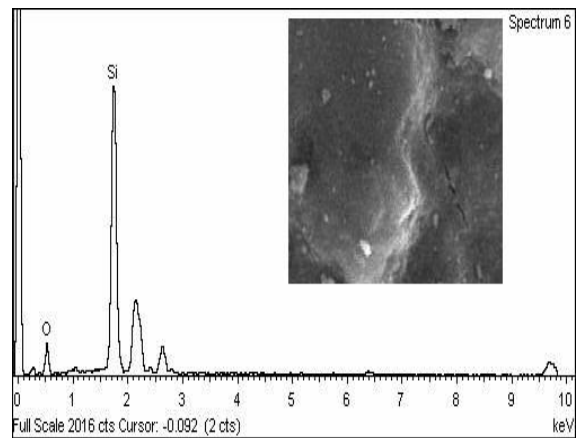
Fig. 4. FTIR spectra of Silica mediated by PEG, PEI and PEO before biological treatment (G, I and O) and after biological treatment (Gb, Ib and Ob) denoted as a, b and c respectively.

### 3.4.2. Scanning electron microscope with EDX analysis

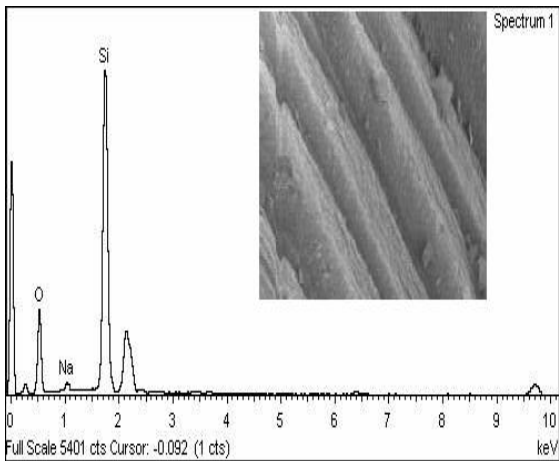
According to SEM/EDX analysis, were performed using Scanning Electron Microscope (JEOL JSM 6360LA, Japan) which is presented in fig. 5, the silica prepared with the use of PEG, PEI and PEO before biological treatment denoted G, I and O, show the Silica and oxygen which form composition of pure Silica. The other peaks observed are attributed to carbon (C) and gold (Au), due to the carbon grid of the sampling and sputtered gold layer. There is a peak corresponding to sodium, which may be attributed to the precursor used. The samples after biological treatment denoted Gb, Ib and Ob show in addition to the silica composition Calcium and Phosphorus due to the precipitation of calcium phosphate from SBF, but this precipitation appeared strong in case of using PEO as mediating polymer and appeared as traces in case of using PEG and PEI. This finding indicate that the Silica produced from PEO as mediating agent has the highest biological activity among the produced silica, this could be explained by the fact that PEG and PEI have OH and NH function groups which ionized leaving negative charge on the surface of the polymer that may affect the produced silica by applying negative charge on their surface. This negative charge resists the precipitation of the phosphate group due to the repulsive forces.



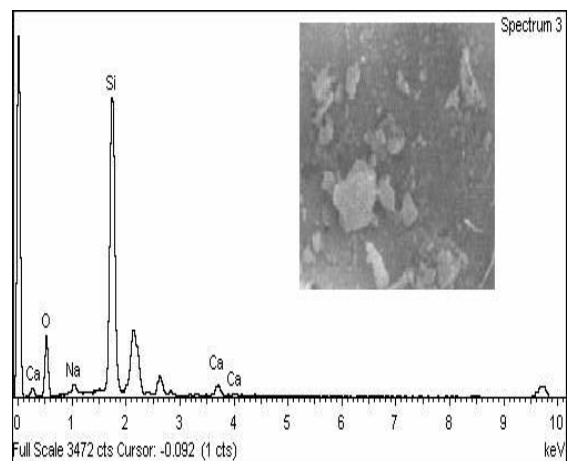
I



O



G



Gb

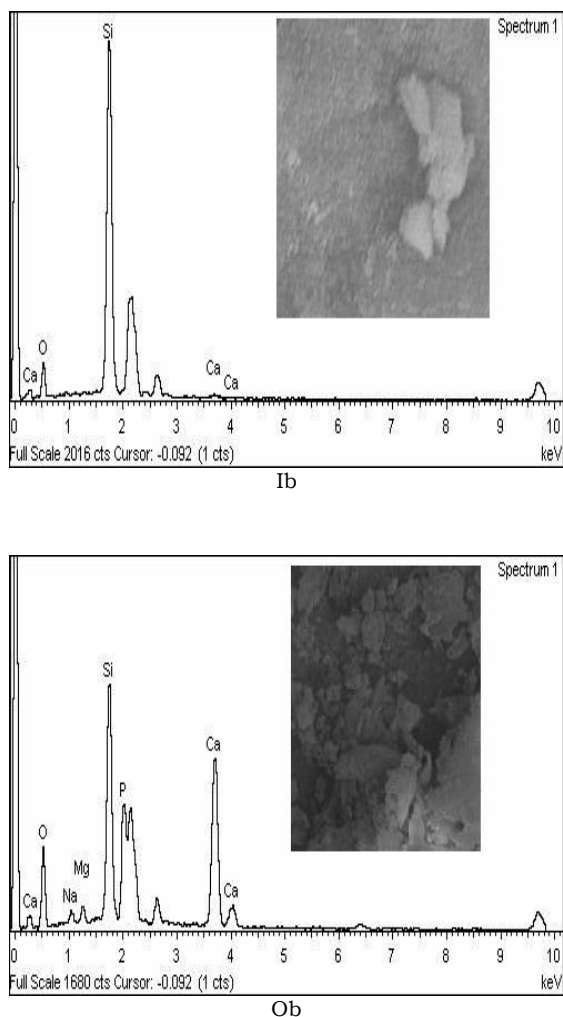


Fig. 5. EDX spectra of Silica mediated by PEG, PEI and PEO before biological treatment (G, I and O) and after biological treatment (Gb, Ib and Ob) denoted as a, b and c respectively.

#### 4. Conclusions

The biomimetic approaches for silica formation as summarized above are very encouraging, as they strongly suggest that the use of principles learned from Nature and in particular from diatom biomineralization will enable the elaboration of novel silica-based materials. These materials could be controlled in their shape and physical structure to serve certain purpose by controlling the mediating agents. The nature of the polymers used as their molecular weight, the charge they have, their hydrophilicity etc., control the product they mediate.

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