Research Article **Open Access**

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Investigation of Antibacterial Properties of Ag Doped TiO_2 Nanofibers Prepared by Electrospinning Process

https://doi.org/10.1515/chem-2018-0081
received January 30, 2018; accepted April 10, 2018.

Abstract: In this study, undoped and 1, 2, 3, 4, and 5 wt % Ag-doped TiO_2 nanofibers have been fabricated by the electrospinning method applying 20 kV voltages at 8 cm height with a flow rate 0.1 mL/h. The antibacterial properties of undoped and doped Ag/TiO_2 nanofibers were tested on *Staphylococcus aureus* bacteria. The antibacterial effect of these fabricated nanofibers has been determined by disc diffusion and Baird parker methods. The results have shown that Ag/TiO_2 nanofibers have an excellent antibacterial effect on this bacterium compared to pure TiO_2 nanofibers. As a result, developed nanofibers can easily be applied in various fields such as biomedical sector and tissue engineering. In addition, the chemical components, morphology, and crystal structure of the nanofibers have been performed by scanning electron microscopy energy dispersive analysis (SEM-EDX), differential thermal analysis/thermal gravimetric analysis (DTA/TG), and X-ray diffraction (XRD) methods.

Keywords: Nanofiber; Characterization; Antibacterial; Ag, TiO_2; sol-gel.

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1 Introduction

Recently, researchers have begun focusing much attention on the fabrication of organic-inorganic nanostructured composite materials used in different fields, such as photovoltaic devices, opthoelectronic circuits, solar cells, lithography, sensors, and medicine [1-7]. Electrospinning is an important method used extensively among the many methods for fabricating nanofiber composite materials due to their simple structure and low cost. Due to these features, recently, it is used extensively to fabricate nanofiber materials in the tapes used for healing the wounds caused by bacteria and viruses in medical [8-12]. In these studies, the most used compound by researchers is TiO_2 which is one of the inorganic- compound with a photocatalytic property causing the disintegration of organic materials under the sunlight UV. So, this compound has been studied in detai[13-15]. On the other hand, some researchers have fabricated TiO_2 nanofibers doping it with different metals such as aluminum, cerium, platinum, silver etc. because of its being a semiconductor with broadband range achievable by doping with these metals. Among these elements, silver is the most used material doped into TiO_2. Many researchers have studied Ag-doped TiO_2 nanofibers because they are antibacterial agents in biomedical materials owing to their powerful antibacterial activity [16-21].

In this report, we have fabricated undoped and doped with different percent of Ag- TiO_2 nanofibers by the electrospinning method. Then, we have investigated the anti bacterial activities of the undoped and Ag-doped TiO_2 nanofibers on *S. aureus* bacteria. In addition, to explain the photocatalytic properties of the fabricated nanofiber samples have been characterized by scanning electron microscopy energy dispersive analysis (SEM-EDX), differential thermal agency/thermal gravimetric analysis (DTA/TG), and X-ray diffraction (XRD) methods.

2 Experimental

2.1 Fabrication of pure and Ag-doped TiO_2 nanofibers

To prepare pure and with different content Ag-doped TiO_2 nanofibers (1%, 2%, 3%, 4%, 5%), we used precursor materials of $AgNO_3$ (Merck, 99.9%, Mw = 62.004 g/mol), $Ti[OCH(CH_3)_2]_4$ (Aldrich, 99.999%, Mw = 284.22 g/mol), CH_3CH_2OH (Sigma-Aldrich absolute, 99:8% Mw = 46.07 g/mol), and PVP (Aldrich, $(C_6H_9NO)x$, Mw = 1,300,000). First, we prepared TiO_2 solution which we will use as a stock solution and also to fabricate pure TiO_2 nanofibers. In order to prepare stock TiO_2 solution, first, 3.6 mL of titanium isopropoxide and 2.4 mL of diethanolamine were added into 100 mL of ethanol. Then, we began to stir this solution with a magnetic stirrer and added 0.24 mL polyethylenglycol dropwise for 1 hour. In the second step, we prepared the solution which will be used for the fabrication of Ag-doped TiO_2 nanofibers. We dissolved 10 g of PVP into 100 mL ethanol and stirred this solution (stock PVP solution) about 1 hour with a magnetic stirrer until an homogeneous solution was obtained. Then, 1%, 2%, 3%, 4%, 5% $AgNO_3$ were dissolved into DMF solutions contained in five separate beakers and stirred half an hour with a magnetic stirrer to obtain homogeneous solutions. After preparing these two solutions, we prepared five different solutions as follows; to obtain the solution that we will use for the fabrication of the 1% Ag-doped TiO_2 fibers, we took 2.41 g from the stock TiO_2 solution and 12.46 g from the stock PVP solution added them together and stirred with the magnetic stirrer until it was homogeneous. Similarly, the other solutions that will be used to fabricate 2%, 3%, 4%, 5% Ag-doped TiO_2 nanofibers were obtained following the same processes. Finally, loading these obtained homogeneous solutions and the pure TiO_2 solution obtained before into a plastic syringe of the pump of the electrospinning set-up, we fabricated Ag-doped and pure TiO_2 nanofiber samples, respectively. All samples have been produced at constant voltage, 20 kV, at constant heights, 8 cm, and with a constant flow rate, 0.1 mL/h.

