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Synthesis, Electron Microscopy and Anti-Microbial Property of Fe₃O₄-Ag Nanotubes

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Abstract

Electrodeposition was used for synthesizing 200 nm diameter Fe₃O₄-Ag nanotubes. Compositional analysis at the single nanotube level revealed a fairly uniform distribution of component elements in the nanotube microstructure. As-synthesized Fe₃O₄-Ag nanotubes were superparamagnetic in nature. Electron diffraction revealed the ultrafine nanocrystalline microstructure of the nanotubes. Effect of Ag on anti-microbial response of the nanotubes was investigated by comparing the effect of sulphate reducing bacterial (SRB) on Fe₃O₄-Ag and Fe₃O₄ nanotubes. Fe₃O₄ nanotubes were also electrodeposited in the present study. It was observed that the Fe₃O₄-Ag nanotubes exhibited good resistance to sulphate reducing bacteria which revealed the anti-microbial nature of the Fe₃O₄-Ag nanotubes.

Keywords: Nanotubes, Anti-bacterial behaviour, ferrofluids, Electron Microscopy.

Introduction

Iron containing nano-solids have found potential application in several fields such as biomedical imaging¹, biomedical drug delivery and diagnostics², catalysis³, ferrofluids⁴ etc. With respect to their application as ferrofluids, iron containing nanosolids are expected to exhibit three important characteristics: (a) they should be superparamagnetic. This property can inhibit their agglomeration due to mutual magnetic attraction. Superparamagnetism can be achieved by decreasing the grain size of ferromagnetic systems to ultrafine sub-10 nm levels⁵, (b) their

geometry should be such that a foreign material can be packed within their volume so that the contained material can be transferred using a magnetic field through a fluid medium. One such geometry is nanotube geometry. This characteristic can be particularly helpful in drug delivery application and (c) they should possess resistance to degradation in the fluid medium. This degradation can be due to various micro-organisms that are present in the fluid medium. One possible way to avoid bio-corrosion is to incorporate Ag into the nano-solids. Ag is well known for its antimicrobial properties⁶.

This work provides an electrochemical based methodology for synthesizing superparamagnetic Fe₃O₄-Ag nanotubes with 200 nm diameter and high aspect ratio. This work also illustrates the superior antibacterial property of as-synthesized Fe₃O₄-Ag nanotubes by comparing it with the anti-bacterial response of Fe₃O₄ nanotubes which were also synthesized in this work. Anti-bacterial response of nanotubes was investigated by exposing them to sulphate reducing bacteria (SRB). SRBs are one of the most prevalent bacteria responsible for bio-fouling and also there is no reported study on the establishment of antibacterial and antifouling properties of Ag in the presence of SRBs.

Materials and Methods

Synthesis of Nanotubes

Fe₃O₄-Ag and Fe₃O₄ nanotubes were synthesized by the conventional electrodeposition technique. Anodic alumina membrane having cylindrical pores with an average diameter of 200 nm, a nominal thickness of 60 μ m and density of 10⁹ pores/cm² was used as template for obtaining nanotube morphology. A platinum foil was used as anode. A copper foil attached to the alumina disc using an adhesive tape was used as cathode. A schematic showing the arrangement of electrodes in the electrochemical cell is provided in Fig. 1.

Synthesis of Fe_3O_4 -Ag nanotubes: Electrolyte used was prepared by dissolving 0.169 g of AgNO_3 , 2.4 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 0.17 g H_3BO_3 and 1.7 g of $\text{CH}_4\text{N}_2\text{S}$ in 100 mL of distilled water. pH of the solution was maintained at 2.5 using NaHCO_3 . A DC current of 4 mA was applied for 15 minutes for electrodeposition which was carried out at ambient temperature.

Synthesis of Fe_3O_4 nanotubes: Electrolyte used was prepared by dissolving 2.4 g of $\text{Fe}(\text{NO}_3)_3$, 0.17 g of H_3BO_3 in 100 mL distilled water. pH of the solution was maintained at 2.5 using NaHCO_3 . A current of 4 mA was applied for 15 minutes for electrodeposition which was carried out at ambient temperature.

After the electrodeposition experiment, the alumina template containing nanotubes was immersed in 1M NaOH solution and left there for 3-4 hours to dissolve the alumina and release the nanowires. As-synthesized nanowires were then washed several times in distilled water for further analysis.

Characterization

Quanta ESEM scanning electron microscope (SEM) fitted with an energy dispersive spectroscopy (EDS) detector was used to determine the morphology and composition of as-deposited samples. A 300 keV field emission FEI Tecnai F-30 transmission electron microscope (TEM) was used for obtaining bright field images, selected area electron diffraction (SAD) patterns and compositional information from as-synthesized nanotubes. Samples for the TEM based analysis were prepared by drop-drying a highly dilute dispersion of as-synthesized nanotubes on an electron transparent carbon coated Cu grid. Scanning transmission electron microscopy-energy dispersive spectroscopy (STEM-EDS) technique which uses a ~ 2 nm sized electron probe was used for obtaining compositional line profiles and elemental maps from individual nanotubes. Room temperature magnetic characterization of as-deposited nanotubes

was performed using Lakeshore VSM using an applied field upto 2 Tesla. X-ray photoelectron spectroscopy (XPS) profile was obtained from the as-synthesized samples using AXIS Ultra DLD (KRATOS ANALYTICAL) instrument.

For determining the anti-microbial response, a mixed culture of sulphate reducing bacteria (SRB) containing *DESULFOTOMACULUM nigrificans* and *DESULFOVIBRIO desulfuricans* was used. Postgate medium⁷ used for culturing the SRB strains was made up of 10 g of tryptone, 1 g of sodium sulfite, 1 g of sodium sulfate and 0.5 g of ferric citrate dissolved in 1 litre of de-ionised water. Full growth of inoculated SRB in the medium was achieved in four days. After growth, the bacterial cells were separated using filtration. As-synthesized Fe₃O₄ and Fe₃O₄-Ag nanotubes were drop-dried on Cu plates. These plates were then exposed to the medium containing fully grown bacterial cells. Cu plates were withdrawn from the medium after 15 days for microscopic analysis of bio-films. The nanotubes on these plates were sponged all over the surface and rinsed with ethanol. Bacterial count in the medium exposed to the nanotubes on Cu plates was determined using the PETTROF-HAUSSER Cell Counting Chamber (Hemocytometer). Absorbance of the bacterial growth medium was determined using the SYSTRONICS-117 UV-VIS Spectrophotometer at a wavelength of 420 nm in order to confirm bacterial population in the presence and absence of exposure to these nanotubes.

