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Bioactive Glass For Synthesis Nanofiber As A Bone Replacement Graft Material

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1. Abstract

Bioactive glass, a biologically useful material that provides a strong bond with vital tissues of the body such as bone tissue and teeth, has attracted the attention of doctors and researchers.
nanofiber network of a bioactive glass/carboxymethyl cellulose/β-cyclodextrin (BAG/CMC/β-CD) nanoceramic is fabricated by electrospinning for application as a bone replacement graft material.The diameter of this as-prepared nanofiber is 10nm. Nanocomposites and nanofiber have been characterized by Fourier transform infrared (FT-IR) spectroscopy, Powder X- ray diffraction (XRD), Scanning Electron Microscopic images (SEM), Energy dispersive X-ray (EDX) analysis and Transmission electron microscopy (TEM).

2. Keywords:
Bioactive Glass, CMC, B-Cyclodextrin, Electrospinning, Nano Ceramic, Nano Fiber

3. Introduction

Bioactive glasses have higher dissolution rate than conventional glasses thus faster degradation in body fluid (BF)[1,2]. This property has led to their use as hard tissue implants for bone tissue engineering [3]. Sol-gel glasses possess higher porosity, apatite-forming ability, and increased surface area compared to the melt-quenched glasses, which have the advantage of higher mechanical properties [4,5]. Bone consists mainly of mineralized tissue in the form of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ [6]. Degradation of BAG takes place via hydrogen exchange in BF and leads to the alkalinity of the environment and the formation of $Si(OH)_4$ [7]. This process leads to the formation of a gel on the bone as a matrix for hydroxyapatite and improves bone tissue repair[7,8]. In addition, raising the pH of the solution causes the use of bioactive glass as an antibacterial agent [9]. Sol-gel processing is currently method to achieve BAG with higher bioactivity[10]. Water insolubale β-cyclodextrin (β-CD) is a polysaccharide derived from starch, which is of special interest in biomedicine due to its high tendency to form complexes with many molecules [11, 12]. Esfanjani et. al used nanoalumina/cyclodextrin as an absorbent of toxic metals and showed that the synthesized nanocomposite has high stability and efficiency[13]. On the other hand, The β-cyclodextrin/surfactant interaction is an important factor in drug delivery[14], food and agricultural industries [15].

Carboxymethyl cellulose (CMC) is one of the derivatives of cellulose (carboxylation of OH groups on cellulose), which is widely used in the food, pharmaceutical and biomedicine areas due to its excellent properties[16]. According to the results of several research groups, the great tendency of CMC to be complex with other molecules due to the presence of carboxymethyl group solves the problem of its insolubility in aqueous medium[17]. Naderi et.al investigated the effects of CMC/β-cyclodextrin/chitosan(CMC/β-CD/CS) as nanocarrier for methotrexate drug delivery[18]. They showed the maximum drug release in CMC/β-CD/CS hydrogel were obtained 92.7 at pH 7.4. The studies illustrated that nanocomposite could be used for novel methotrexate drug delivery systems.

Nanofibers can be generated from different polymers and hence have many possible commercial and medical applications such as in tissue engineering[19, 20], drug delivery [21] and cancer diagnosis[22]. Electrospinning is the most commonly used method to generate nanofibers because of the ability to generate ultrathin fibers with controllable diameters, compositions, and orientations. The production of artificial fibers using electrostatic forces was first reported by Formahals in 1934 and then completed by other researchers by removing its limitations [23,24]. In this paper, we present the synthesis of BAG/CMC/ β-CD nanoceramic via sol-gel method. The characterization results will be discussed. By controlling pH, temperature and stirring rate in the reaction as well as the speed of exiting the nanoceramic solution in the electrospinning process, biocompatible nanofibers with the ability to be implanted in the body were synthesized.