2.2 Determination of the antibacterial effect of nanofibers

After completing the fabrication of the nanofiber samples, we applied two different methods to see their antibacterial effect on *S. aureus* bacteria known as Disk Diffusion Method and Baird Parker Agar Plate. We, first, applied the disk diffusion method to see the effect of the concentration of Ag on the bacteria as following: To do this, 1% and 5% silver-doped TiO_2 solutions were immersed on each of the cellulose and silicon substrates. Then, 100 µl 0.5 Mc Farland turbidity of *S.aureus* (ATCC 25923), suspensions were spread on to petri plates. After this step, all samples which will be tested were cut into 6x6 mm pieces and placed on to the plates and incubated aerobically for 24 hours at 35±2°C. After incubation, growth inhibition zone diameters around the samples were measured with a ruler. Different diameters of the inhibition zones around the discs have demonstrated the antimicrobial effects of the tested substance.

After completing this measurement, we applied the Baird Parker Agar Plate method for the fabricated Ag-doped nanofibers. To do this we used the following procedure: Counting of *S. aureus* bacteria was carried out using Baird Parker agar with spread plate method. Firstly *S. aureus* ATCC 6538 strain was reproduced in nutrient broth for 24 hours at 37°C. Ag-doped TiO_2 nanofiber samples produced on lamella. After, each sample surface was inoculated to be 10^6 kob / mL bacteria in cm^2. The sample from reproduced microorganism was spread homogeneously to the surface with a sterile pipette. After the samples are kept in the appropriate time and environment, samples were taken from the surface with the help of a sterile swap of each sample and the swaps were transferred into tubes with sterile 10 mL ringers in the tubes. After preparing serial dilutions, Baird Parker agar plates were inoculated with each of the dilution of bacteria with the help of a sterile drigalski spatula so sowing with each pair being parallel to each other and spread homogeneously.

The broth to be used in the analyzes was sterilized in an autoclave at 121°C for 20 minutes at 1 atmosphere pressure, then cooled to room temperature. In this way, for each sample to be plated, 3 dilutions of darkness, sunlight, and UV were prepared. After the media was expected to absorb the sample, the media was allowed to incubate for 24 h at 37°C. After incubation, black colonies with white zones around 0.5 mm denier were counted and the number of bacteria in cm^2 of samples was calculated according to the following formula [22-24].

$$N=C/[V\times (n1+0.1\times n2)\times d\,]$$

Here; N; , the number of microorganisms in 1 gram or 1 milliliter; C, Total number of colony counted in all petri plates; V; volume transferred to petri plates counted (mL); n1, the petri plate where the first dilution is done is counted: n2, The petri plate which is counted in the second dilution; d, the dilution ratio of the two consecutive dilutions of the census.

2.3 Characterization

Morphology, crystal structure, and thermal properties of the fabricated fibers were performed by scanning electron microscope (SEM) (Leo 1430 VP), X- ray diffraction (XRD) (XRD 6000- Shimadzu) with CuKα radiation (λ = 1.5418 **Å**) at 40 kV and 100 mA, and differential thermal analysis/thermal gravity (DTA/TG) (Netzsch STA449F3) measurements.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 SEM measurements

SEM images of 1, 2, and 3, 4, 5 wt % Ag-doped TiO_2 nanofibers and their measured average thicknesses are given in Figure 1 and Table 1, respectively. As seen from Figure1, all nanofibers are straight, smooth, and uniform long fibers and their thicknesses are at the nanometer scale. Images of nanofibers taken with scanning electron microscopy (SEM) have been analyzed to study the fiber morphology and to determine distributions of the diameter fiber thicknesses using FibraQuant 1.3 Software.