Results and Discussion

A representative SEM micrograph of as-synthesized Fe₃O₄-Ag nanotubes is shown in Fig. 2(a). It can be seen that the electrodeposition experiment has produced high yield of uniform diameter nanotubes. Outer diameter of the nanotubes was found to be approximately 200 nm which is expectedly similar to the diameter of the pores in the alumina template. Surface area of the nanotube was calculated to be approximately $12.5 \times 10^{-6} \text{ mm}^2$. For calculating the surface

area, length of the nanotube was considered to be 20 μm . Compositional analysis of the as-synthesized nanotubes using SEM-EDS technique revealed the presence of Fe, Ag and O element in the nanotubes. Relative atomic percentage of Fe and Ag in the nanotube was found to be 85 at% Fe and 15 at% Ag. Representative low and high magnification TEM bright field image of Fe_3O_4 -Ag nanotubes is shown respectively in Fig. 2(b) and 2(c). Fig. 2(b) reveals a population of uniform diameter nanotubes. Hollow geometry of the nanotubes is clearly visible in the high magnification images in Fig. 2(c). A representative SAD pattern obtained from an agglomerate of nanotubes is shown in Fig 2(d). Presence of broad diffraction rings in the SAD pattern clearly indicates that the nanotubes are polycrystalline with extremely fine grain sizes. Indexing of the SAD pattern clearly revealed the presence of Fe_3O_4 phase in the nanotubes. The interplanar spacings ('d' spacings) and corresponding crystallographic planes of the Fe_3O_4 crystal are indicated in Fig. 2(d). The SAD pattern does not reveal diffraction signature corresponding to the pure Ag phase. This indicated mixing of Ag into the Fe_3O_4 lattice. XPS spectrum obtained from the nanotubes showing $\text{Ag}3d_{5/2}$ and $\text{Ag}3d_{3/2}$ peaks is provided in Fig. 2(e). XPS based analysis revealed that Ag was present in zero valent state in the nanotubes⁸.

STEM-EDS compositional analysis was conducted to investigate the compositional uniformity in the as-synthesized nanotubes. A representative compositional mapping result is shown in Fig. 3(a). The compositional mapping result clearly reveals that all the three component elements are co-present in all the parts of the nanotube microstructure and there is no preferential segregation of any of the component atom(s). To further confirm the compositional uniformity, compositional line profile analysis was conducted across the nanotube diameter. A representative line profile analysis result is shown in Fig. 3(b). Insert in Fig. 3(b) is the STEM image of the nanowire from which the line profile data was obtained. Two important observations that can be

made from Fig 3(b) are: (a) all the three component atoms are present at every analysis point along the nanotube diameter and (b) a non-bell shaped counts vs distance curve is obtained across the nanotube diameter. A fairly flat curve clearly indicates that the volume of the material along the analysis line is fairly constant. For a cylindrical geometry this is only possible if the cylinder is hollow from inside. A filled cylinder would generate a bell shaped counts vs distance (along the diameter) curve as the amount of material will increase towards the center of the cylinder⁹. The compositional line profile analysis further confirmed the hollow nanotube geometry of the electrodeposited mass.

A representative SEM micrograph of as-deposited Fe₃O₄ nanotubes is provided in Fig. 4(a). It can be seen that the electrodeposition method has produced a high yield of 200 nm diameter nanotubes. SEM-EDS analysis expectedly revealed the presence of only Fe and O in the nanotubes. Representative high and low magnification of TEM bright field image of Fe₃O₄ nanotubes is shown respectively in Fig. 4(b) and (c). It can be seen in Fig. 4(b) that uniform diameter hollow Fe₃O₄ nanotubes have formed. Hollow geometry of the nanotubes is clearly visible in the high magnification image in Fig. 4(c). SAD pattern obtained from an agglomerate of nanotubes is shown in Fig 4(d). Presence of broad diffraction rings in the SAD pattern clearly indicates that the nanotubes are polycrystalline with extremely fine grain sizes. Indexing of the SAD pattern clearly revealed the presence of Fe₃O₄ phase in the nanotubes. The interplanar spacings ('d' spacings) and corresponding crystallographic planes of the Fe₃O₄ crystal are indicated in Fig. 4(d). Compositional line profile analysis of individual Fe₃O₄ nanotubes was conducted using the STEM-EDS technique. A representative line profile analysis result is shown in Fig. 4(e). Insert in Fig. 4(e) is the STEM image of the nanowire from which the line profile

data was obtained. It can be observed in Fig. 4(e) that both Fe and O are fairly uniformly distributed across the nanotube diameter.

Magnetic hysteresis loop obtained from as-synthesized $\text{Fe}_3\text{O}_4\text{-Ag}$ and Fe_3O_4 nanotubes are shown respectively in Fig. 5(a) and (b). Both the magnetic hysteresis loops in Fig. 5 are characterized by extremely low magnetic coercivity and no magnetic saturation till 2 tesla field. Both these attributes reveal superparamagnetic nature¹⁰ of the nanotubes. At all values of the applied magnetic field, a decrease in the magnetization value for the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes when compared to the magnetization value of the Fe_3O_4 nanotube confirmed the presence of diamagnetic Ag in the lattice of the Fe_3O_4 phase in the case of the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes.

SEM micrographs of $\text{Fe}_3\text{O}_4\text{-Ag}$ and Fe_3O_4 nanotube film after exposure to the SRB containing medium are shown respectively in Fig. 6(a) and (b). It can be observed that biofilm has formed on both Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes. It is however clearly evident from the SEM micrographs that between the two nanotubes extent of biofilm formation is considerably less in case of the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes. Fe_3O_4 nanotubes are not visible as they are completely covered by the biofilm whereas $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes are still visible. Representative TEM micrographs of individual $\text{Fe}_3\text{O}_4\text{-Ag}$ and Fe_3O_4 nanotube after exposure to the SRB are shown respectively in Fig. 7(a) and (b). Supporting the SEM observation, Fig. 7 clearly reveals that the amount of biofilm formed on the Fe_3O_4 nanotubes is considerably greater than the amount of biofilm on the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes.

Compositional analysis of the nanotube film after exposure to SRB revealed that the Fe_3O_4 nanotube film contained greater amount of bacterially reduced sulfide than the sulfide content in the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotube film. Both the above observations strongly indicate that the extent of biofilm formation is higher in case of non-Ag containing nanotubes. Bacterial count in

the solution exposed to Fe_3O_4 , and $\text{Fe}_3\text{O}_4\text{-Ag}$ was determined using a hemocytometer. Bacterial counts were taken from 16 square boxes, 0.25 mm long and 0.1 mm deep. SRB count obtained from the control, solution exposed to Fe_3O_4 nanotubes, and solution exposed to $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes respectively were 8.0×10^9 , 3.2×10^9 , and 1.4×10^9 cells/mL respectively. A significant reduction in the SRB count in solution exposed to $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes when compared to the bacterial count in control and Fe_3O_4 nanotubes cases clearly illustrated the role of Ag in inhibiting bacterial growth and thus reducing the extent of biofilm growth. Spectrophotometry was also carried out on the control, solution exposed to Fe_3O_4 nanotubes and $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes. De-ionized water was used as reference for these measurements. Absorbance values determined were 0.560, 0.425, and 0.300 respectively for the control, Fe_3O_4 nanotubes and $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes cases. Lower optical density corresponds to less scattering of light due to lower density of bacteria. This result also clearly indicated the role of Ag in inhibiting the bacterial growth. The above results clearly illustrated the high anti-microbial property of as-synthesized superparamagnetic $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes.