4. Experimental

4.1. Materials and Methods

Polyvinyl alcohol(PVA, C_2H_4O) n, tetraethylorthosilicate($C_8H_{20}O_4Si$), calcium nitrate ($Ca(NO_3)_2 \cdot xH_2O$), sodium nitrate ($NaNO_3$), ammonium dihydrogen phosphate ($NH_4(H_2PO_4)$), β-cyclodextrin ($C_8H_{15}NaO_8$) and

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carboxymethylcellulose ([$C_6H_{10}O_5$]-n) was purchased from Merck. The formation of the nano ceramics and nano fibers was identified and confirmed using Fourier transform infrared spectroscopy (FTIR) (Spectrum two, Perkinelmer) in the range of 4000–400 cm^{-1}.Transmission electron microscopy (TEM) (Zeiss-EM10C-100 KV , Germany) was used to study the size and shape of bioactive glass nanostructures. To study the structure and phase present in the prepared bioactive glass/CMC/β-CD nano fibers, X-Ray diffraction (XRD) technique (X' pertpro, Panalystical, at the wavelength of λ=1.54 A° with Cu-Kα radiation (30 kV, 30 mA) in the 2θ range from 5° to 80°) was utilized.

Reference	Title
(25)	Effects of parameters on nanofiber diameter determined from electrospinning model Bioactive glass fiber fabrication *via* a combination of sol-gel process with electro-spinning technique
(26)	Electrospinning and Electrospun Nanofibers: Methods, Materials, and Applications
(27)	Preparation and Characterization of Carboxymethyl Cellulose-Based Bioactive Composite Films Modified with Fungal Melanin and Carvacrol
(28)	Incorporation of inorganic bioceramics into electrospun scaffolds for tissue engineering applications: A review
(29)	Bioceramic fibrous scaffolds built with calcium silicate/hydroxyapatite nanofibers showing advantages for bone regeneration
(30)	Synthesis and characterization of electrospun cerium-doped bioactive glass/chitosan/polyethylene oxide composite scaffolds for tissue engineering applications
(31)	

The morphology of the surface of the nano bioceramics and nano fibers were performed by the scanning electron microscopy (SEM; VEGA/TESCAN, Czech) equipped with EDS (Rantek, America). Electrospinning, a viable technique to produce nanofibers, was carried out by ES 1000.

4.2. Preparation of Bio Active Glass/Carboxymethyl Cellulose (BAG/CMC) nanobioceramic

The bioceramic BAG/CMC was prepared by using the sol-gel method[32-34] via dispersing carboxymethyl cellulose (Mw =250,000) (1 g) in deionized water and stirring for 1h at 80ºC and pH 2. 20ml of tetraethylorthosilicate (TESO) was added to the solution and stirred at room temperature. After 1h, 8.75g calcium nitrate, 6.75 g sodium nitrate and 1.2g ammonium dihydrogen phosphate were added the solution, subsequently and stirred for 1h. After ultrasound sonication processing, the obtained gel dried in oven at 100ºC.

4.3. Preparation of Bio Active Glass/ β-Cyclodextrin (BAG/ β-CD nano bioceramic

β-Cyclodextrin(2g) was dispersed in deionized water and stirred for 1h at 80ºC and pH 2. As the previous step, 20ml of tetraethylorthosilicate (TEOS) was added to the solution and stirred at room temperature. After 1h, 8.75g calcium nitrate, 6.75 g sodium nitrate and 1.2g ammonium dihydrogen phosphate were added the solution, subsequently and stirred for 1h. Finally, the obtained gel dried in oven at 100ºC.

4.4. Preparation of Bio Active Glass/ Carboxymethyl Cellulose/β-Cyclodextrin (BAG/CMC/ β-CD nano bioceramic

For synthesis of BAG/CMC/β-CD, β-Cyclodextrin (2g) and Carboxymethyl Cellulose (2g) were dispersed in deionized water and all the steps to prepare the nano bioceramic are the same as the previous description.