As seen Table 1 and Figure 1, diameters of Ag/TiO_2 nanofiber samples are at the nanometer scale. The measured average diameter of these nanofibers depends on the concentration of Ag. The diameters of the nanofibers increased with increasing Ag concentration (up to 3%). Similar mechanisms and results were reported in our previous study [16].

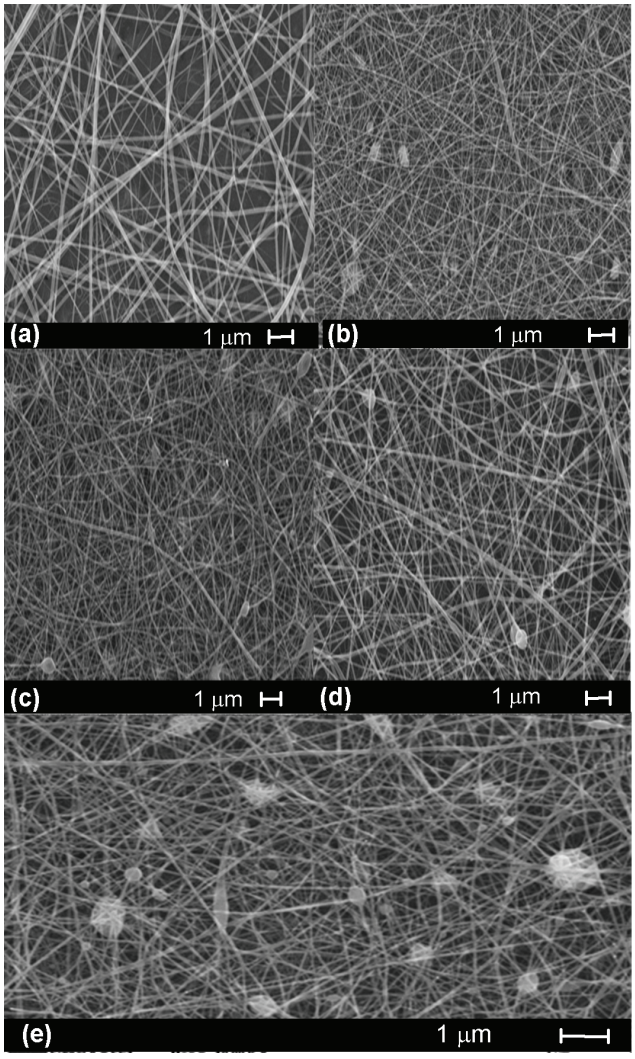

Figure 1: SEM Image of Ag-Doped TiO_2 Nanofibers (a) 1 % Ag, (b) 2% Ag, (c) 3% Ag, (d) 4% Ag, (e) 5% Ag.

Table 1: Average diameter thickness, standard deviation, mean value, number of the measured nanofibers, and image analysis of 1, 2, and 3, 4, 5 wt % Ag-doped TiO_2 nanofibers.

Ag wt%	Fiber Diameter (μm)			Measure-ments	Image Area Analyzed (%)
	Average	Std. Dev.	Mean		
1	0.333	0.134	0.318	357	100
2	0.464	0.228	0.557	20	100
3	0.562	0.194	0.626	19	100
4	0.204	0.124	0.164	30	100
5	0.439	0.239	0.436	53	100

3.2 XRD measurements

The XRD patterns of the pure TiO_2 nanofibers and 5% Ag doped TiO_2 nanofibers thermally treated at 500°C are shown in Figure 2 a, b, respectively. As it is known from the literature that pure TiO_2 nanofibers thermally treated below 500°C have the crystalline both anatase and rutile [16] . As seen from Figure2, there is a decrease in the rutile structure in 5% Ag-doped TiO_2 nano fiber compared to pure TiO_2 nanofiber.