Conclusions

200 nm diameter $\text{Fe}_3\text{O}_4\text{-Ag}$ and Fe_3O_4 nanotubes were synthesized using the electrodeposition method. Anodic alumina template was used to obtain the nanotube morphology. The electrodeposition condition adopted produced a high yield of uniform nanotubes. Compositional mapping and compositional line profile analysis results clearly indicated a fairly uniform distribution of component elements in the nanotube microstructure for both the cases. SAED pattern indicated that both $\text{Fe}_3\text{O}_4\text{-Ag}$ and Fe_3O_4 nanotube contained randomly oriented ultrafine nano-crystalline grains. Magnetic measurements revealed a superparamagnetic nature for the $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes. Exposure of the as-synthesized nanotubes

to SRB revealed the role of Ag in inhibiting the microbial growth. The extent of biofilms formation was considerably greater for the non-Ag containing Fe₃O₄ nanotubes.

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Figure captions

Fig. 1 A schematic showing the arrangement of electrodes in the electrochemical cell.

Fig. 2 (a) Representative SEM micrograph of $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes, (b) low and (c) high magnification TEM bright field image of $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes, (d) an indexed SAD pattern obtained from an agglomerate of nanotubes revealing the presence of Fe_3O_4 phase, (e) XPS profile obtained from nanotubes revealing $\text{Ag}3d_{5/2}$ and $\text{Ag}3d_{3/2}$ peaks.

Fig. 3. (a) A representative compositional mapping result and (b) a representative compositional line profile analysis along the nanotube diameter result. Insert in Fig. 3(b) provides the STEM image of the nanowire from which the line profile data was obtained.

Fig. 4: (a) A representative SEM micrograph of as-deposited Fe_3O_4 nanotube, (b) low and (c) high magnification TEM bright field image of Fe_3O_4 nanotubes, (d) an indexed SAD pattern obtained from an agglomerate of nanotubes revealing the presence of Fe_3O_4 phase (e) result obtained from compositional line profile analysis along the nanotube diameter. Insert in Fig. 4(e) provides the STEM image of the nanowire from which the line profile data was obtained.

Fig. 5. Magnetic hysteresis loop obtained from as-synthesized (a) $\text{Fe}_3\text{O}_4\text{-Ag}$ and (b) Fe_3O_4 nanotubes.

Fig. 6: Representative SEM micrograph of (a) $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotubes and (b) Fe_3O_4 nanotube after exposure to SRB.

Fig. 7: Representative TEM micrograph of (a) $\text{Fe}_3\text{O}_4\text{-Ag}$ nanotube and (b) Fe_3O_4 nanotube after exposure to SRB.

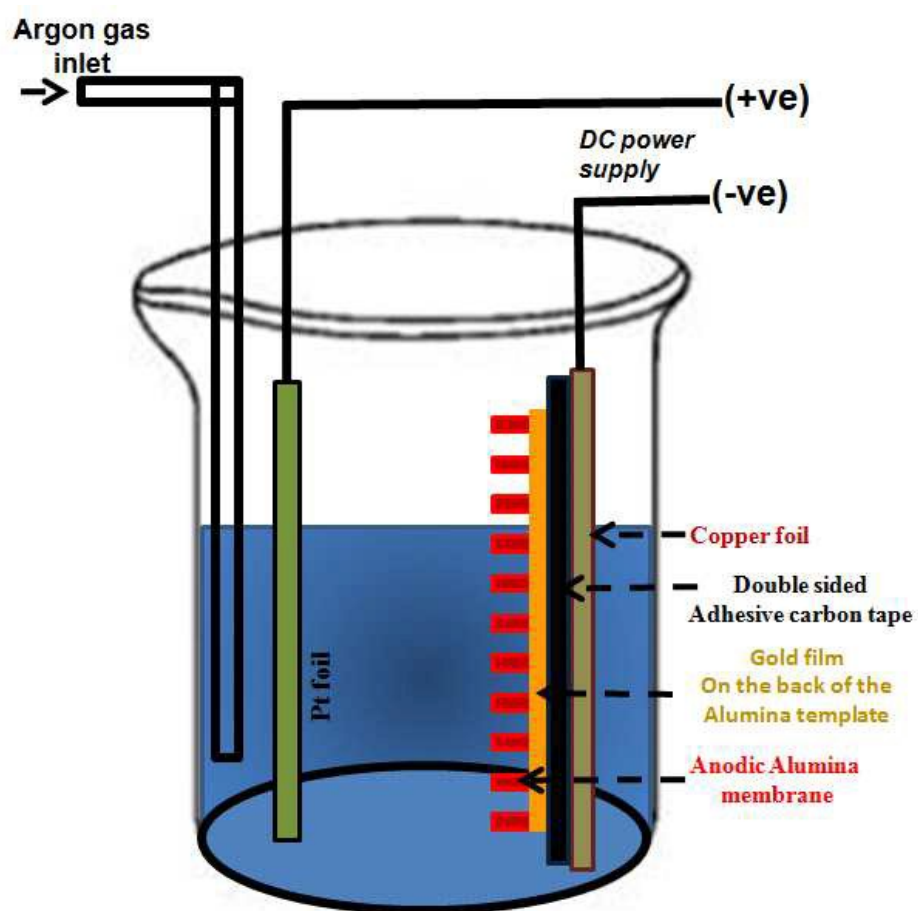


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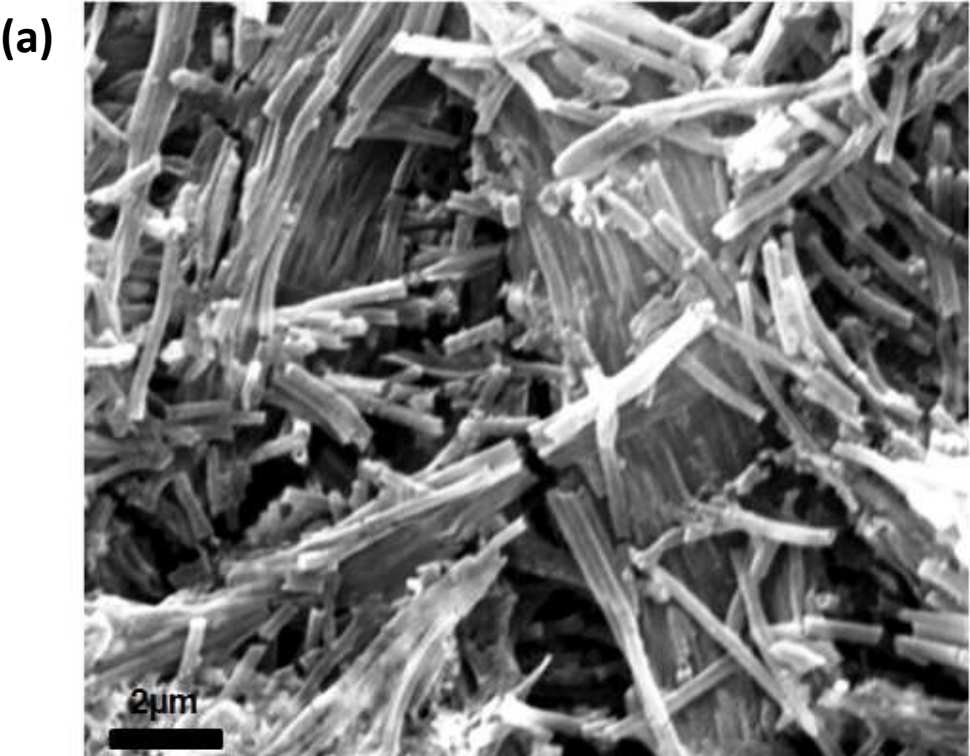