4.5. Preparation of Bio Active Glass/ Carboxymethyl Cellulose/β-Cyclodextrin (BAG/CMC/β-CD nano fiber

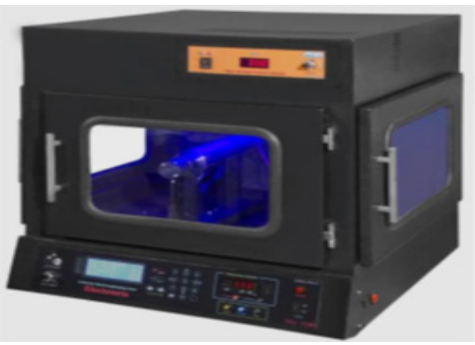
Each of the prepared nano bioceramic solutions was placed in 5ml syringes. Then the syringes were applied for electrospinning process. The electrospinning process involves the application of a strong electrostatic field to a capillary connected with a reservoir containing a nano ceramic solution [27]. It consists of a syringe with a metal capillary (diameter 2 mm) and a pressure supply on the piston of the syringe.

The electrospinning settings are as follows:

The distance between the tip of the capillary and the counter electrode was typically of the order of 11-12 cm, the applied voltages were in the range from 3.2 kV to 7.7 kV. The speed of exiting the solution from the syringe was selected 0.4 ml/h. Nano fibers were formed on aluminum foil. The nanofibers were exposed to steam for 24 hours and then kept in an incubator.

5. Results and Discussions

The Infrared Absorption Spectra (FT-IR) was used to characterize nano bioceramics and nano fibers. The FTIR spectra of BAG/CMC, BAG/β-CD and BAG/CMC/β-CD nano composites were shown in Fig 2. The peak at 3431.34cm^{-1} is due to OH stretching vibration. The peaks at 1635.47 and 1382.48 cm^{-1} indicated the existence of C-O and P-O groups in the structure. The symmetric stretching vibration bands of Si-O were observed at 1051.63, 8336.40 and 829.60 cm^{-1} [35]. Also, the band at 575.48 cm^{-1} indicated PO_4^{-3}. Table 1 summarized the characterization bands in BAG/β-CD FT-IR spectra.



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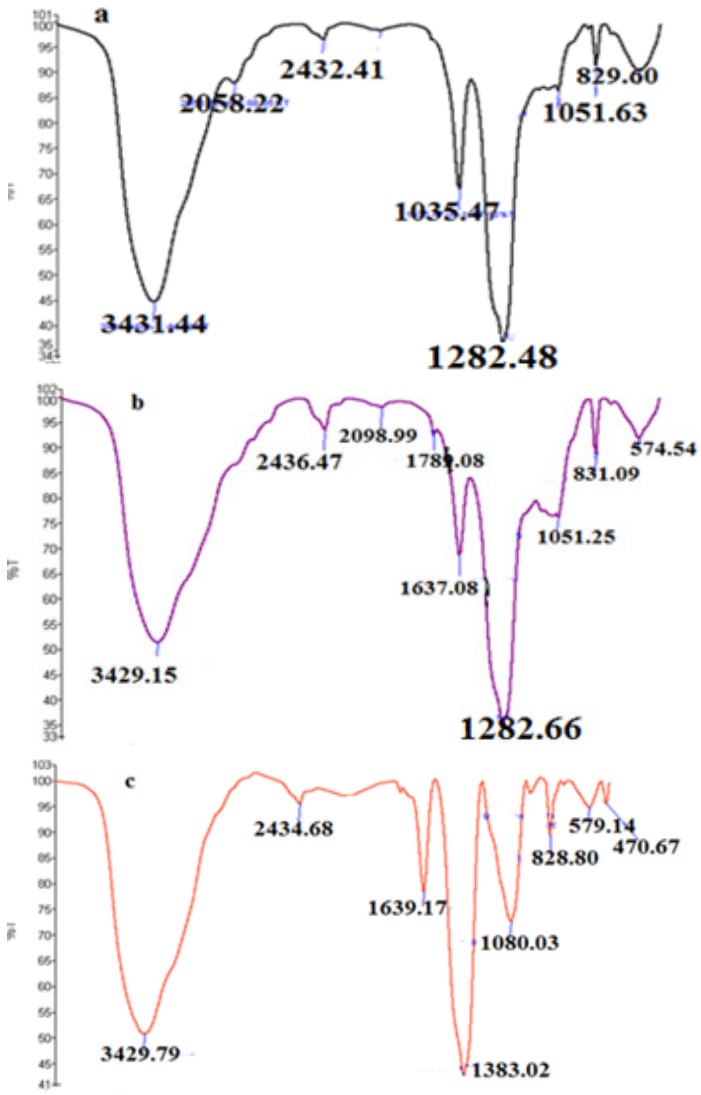
Characterization bond	Wavenumber
O-H stretching vibration	3429.15 cm^{-1}
CH_2	2436.47 cm^{-1}
SiO_4 stretching	1051.25, 831.09 cm^{-1}
Si-O-Si	574.57