3.3 DTA /TG measurements

Chemical properties of the sample materials have been determined by DTA/TG measurements. DTA and TG analysis of pure TiO_2 and 5 wt % Ag doped TiO_2 nanofibers show in Figure 3. As seen from these figures, mass losses of nanofibers are different. The broad endothermal peak in the range of 400-500^{0}C corresponded to the enhanced crystallization of anatase rather than an anatase→rutile phase transition. The TG curve recorded 62.9% significant

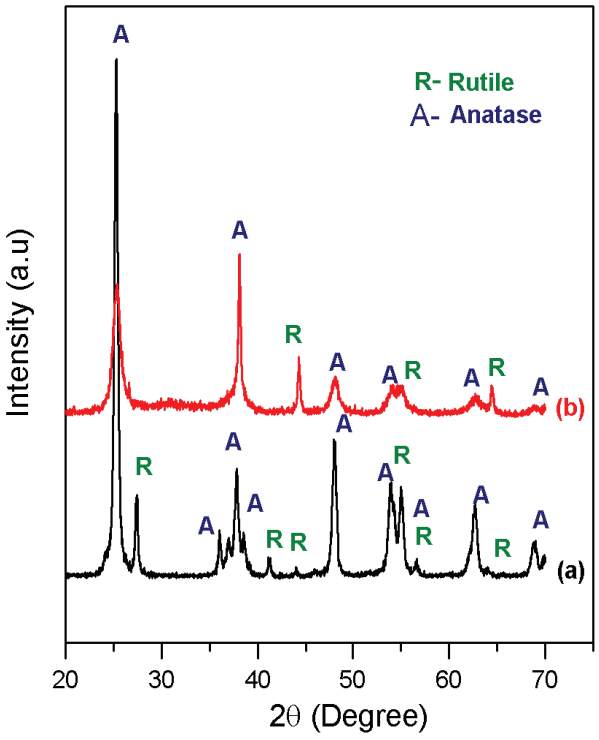

Figure 2: XRD Patterns of (a) Pure TiO_2 and (b) 5 % Ag-doped TiO_2.

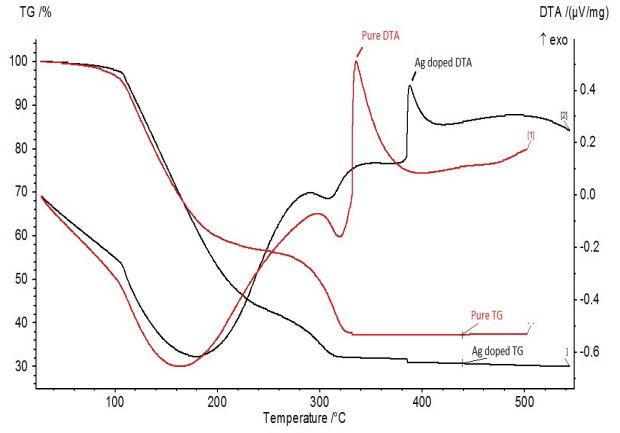

Figure 3: DTA and TG analysis of pure TiO_2 and 5 % Ag Doped TiO_2 nanofibers.

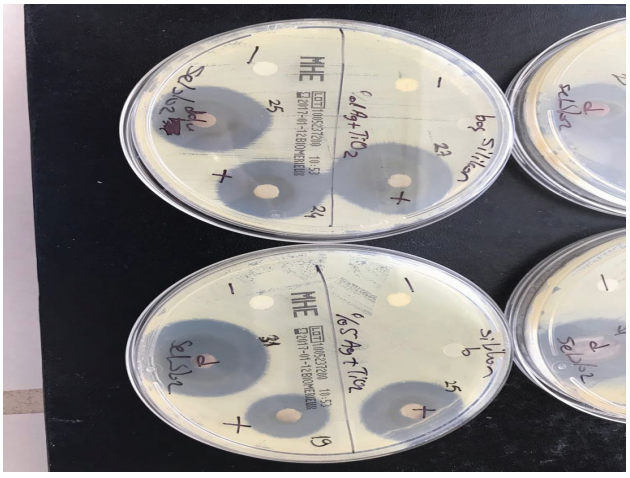

Figure 4: Image of the zones showing Inhibitory Effect of Ag /TiO_2 Nanofibers against *S. Aureus*.

weight losses in the temperature range 25-600°C, which are mainly due to dehydration of physically absorbed water, volatile organic solvent and crystallization water in the as-prepared Ag–TiO_2 samples.

3.4 Disc diffusion method and baird parker agar plate method measurements

The experimental results of diameters of inhibitory zones against bacteria obtained from disc diffusion method for 1% and 5% Ag-doped nanofibers are given in Table 2.