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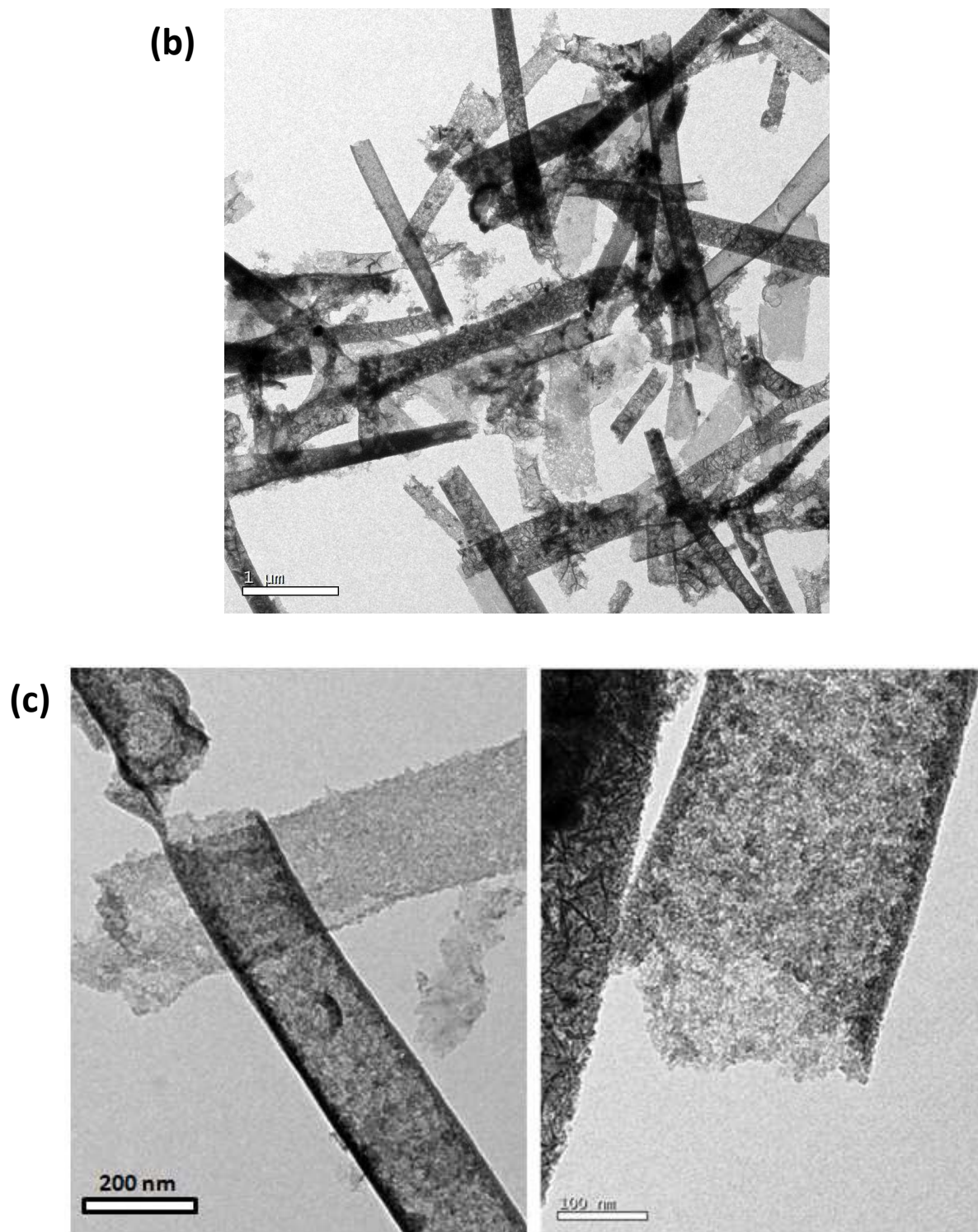


