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**EVALUATION OF THE PROTEIN OF NANOSTRUCTURED SILICA BASED  
BIOMATERIAL**

**AFROOZEH A<sup>1\*</sup>, ZEINALINEZHAD A<sup>2</sup>, POURMAND S. E<sup>3</sup>**

**1:** Young Researchers and Elite Club, Jahrom Branch, Islamic Azad University, Jahrom, Iran

**2:** Chemistry Department, Anar Branch, Islamic Azad University, Anar, Iran

**3:** Department of Optics and Lasers Engineering, Estahban Branch, Islamic Azad University,  
Estahban, Iran

**ABSTRACT**

This paper is focussed on the evaluation of the protein binding capability on silica based material containing  $\text{CaMoO}_4$  and HAP nanocrystals. This sample was considered in the present study, since the presence of small nanocrystalline apatite crystallites in size is required in a biomaterial for ensuring the both protein adsorption and samples bioactivity. The evaluation of the atomic composition and protein surface coverage was examined by achieving information from the outermost few nanometres surface layer by XPS. The ratio between carbon and nitrogen on the material's surface after protein adsorption was found to be reasonably close with the value obtained from the measurement of pure BSA, this being a direct consequence of protein adherence. The comparative analysis of FTIR spectra recorded from lyophilized proteins and from biomaterial surface after protein adsorption was considered in order to obtain information about the secondary structure of the proteins. The protein surface coverage was also confirmed by SEM morphological characterization. The results obtained by in vitro tests indicate that the nanostructured  $\text{SiO}_2\text{-CaO-P}_2\text{O}_5\text{-MoO}_3$  system has a great potential for bone tissue engineering with respect to the ability to bind to protein.

**Keywords: BSA Functionalization, Protein Adsorption, Biomaterial**

## INTRODUCTION

This study reports the bovine serum albumin (BSA) interaction with the surface of molybdenum doped  $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$  sample, based on the properties of this protein to inhibit thrombus formation [4] and to passivate the surface and reduce significantly the acute inflammatory response of the biomaterial [4-6]. Bioactive glasses containing molybdenum oxide attracted increasing interest in biomaterials field because they join the bioactivity and biocompatibility of glass to the possibility of releasing molybdenum ions in a specific body site [6, 7]. Molybdenum is an essential element required for growth of most organisms (i.e. humans, microbes, animals and plants) [8] with a functional role as an active cofactor for several enzymes involved in oxidation/reduction reactions [9]. The apatite mineralization on a bioactive glass is involved in the protein adsorption on the surface. Moreover the calcium molybdate crystalline phase positively influences the biological performance of silica based materials [6]. In order to evaluate the BSA affinity there were performed Scanning Electron Microscopy (SEM) morphology investigation and FTIR study of the protein secondary structure within the adsorbed layer. Moreover the evaluation of the atomic

composition and protein surface coverage was examined by achieving information from the outermost few nanometres surface layer by X-ray Photoelectron Spectroscopy (XPS).

## METHODOLOGY

The  $60.6\text{SiO}_2\cdot 30.3\text{CaO}\cdot 9.1\text{P}_2\text{O}_5$  (mol %) doped with 1%  $\text{MoO}_3$  amorphous sample was synthesized using tetraethoxysilane ( $\text{Si}(\text{OC}_2\text{H}_5)_4$  - TEOS) in distilled water-ethanol solution (TEOS:H<sub>2</sub>O:EtOH=1:2:0.5), triethyl phosphate ( $\text{C}_6\text{H}_{15}\text{O}_4\text{P}$  - TEP), calcium nitrate ( $\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$ ) and ammonium molybdate ( $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ) dissolved in distilled water. The nitric acid was chosen as catalyst. The gel obtained after 3 days was allowed to age for another 3 days at room temperature, and after aging it was dried at 110 °C for 24 hours. 600 °C thermal treatment has been applied in air for 4h in order to develop HAP and  $\text{CaMoO}_4$  nanocrystals in the amorphous silica based matrices.

The sample was incubated for 5 hours at 37 °C in a bovine serum albumin (BSA) enriched phosphate-buffered solution (1 mg/ml). The more common BSA was used as a model protein in this study since the amino acid sequence and structure of human and bovine serum albumin display a large similarity [6]. Both human and bovine serum albumins are large globular proteins about 66 kDa [12].

Before the biocompatibility evaluation tests the sample was washed three times with buffer solution to remove the detachable protein molecules from the surface, filtered and dried at 37 °C for 24 hours.

The morphology of the samples before and after protein surface functionalization was explored by SEM using a FEI QUANTA 3D FEG dual beam in high vacuum work mode. FTIR spectroscopic analyses were realized with a JASCO 6200 FTIR spectrometer with a spectral resolution of 4 cm<sup>-1</sup>. The FTIR spectra were recorded from powder samples mixed with KBr and pressed into pallets. XPS measurements were performed using a SPECS PHOIBOS 150 MCD system equipped with monochromatic AlK $\alpha$  source (250 W,  $h\nu = 1486.6$  eV), hemispherical analyser and multichannel detector. The typical vacuum in the analysis chamber during the measurements was in the range of 10<sup>-9</sup> - 10<sup>-10</sup> mbar. An electron flood gun was used for charge neutralization. The binding energy scale was charge referenced to C 1s photoelectron peak at 284.6 eV. Elemental composition was determined from spectra acquired at pass energy of 100 eV.