Table 1. FT-IR characterization data of BAG/β-CD

In FT-IR spectrum of BAG/CMC/β-CD (Fig 3c), the absorption band of OH shifted to lower wavelengths (3429.79 cm^{-1}) compared to the peak of OH in BAG/CMC (3431.34 cm^{-1}). While C-O and P-O peaks shifted to higher wavelengths (1639.17 cm^{-1} and 1383.02 cm^{-1}). These shifts along with the shifts observed at 1080.03 cm^{-1}, 826.80 and 470.87 peaks related to Si-O-Si stretching and bending bands clearly indicate the interaction between the bio active glass and the biomolecules.



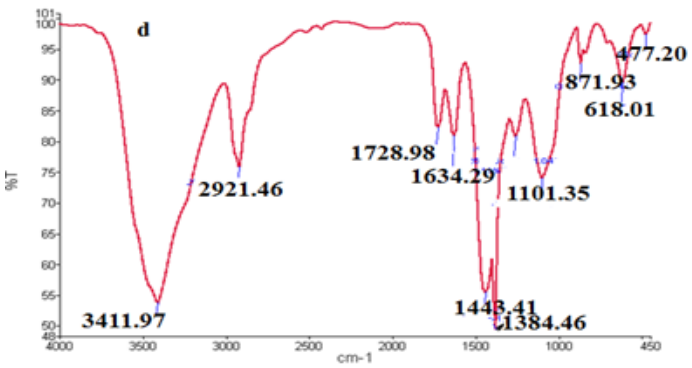


Fig.3 FT-IR of a) BAG/CMC, b) BAG/ β-CD, c) BAG/CMC/ β-CD nano ceramics and d) BAG/CMC/ β-CD nano fiber

In nano fiber, the stretching vibration of OH and CO observed at 3411.7 cm^{-1} and 1634.29 cm^{-1}, respectively. The bands at 871.93 cm^{-1} and 477.20 cm^{-1} related to Si-O-Si. The X-ray Diffraction (XRD) pattern of nano ceramics and nano fiber were shown in fig.4.The 2θ scanning range was set between 8° and 80°. It is noteworthy that the main peak appeared around 2θ=29° determined the size of nano ceramics and nano fiber[34]. This peak is related nano active glass. The CMC exhibits a very small crystallinity (Figure a). The crystalline size of the BAG/CMC nano ceramic was calculated by the Scherrer equation as follows:

$D = K\lambda/\beta 1/2\cos\theta$

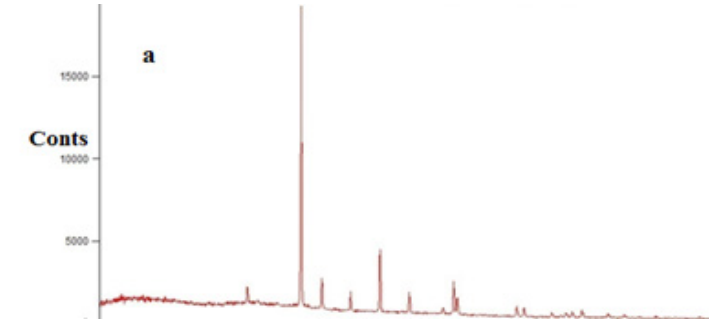


Fig.4 XRD of a) BAG/CMC,

Where D is the average crystal size, K is a constant (here chosen as 1), λ is the wavelength of X-ray radiation (1.54056 A), $\beta_{1/2}$ is the half width of the diffraction peak, and θ (◦) is Bragg's angle. The result of D value, using 2θ=29.45°, was calculated at 23 nm. As can be seen, the XRD pattern of BAG/β-CD (figure 4b) shown peaks at 2θ=29.8°, 32°, 39° and, 48°. The size of the nano ceramic was estimated 20nm.

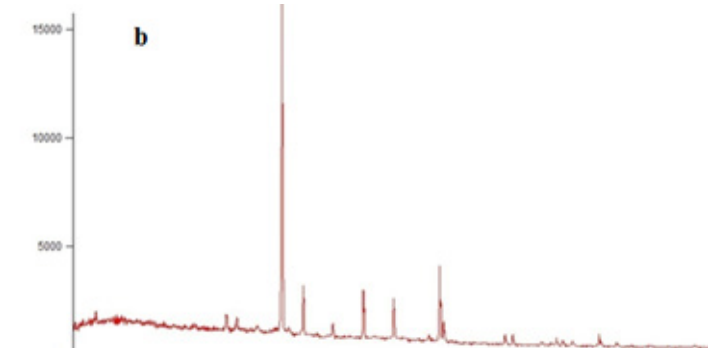


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Fig.4 XRD of b) BAG/ β-CD

The XRD pattern of BAG/CMC/β-CD (figure 4c) shown 3 peaks at 2θ=29°, 39° and, 49°. While in the XRD of nano fiber, only the broad peak at 2θ=20° with the nano fiber size 7nm was seen.

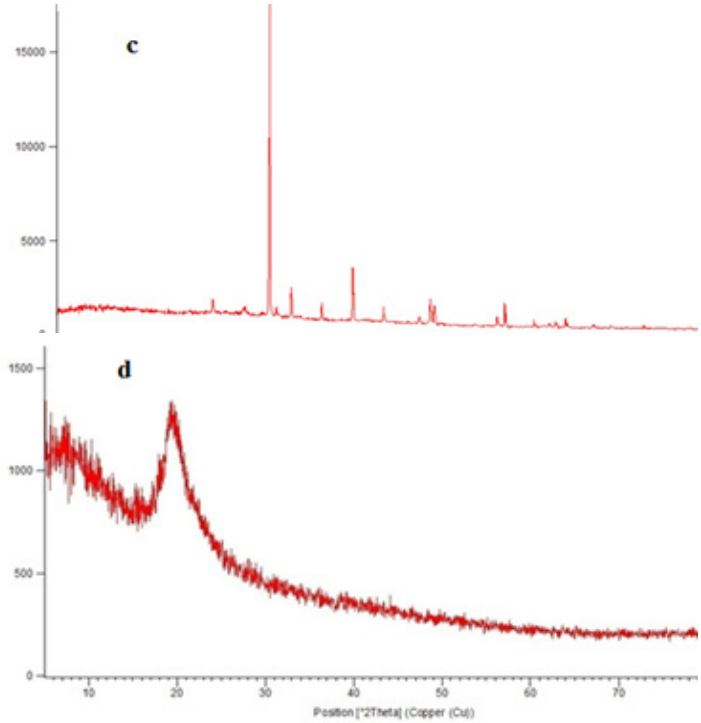
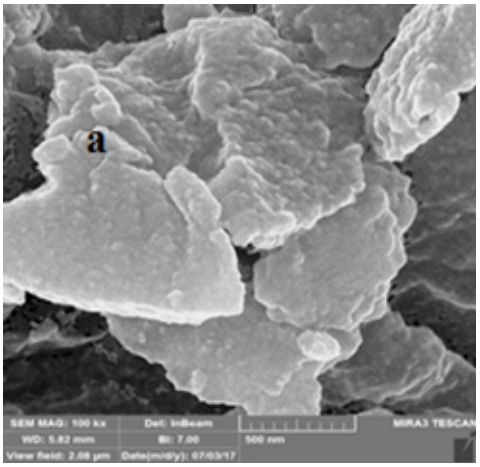