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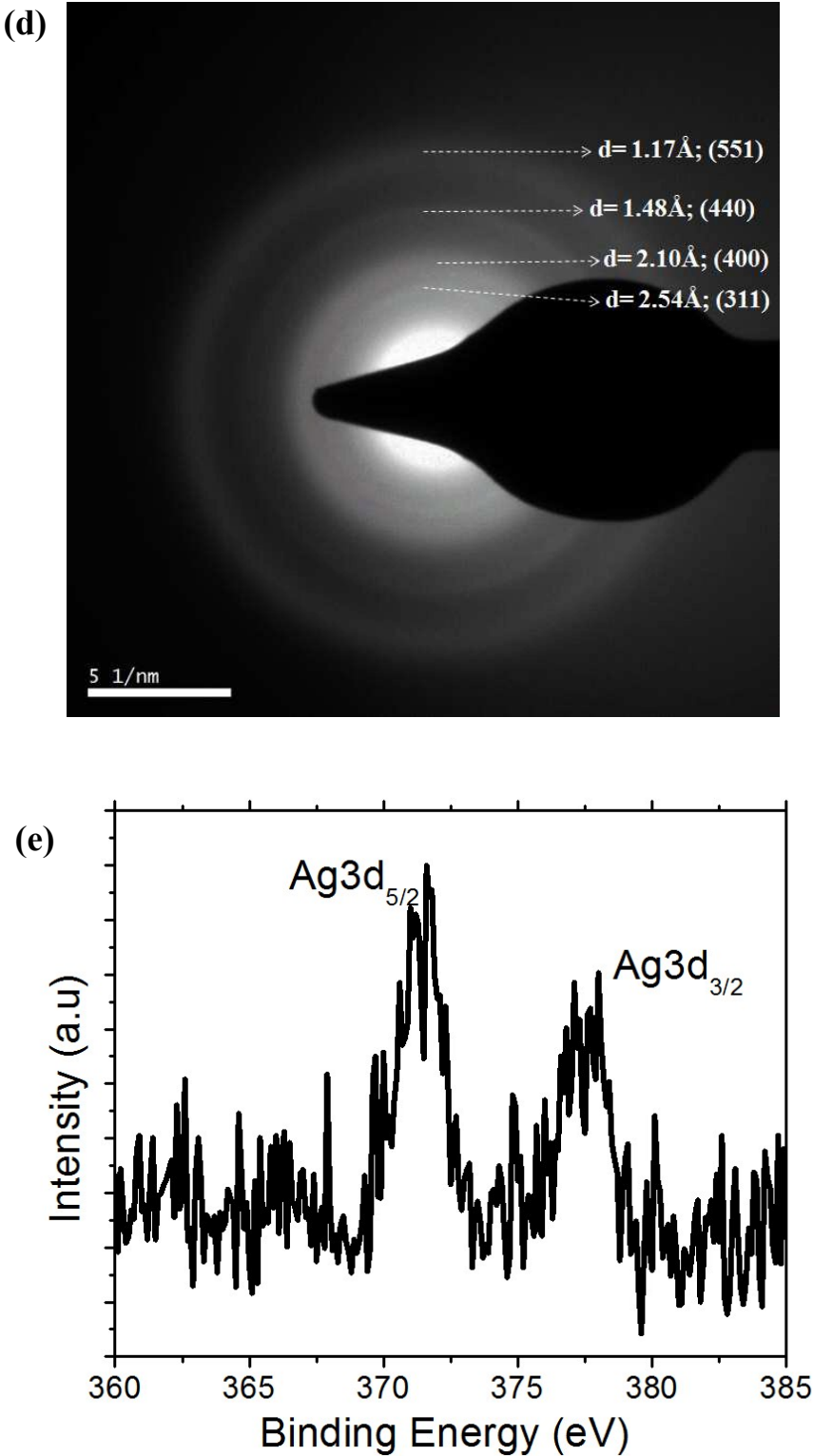


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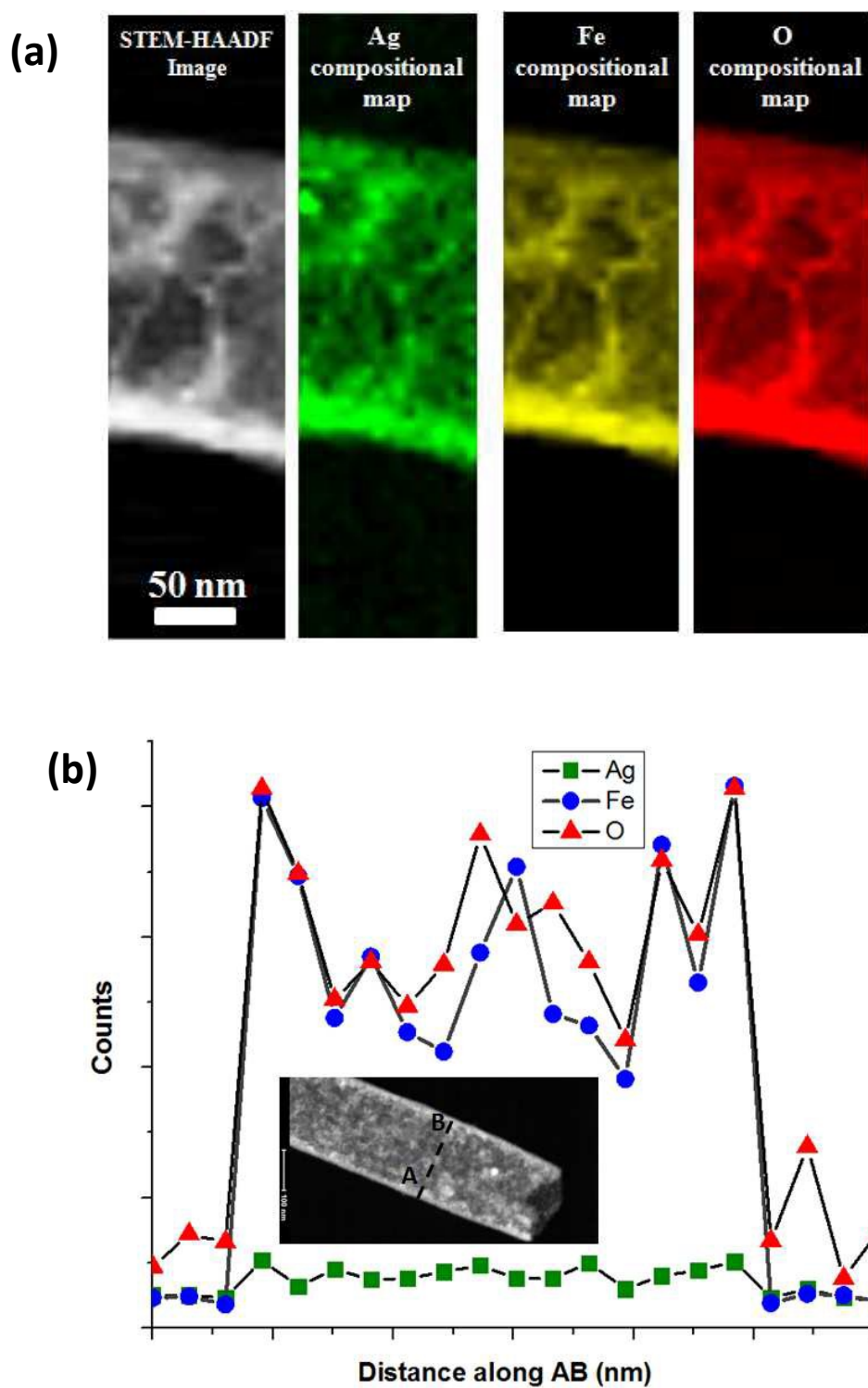