As seen in Table 2, diameters of inhibition zones of Ag-doped TiO_2 nanofibers (1% and 5%) on the silicone substrate for *S. aureus* were measured as 27 mm and 25 mm, respectively. Similarly, diameters of inhibition zones of Ag-doped TiO_2 nanofibers (1% and 5%) on the cellulose substrate for *S. aureus* were measured as 25 mm and 31 mm, respectively. The largest zone diameter (31 mm) was obtained for 5% Ag-doped on the cellulose substrate. The inhibition zone diameters for all samples on culture plates are seen in Figure 4. According to the results, excellent antibacterial effects were obtained against *S. aureus*.

The experimental results of the number of bacteria obtained from Baird Parker Agar Plate method for Ag-doped TiO_2 nanofibers 1%, 2%, 3%, 4% and 5% are given in Table 3. As seen in Table 3, the number of bacteria decreases with increasing silver content under UV, sunlight and dark, until it goes completely to zero. As a result, we can say that Ag-doped TiO_2 nanofibers have very good antibacterial effect on *S. aureus* bacteria.

Table 2: Diameter of Inhibitory Zone against Bacteria.

(w)% Ag/TiO_2	Diameter of Inhibitory Zone Against Bacteria (mm)		
	Silicone	Cellulose	Empty disc
Pure	100 µl 0.5 Mc Farland		
1	27 mm	25 mm	-
5	25 mm	31 mm	-

Table 3: Bacterial Amount (kob/cm²).

(w)% $AgTiO_2$	Bacterial Amount (kob/cm²)		
	Dark	UV	Sunlight
Pure	$1.85.10^5$		
Undoped TiO_2	$7.1.10^4$	$7.0x10^2$	$5.1.10^3$
1% Ag	$1.3.10^3$	0	0
2% Ag	0	0	0
3% Ag	0	0	0
4% Ag	0	0	0
5% Ag	0	0	0

4 Conclusions

Undoped and Ag- doped TiO_2 nanofibers were produced by using the electrospinning method. The results obtained from the characterization measurements of the samples are given as following;

The antibacterial properties of the produced nanofibers were investigated by using two methods (Baird Parker agar plate and disc diffusion). *S. aerus* (ATCC 6538) strain was used as the Baird Parker agar plate method and *S. aerus* (ATCC 25923) strain was used as the disc diffusion method. Increasing silver content, almost all of the bacteria have disappeared under UV and sunlight and the dark.

This was measured using the inhibition zones diameters for all samples on culture plates. According to the results, good antibacterial effects were obtained. The biggest zone diameter was obtained with 31 mm for *S. aureus*.

The morphology and structure of undoped and Ag-doped TiO_2 nanofibers was confirmed by SEM, XRD, and DTA/TG techniques.

Acknowledgements: This work was supported by Suleyman Demirel University via Research Project Coordination Unit [BAP PROJECT- 4196-YL1-14].

Conflict of interest: Authors state no conflict of interest.

References

[1] Li L., Sui H., Zhao,K., Zhang W., Li X., Liu S., et.al., Preparation of carbon nanofibers supported MoO_2 composites electrode materials for application in dye-sensitized solar cells, Electrochim. Acta, 2018, 259, 188-195.

[2] Krysovaa H., Trckova-Barakovab J., Prochazkaa J., Zukala A., Maixnerc J., Kavana L., Titania nanofiber photoanodes for dye-sensitized solar cells, Catal. Today, 2014, 230, 234–239.

[3] Yamada K., Suwa Y., Katagiri C., Nakayama K., High vertical carrier mobility in the nanofiber films of a phthalocyanine derivative and its application to vertical-type transistors, Org. Electron, 2018, 53, 320–324.

[4] Ai Y,, Loua Z., Chena S., Chen D., Wang M. Z., Jiangd K., Shena G., All rGO-on-PVDF-nanofibers based self-powered electronic skins, Nano Energy, 2017, 35, 121–127.

[5] Massagli G., Quaglio M., Semiconducting nanofibers in photoelectrochemistry, Mat. Sci. Semicon. Proc., 2018, 73,13–21.

[6] Tanveera M., Habib A., Khan B. M., Improved efficiency of organic/inorganic photovoltaic devices by electrospun ZnO nanofibers, Mater. Sci. Eng. B, 2012,177,1144–1148.

[7] Ramesh S., Kim S. H., Kim J.H., Cellulose–Polyvinyl Alcohol–Nano-TiO2 Hybrid Nanocomposite: Thermal, Optical, and Antimicrobial Properties against Pathogenic Bacteria, Polym. Plast. Technol. Eng., 2018, 57, 669–681.