Fig.4 XRD of c) BAG/CMC/ β-CD nano ceramics and d) BAG/CMC/ β-CD nano fiber

Scanning Electron Microscopic images (SEM) from the surface of the synthesized nano ceramics and nano fiber showed that the nano ceramics are formed in mass structure while nano fibers are formed in strand structure with a diameter of 10nm. As can be seen in figure 5, studying the surface morphology of nano fiber with magnification of 200nm proved the existence of nanofiber without crosslinking [19, 36]. As a results, obtained nanofiber structure is very important for medical applications.



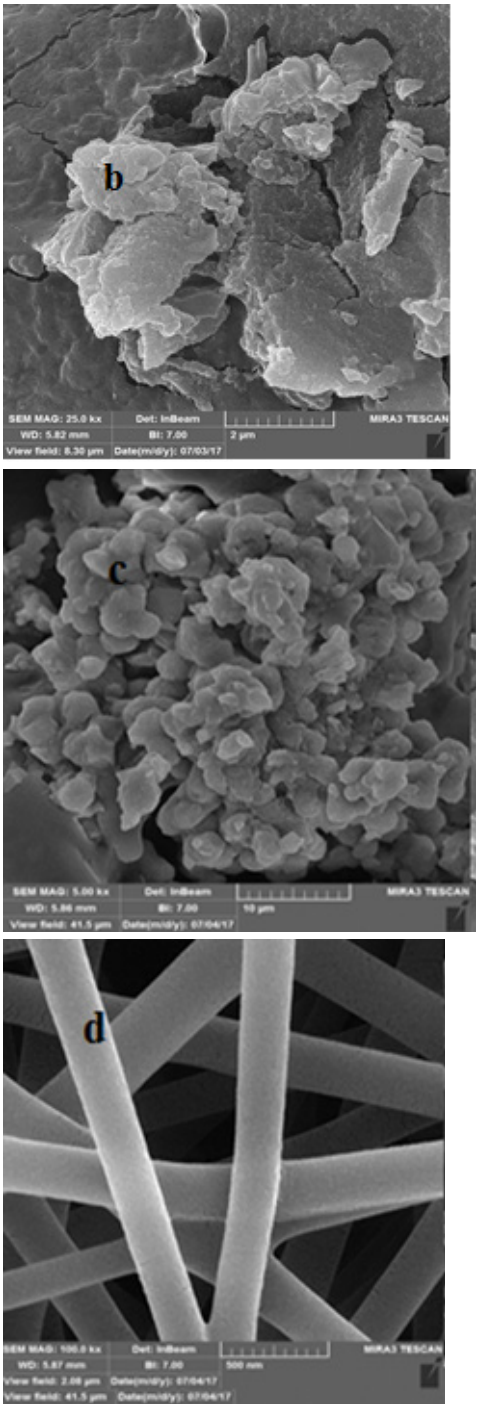


Fig. 5 SEM images of a) BAG/CMC, b) BAG/ β-CD, c) BAG/CMC/ β-CD nano ceramics and d) BAG/CMC/ β-CD nano fiber