Figure 3

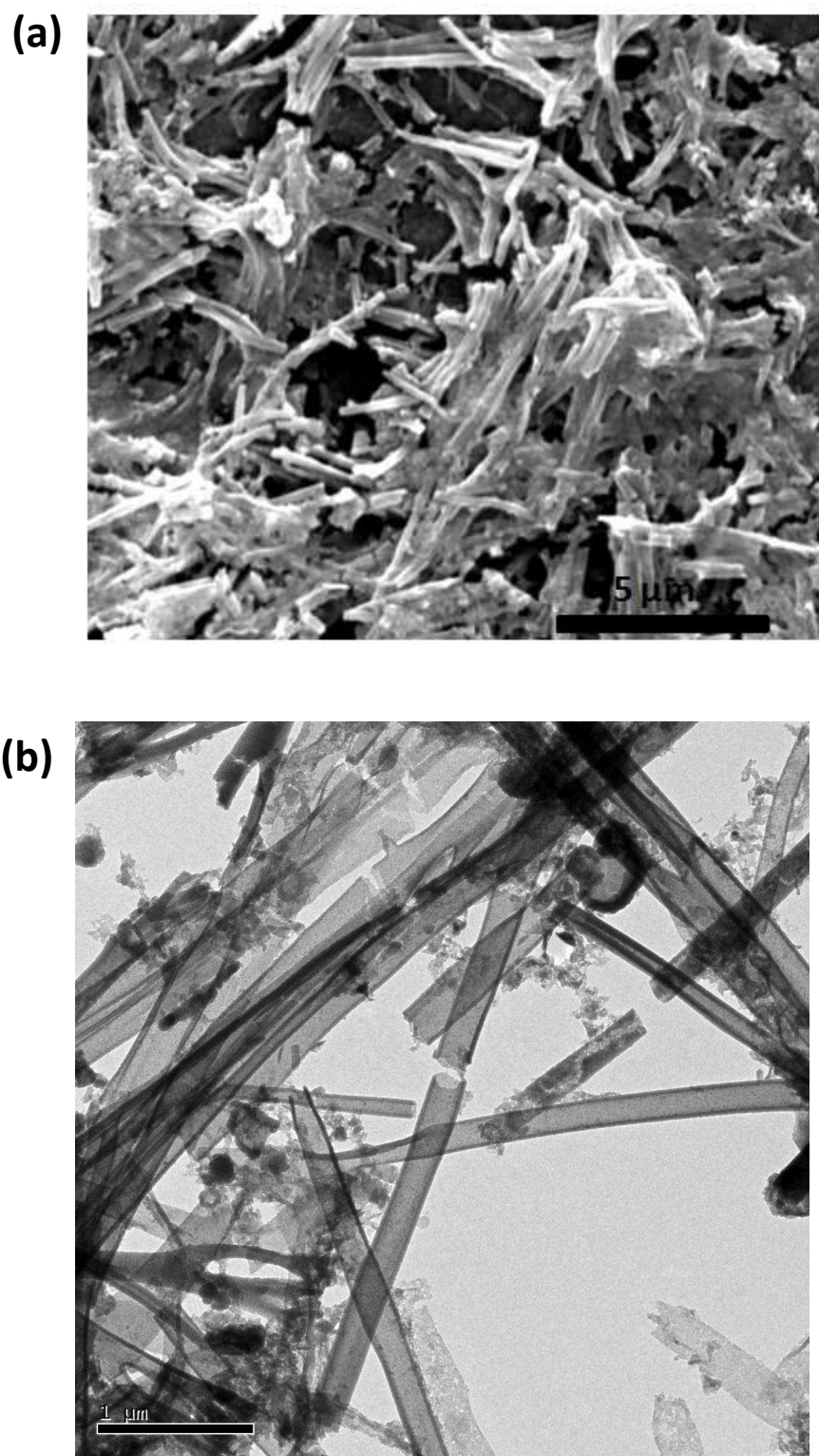
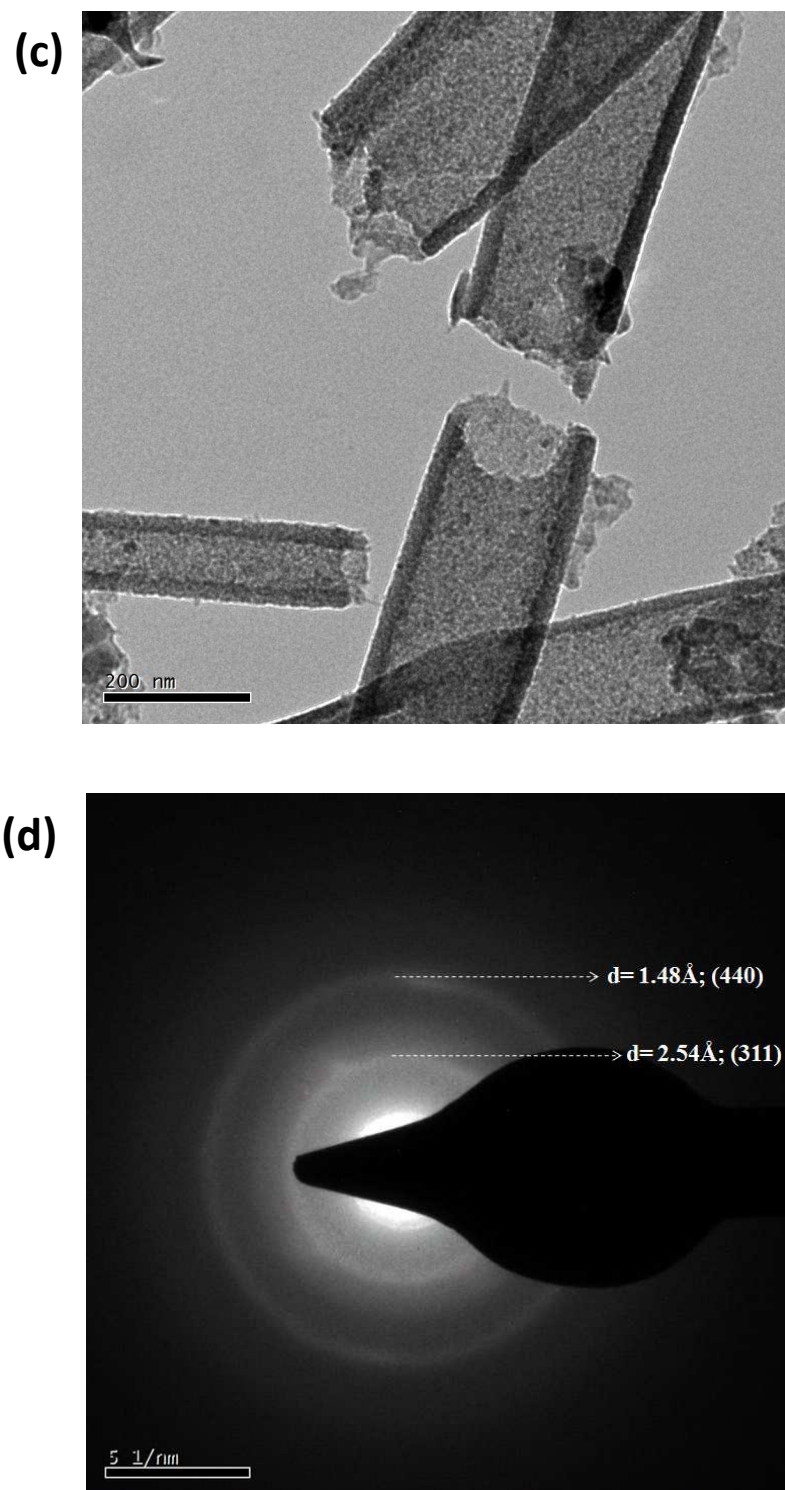


