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Nano-silver embeded nanofibrous face-mask for infection control

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ABSTRACT

Many bacterial or virus diseases are harmful for human organism as well as dust impurities and the large spectrum of micro-organisms in the surrounding air. The boom of nanotechnology demands the exposure to nanoparticles which may be dangerous if inhaled. Our research team is trying to get used of nanoscience to be protected of its side effects. The objective of the proposed facemask has been reached through a filter which contains at least one couple of nanofibrous layers, out of which in the direction of passage of the filtrated media, to be loaded with nanosilver particles and then study their anti-viral properties to capture the nano sized impurities and control infection. As the nanometerials can have side effects of inhaling them so, we thought about fabricating a new nanofibrous face-mask to be worn by the personnel dealing with nanoparticles as well as against common pollutions. At the same time nanofibrous can be loaded with nanosilver for the purpose of anti-viral infection as in the case of medical usage.

1. INTRODUCTION

At present there is known a large quantity of various types of screens, respirators, gas masks, filters and similar equipment for cleaning of breathed in air, while the entire majority of the known solutions of these means concentrates first of all to removing of dust particles from the inhaled air. Their principle consists especially in creation of more or less complex labyrinth (e.g. of fibres) so that there is the highest possible probability that the dust particles or similar corpuscular impurities are caught. The disadvantage of such designed means nevertheless is that in spite of their relative complicated structure they mostly do not act on micro-organisms/nanoparticles being present in the passing air and these after then easily penetrate into the airways of the user, possibly they are caught in the structure of the said means, where they quietly exist and they may, even after a relatively long time since bringing the first micro-organisms/nanoparticles, become a source of infection or contamination (Brown 1992).

Polymeric nanofibers layer is produced by Electrospinning technique. Electrospinning is an old technique. It was first observed by Rayleigh in 1897, studied in detail by Zeleny in 1914 (on electrospraying), and patented by Formhals in 1934. In particular, the work of Taylor and others on electrically driven jets has laid the groundwork for electrospinning. Electrospun nonwoven webs, as currently fabricated, are accumulations of randomly deposited nanofibers. These webs have extremely high surface to volume ratios and porosities, as well as nanoscale features, and they have proven useful in filtration systems and show promise as scaffold materials in tissue engineering (Huang, 2003), (Tsai, 2002).

The nonwoven structure has unique features, including interconnected pores and a very large surface-to-volume ratio, which enable such nanofibrous scaffolds to have many biomedical and industrial applications (Said, 2011-2012). The chemical composition of electrospun membranes can be adjusted through the use of different polymers, polymer blends, or nanocomposites made of organic or inorganic materials. In addition to the control of material composition, the processing flexibility in maneuvering physical parameters and structures, such as fiber diameter, mesh size, porosity, texture, and pattern formation, offers the capability to design electrospun scaffolds that can meet the demands of numerous practical applications (Elaufy, 2005). Electrospun nanofibrous layers have very high porosities which allow them to be highly permeable to water vapor, but they also have extremely small pore sizes, which makes them extremely efficient filters and can be used in facemask production (Podgórski, 2006), (Barhate, 2008).

However, thanks to emerging nanotechnology applications, silver is making a comeback in the form of antimicrobial nanoparticles. As even the most powerful antibiotics become less and less effective, researchers have begun to re-evaluate old antimicrobial substances such as silver and as a result, antimicrobial nano-silver applications have become a very popular early commercial nanotechnology product (Chen, 2008).

In this antibacterial medicated facemask, each component serves a different function: nanofibers as the adsorption material for capturing bacteria/nanoparticles, and nano-silver as the release-active antibacterial agent. The morphology of propsed face-mask will be studied as well as investigating the antibacterial behavior of the nanosilver effect.

2. MATERIALS & METHODS

2.1 Materials

The following materials were used: Nylon 6, Formic acid ~95%, Silver nitrate AgNo3 & Sodium borohydride were purchased and used as it is (Sigma Aldrich, USA). Mueller Hinton agar and Triptych soy broth TSB were used for cell culture for antibacterial effect study. (Oxoid Ltd; Basingostok; Hampshire, England).

2.2. Development and Electrospinning of Nanofibers.

Nylon6 was dissolved in formic acid with three different concentrations (20, 24, 28 w/w%) and stirred for 20 min till complete homogenous mixture is obtained using magnetic stirrer (USA made). Nylon6 nanofibers were prepared using the electrospinning technique in an air-conditioned laboratory at an ambient temperature of 22 ^{o}C and relative humidity of <65%. The electrospinning apparatus was equipped with a high-voltage DC power supply (Gamma high voltage, Inc., USA) set to 22 kV and a syringe with a blunt-tip stainless steel spinneret (0.9 mm diameter). The distance between the spinneret and the fiber collector was kept constant at 10 cm. Nylon6/formic acid solution (5 ml) was gravity-fed to the spinneret. A copper collector covered with aluminum foil was used as the collector of nanofibers.