[8] Malwala D., Gopinath P., Efficient adsorption and antibacterial properties of electrospun CuO-ZnO composite nanofibers for water remediation, J. Hazard. Mater., 2017, 321, 611–621.

[9] Zhang Z., Wu Y., Wang Z., Zhang X., Zhao Y., Sun L., Electrospinning of Ag Nanowires/polyvinyl alcohol hybrid nanofibers for their antibacterial properties, Mater. Sci. Eng. C, 2017, 78, 706–714.

[10] Hwang S. H., Song J., Jung Y., Kweon O. Y., Song H., Jang J., Electrospun ZnO/TiO2 Composite Nanofibers as a Bactericidal Agent, **Chem. Commun.**, 2011,**32**, 9164-9166.

[11] Hassan M. S. , Amna T. , Al-Deyab S. S., Kim H. C., Oh T. H. , Khil M. S. , Toxicity of Ce2O3/TiO2 composite nanofibers against S. aureus and S. typhimurium: A novel electrospun material for disinfection of food pathogens, Colloids Surf. A Physicochem. Eng. Asp., 415, 2012, 268– 273.

[12] Nthumbi R. M., Ngila J. C., Electrospun and functionalized PVDF/PAN nanocatalyst-loaded composite for dechlorination and photodegradation of pesticides in contaminated water, Environ. Sci. Pollut. Res., 2016, 23, 20214–20231.

[13] Barakat N. A. M., Kanjwal M. A., Al-Deya S. S., Chronakis I. S., Kim H. Y., Influences of Silver-Doping on the Crystal Structure, Morphology and Photocatalytic Activity of TiO2 Nanofibers, Mater. Sci. Appl., 2011, 2, 1188-1193.

[14] Ramesha S., Kima H. S., Kim J. H., Cellulose–Polyvinyl Alcohol–Nano-TiO2 Hybrid Nanocomposite: Thermal, Optical, and Antimicrobial Properties against Pathogenic Bacteria, Polym-Plast. Technol., 2018, 57, 669–681.

[15] Caratão B., Carneiro E., Sá P., Almeida B., Carvalho S., Properties of Electrospun TiO_2 Nanofibers, J. Nanotechnol., 2014,1-5.

[16] Cicek Bezir N., Evcin A., Kayalı R., Kasıkcı Ozen M., Oktay A., Investigation of structural, electronic and optical properties of pure and Ag-doped TiO_2 nanofibers fabricated by electrospinning, Cryst. Res. Technol., 2016, 51, 65–73.

[17] Evcin A., Ç. Bezir N., Kayalı R., Kasıkçı M., Oktay A., Characteristic Properties of Dy-Eu–Ag co-Doped TiO2 Nanoparticles Prepared by Electrospinning Processes, Acta. Phys. Pol. A, 2015, 128, 303-306.

[18] Mishra S., Ahrenkiel S. P., Synthesis and Characterization of Electrospun Nanocomposite TiO_2 Nanofibers with Ag

Nanoparticles for Photocatalysis Applications, J. Nanomater., 2012, 1-6.
[19] Aboelzah A., Azad A. M., Vijay G., Necrosis of Staphylococcus aureus by the Electrospun Fe- and Ag-Doped TiO2 Nanofibers, Int. Sch. Res. Notices, 2012, 1- 11.
[20] Kanjwal M. A.,Barakat N. A. M., Sheikh F. A., Khil M. S., Kim H. Y., Functionalization of Electrospun Titanium Oxide Nanofibers with Silver Nanoparticles: Strongly Effective Photocatalyst, Int. J. Appl. Ceram. Technol., 2010, 7, E54–E63.
[21] Barakat N. A. M., Kanjwal M. A., Al-Deyab S. S., Chronakis I. S., Kim H. Y., Influences of Silver-Doping on the Crystal Structure, Morphology and Photocatalytic Activity of TiO2 Nanofibers, Mater. Sci. Appl., 2011, 2, 1188-1193.
[22] J. T. Nickerson, A. J. Microbiology of Food and Food Prossesing. American Elsevier Publishing Company. New York. USA. (Sinskey), 1974.
[23 Anonim, Census of milk and products and coliforms. Part 1, Colony Counting Technique at 30°C. General Directorate of Turkish Standards, Ankara, TS 6930, 1989.
[24] K. Halkman, Food Microbiology Applications, Başak Printing and Promotion Services Limited Company, Ankara, 2005.