Figure 4

**Figure 4**

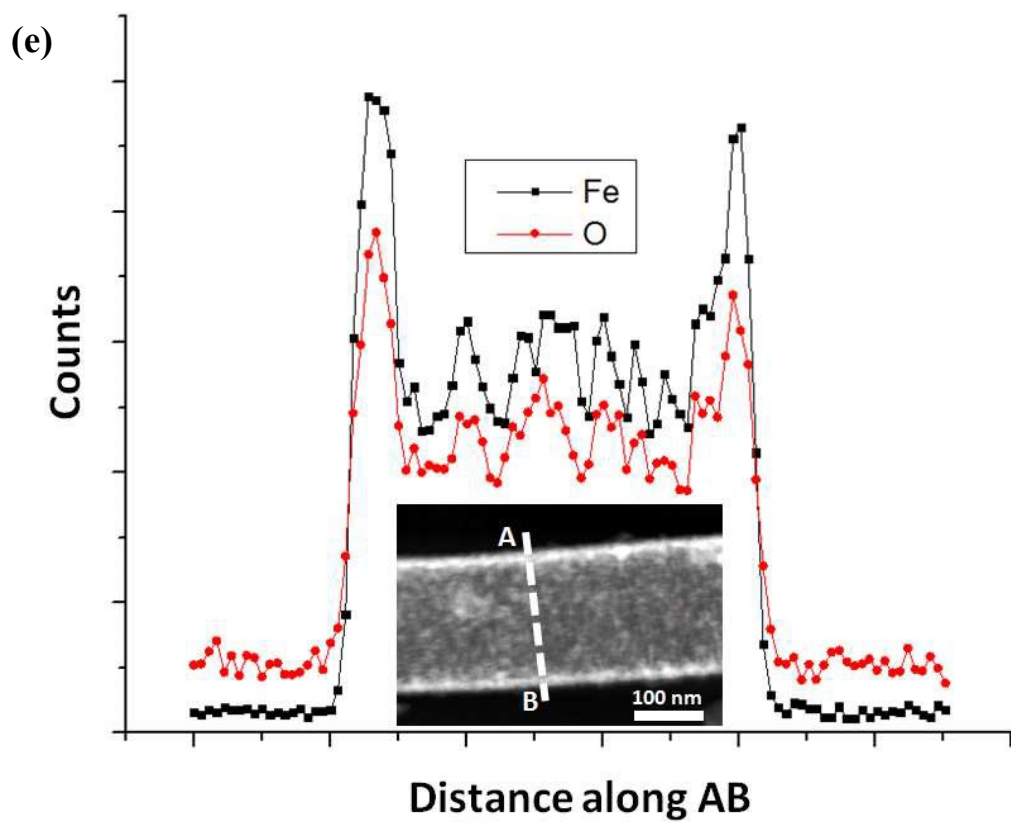


Figure 4

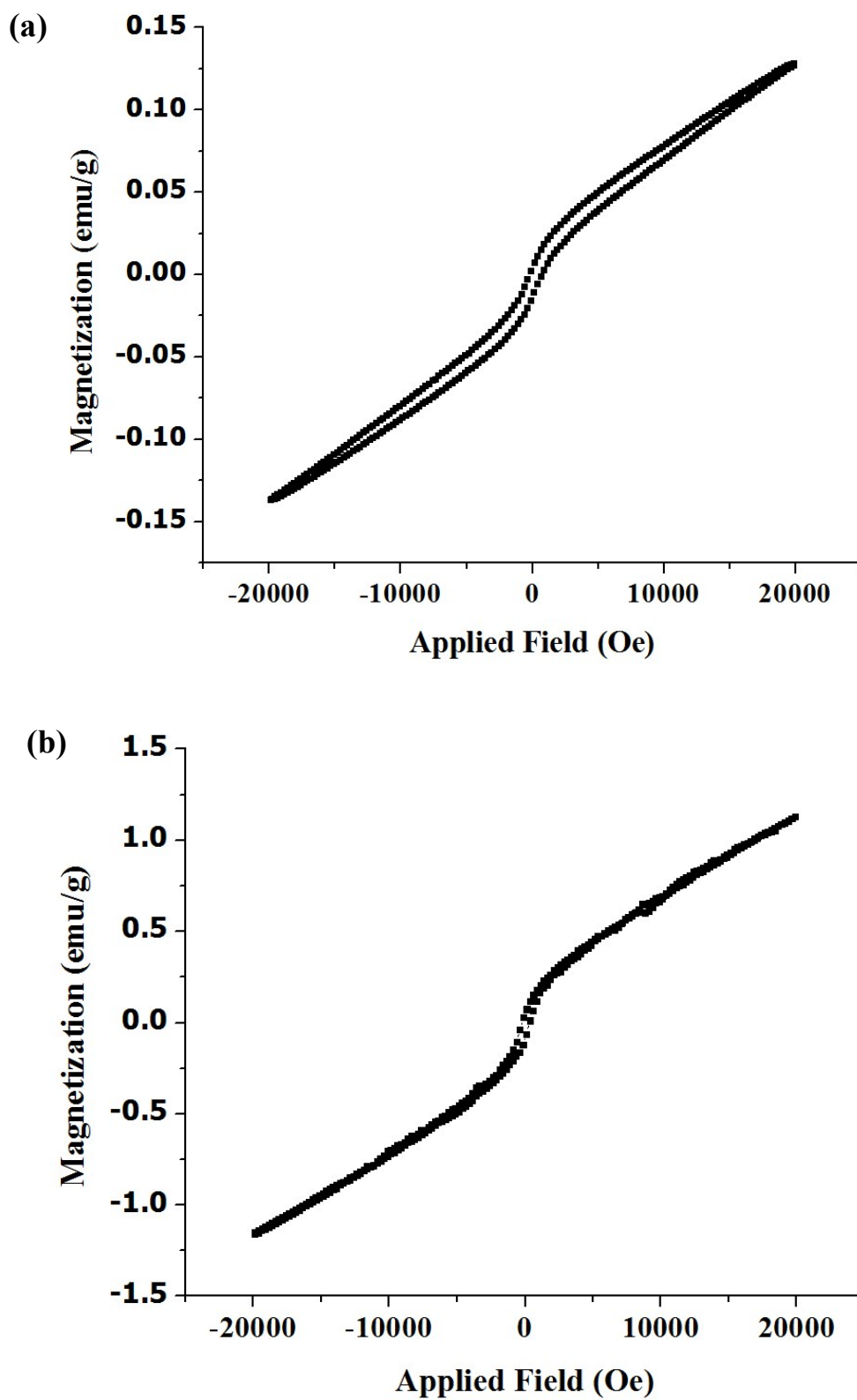