## RESULTS AND DISCUSSION

The **Figure 1** presents the morphology of the investigated sample before and after protein

attachment. The surface morphology, investigated by SEM (**Figure 1a**) evidenced the CaMoO<sub>4</sub> nanocrystals, their shape being the same as the one reported in the literature [13]. The protein layer coating the sample's surface is well evidenced in the SEM image (**Figure 1b**).

The surface analysis is critical because the material surface is in direct contact with biological environments. The ratio between carbon and nitrogen on the material's surface after protein adsorption is comparative with the value obtained from the measurement of pure BSA, this being a direct consequence of protein adherence. Furthermore the shape of O 1s, C 1s and N 1s spectra are similar to the one recorded for pure BSA revealing the presence of peptide carbons and peptidic oxygen on the functionalised sample as a proof of protein loading in great amount. For protein functionalized sample a decrease in silicon and molybdenum photoelectron peaks (**Table 1**) emphasizes that the surface is covered with BSA.

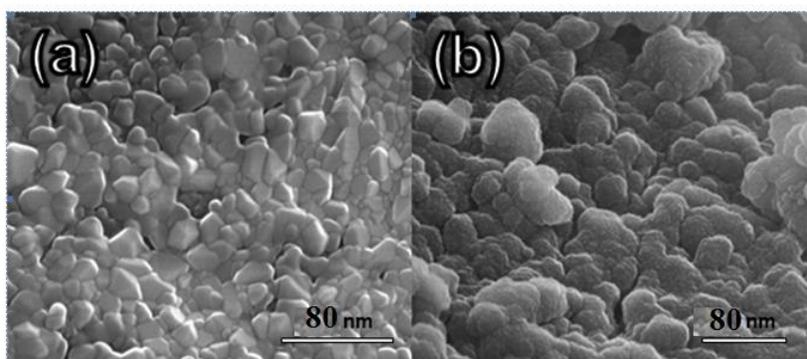
The affinity of BSA to the surface was confirmed also by FTIR spectroscopy (**Figure 2**). In the FTIR spectrum of functionalized sample can be observed a slightly increase in the absorption band centred at 1650 cm<sup>-1</sup>, representative for amide I (C=O) stretching mode. Moreover, the absorption band centred

at  $1550\text{ cm}^{-1}$  can be assigned to the presence of amide II functional group (60% N-H bending and 40% C-N stretching modes) [6, 15]. A proper deconvolution of the protein FTIR spectra, in order to obtain quantitative structural information cannot be done because of the bands around  $1635$  and  $1420\text{ cm}^{-1}$

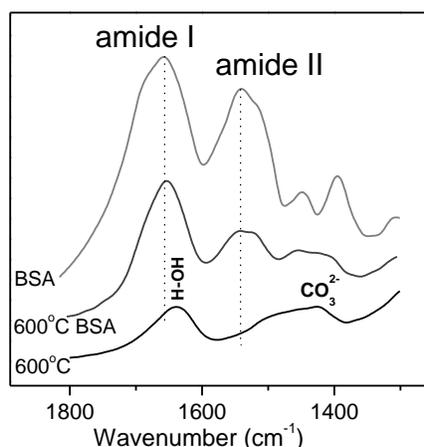
(corresponding to  $\nu_1$  bending vibration of trapped water molecules [16] and  $\nu_3$  asymmetric stretching vibration of  $\text{CO}_3^{2-}$  traces [17, 18], respectively) that are present in the spectrum before the sample being subject to functionalization.

**Table 1: Elemental surface composition estimated from XPS survey spectra before and after immersion in BSA solution, in comparison with lyophilized BSA**

Sample	O	C	Ca	N	Mo	P	Si	C/N
Investigated material	51.83	3.32	11.78	2.67	0.98	6.56	21.72	1.38
BSA on the investigated material	42.9	29.6	10.7	8.2	-	9.5	1.4	3.53
BSA	18.5	64.7	-	16.9	-	-	-	3.88



**Figure 1: SEM images of sample before (a) and after BSA adsorption (b)**



**Figure 2: The FTIR spectra recorded in the amide region for the sample before and after BSA functionalization compared with the pure BSA**

## CONCLUSIONS

The interaction of bioactive material with BSA is clearly pointed out by N 1s and C 1s XPS signals, specific to proteins. After immersion in protein solutions, the O 1s photoelectrons correspond significantly to proteins peptidic oxygen. Moreover the decrease in silicon and molybdenum photoelectron peaks for protein functionalized sample emphasizes the surface coverage by BSA. FTIR spectroscopy confirmed also the affinity of BSA to the surface by the presence of both amide I and II vibration bands. The protein layer coating the sample's surface is well evidenced in the SEM image. In terms of protein adsorption the analyses performed in this study confirm the adherence of BSA on the surface of the nanostructured SiO<sub>2</sub>-CaO-P<sub>2</sub>O<sub>5</sub>-MoO<sub>3</sub> material that is considered beneficial for further interaction with surrounding cells.

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