Energy dispersive X-ray (EDX) analysis was used for the elemental analysis of nano ceramics and nano fiber. Table summarized the EDX data of BAG/CMC nano ceramics and nano fiber data. The size and morphology of nano fiber was studied by transmission electron microscopy (TEM); Fig.6 showed TEM of BAG/CMC/ β-CD nano fiber. The figures confirmed the formation of nano fiber with 10 nm diameter. The results of the field emission electron microscope show the formation of continuous and uniform structures of bioactive glass nanocomposites,

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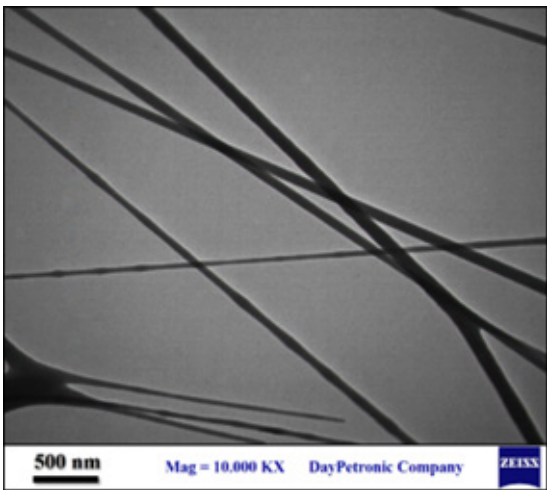
which have no grains and have a relatively uniform diameter. This result confirms that the concentrations used have sufficient viscosity and also the prepared nanofibers have been prepared with a suitable diameter of about 10 nm [37,19].



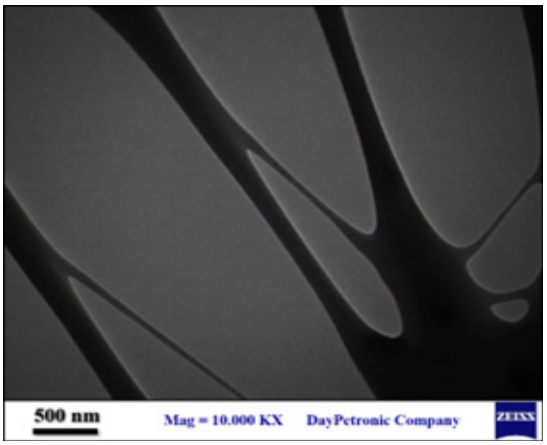


Fig. 6 TEM of BAG/CMC/β-CD nano fiber

6. Conclusion

The remarkable and biodegradable nature of the nanofibers is highly advantageous in widespread applications, significantly in the biomedical field and nanobiotechnology. This paper showed that bioactive glass/carboxymethyl cellulose/nanofiber/β-cyclodextrin (BAG/CMC/β-CD) are easily synthesized via electro spinning method and characterized by FT-IR, XRD, TEM, SEM and EDX techniques. Our results show that synthesized nano fibers are promising candidates as implants and can be used as efficient materials for the drug delivery and tissue engineering.

BAG/CMC/ β-CD nano fiber	BAG/CMC/ β-CD nano ceramic	BAG/ β-CD	BAG/CMC
S; 38.94%,	O; 37.39%	O; 39.71%	O; 39.18%
O; 33.18%;	Na; 26.99%	Ca; 25.56%	Na; 37.67%
Na; 8.72%,	S; 12.11%	P; 24.51%	S; 8.74%
N; 5.31%,	Si; 9.12%	Na; 2.77	N; 8.41%
Ca; 3.06%	N; 7.67%	Si; 2.77%	Si; 3.98%
P 2.99%	Ca; 5.32%	N; 3.95%	Ca; 1.05%
	P 1.40%	S 0.73%	P; 0.61%

Table.1 EDX data of nano ceramics and nano fiber

7. Acknowledgements

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