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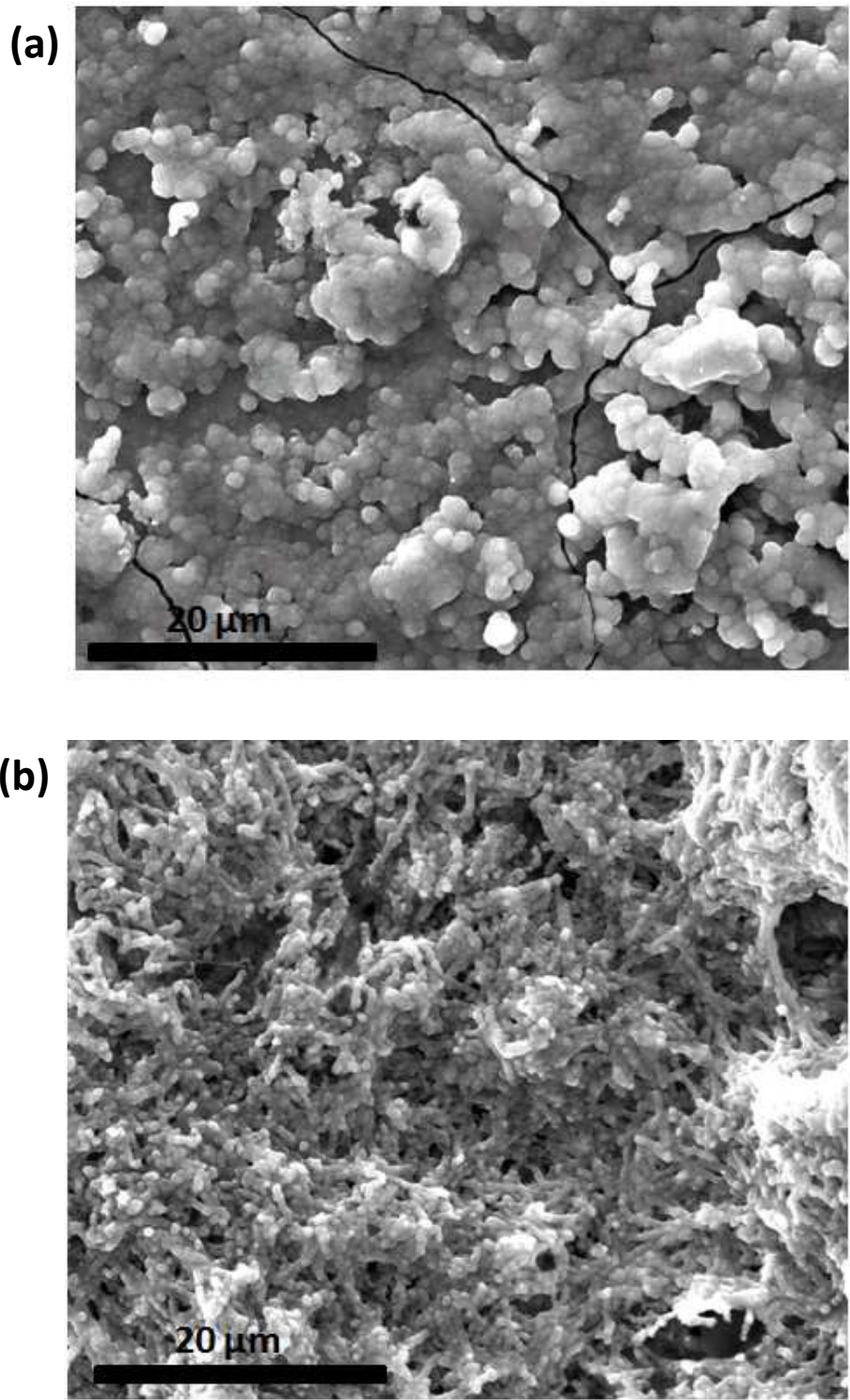


Figure 6

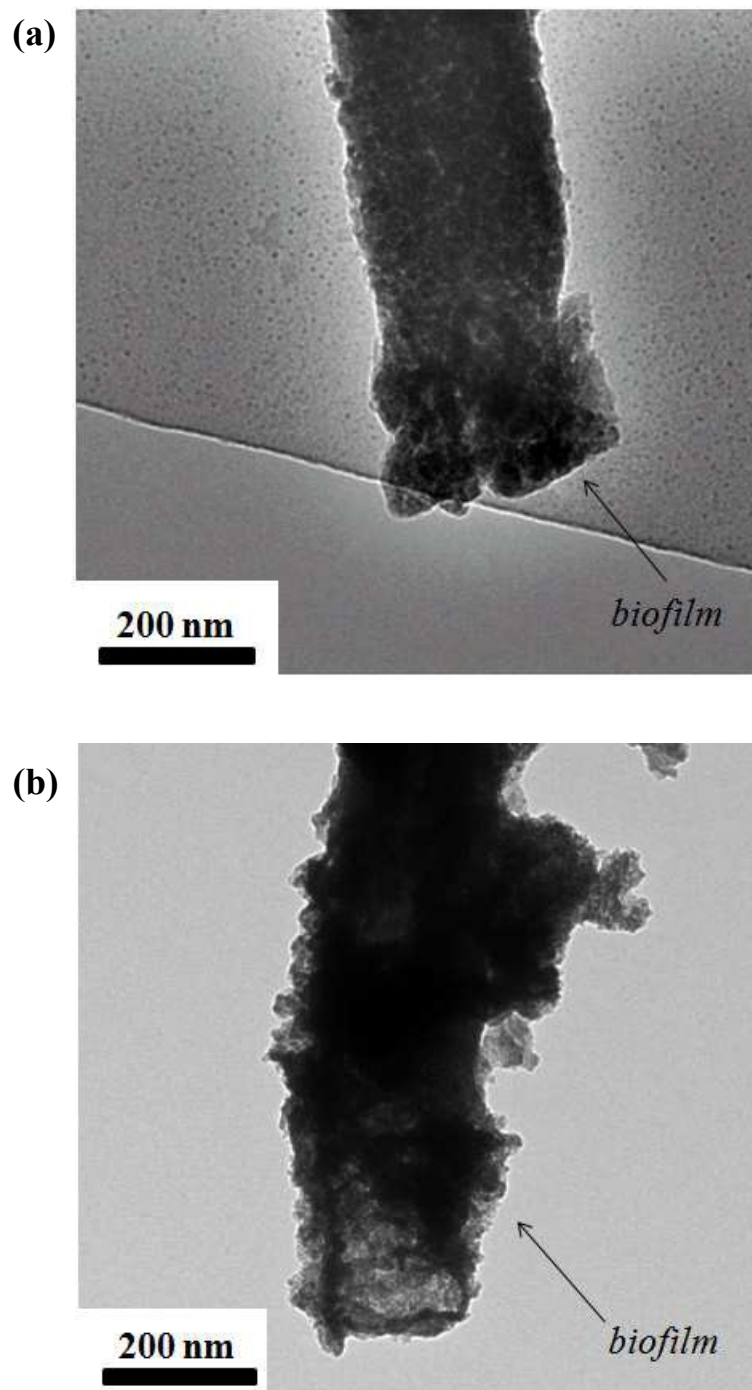


Figure 7