2.3 Preparation of Nano-silver particles

Ag nanoparticles were prepared by the chemical reduction method which agreed with the literature. Briefly, 0.002 mol dm^{-3} sodium borohydride solutions were mixed with 0.001 mol dm^{-3} silver nitrate solution. Using a measuring cylinder, 30 cm^3 of freshly prepared 0.002 mol dm^{-3} sodium borohydride solutions was measured. The solution was poured into a 100 cm^3 conical flask. A magnetic stirrer bar is added and the flask is placed in an ice bath using a magnetic stirrer. The liquid was stirred and cooled for about 20 minutes. 2 cm^3 of 0.001 mol dm^{-3} silver nitrate solution is added to the cold, stirred solution at the rate of one drop per second. The stirring was stopped immediately once the silver nitrate solution has been added. The flask was removed from the ice bath and the colloidal silver has been produced. The preparation steps of Ag nanoparticles are shown in Figure 1.

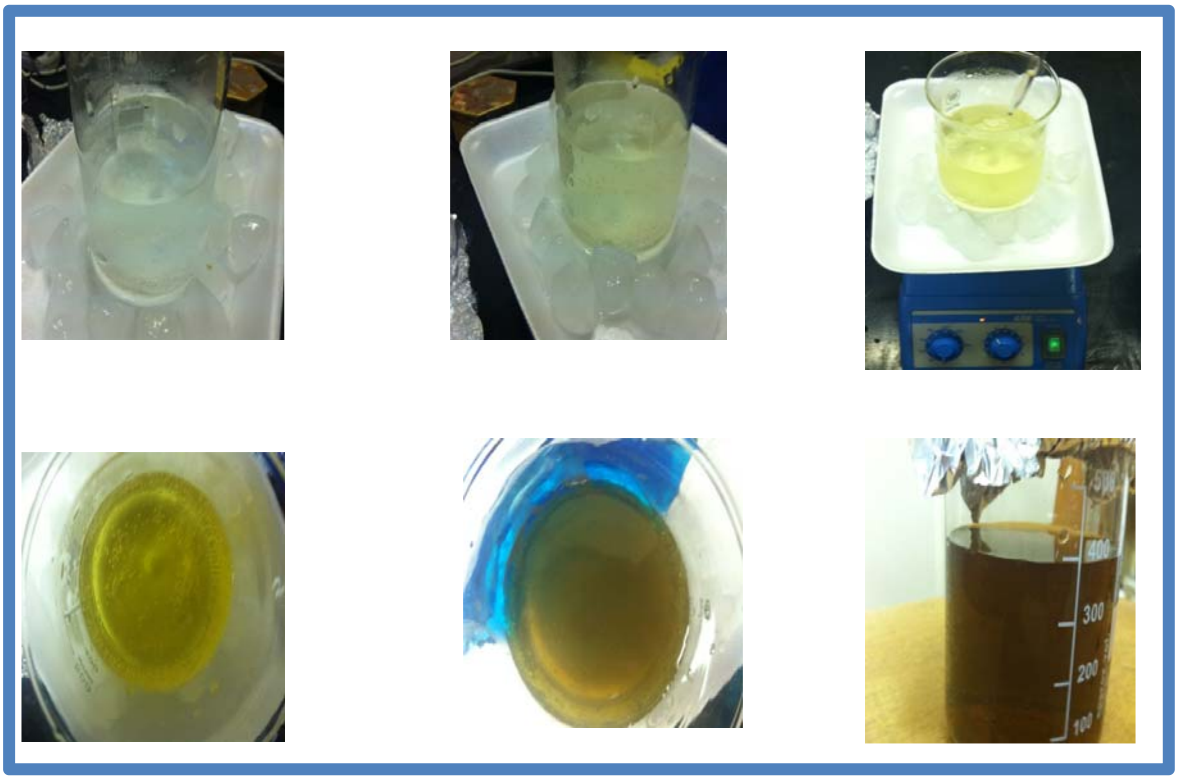

Fig.1 The preparation steps of colloidal solution of Ag nanoparticles.

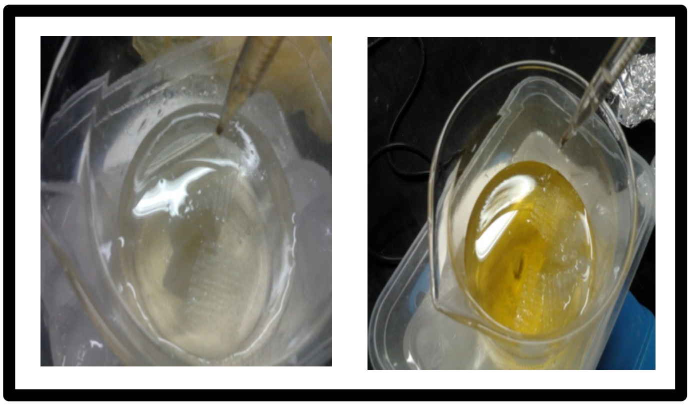

Fig. 2 Soaking the nanofibrous mesh during the preparation of Ag nano particles.

2.3.1 Loading the Nylon nanofibers with Ag nanoparticles

The Nylon nanofibers samples were soaked (as shown in Figure 2) during the chemical reduction by stirring of the sodium borohidryde solution, and then the prepared silver nitrate solution was added at one drop per second rate. Then every an hour one sample of each sample was extracted from the solution. This process allows the Ag nanoparticles to precipitate between the nanofibers & physically bonded to them.

2.4 Characterization of Nylon Nanofibers

Samples of Nanofibers were mounted on metal stubs using double sided adhesive tape onto which the nanofibrous meshes were fixed. The samples were then coated with gold using an ion sputtering coater (JFC-1100E, JEOL, Japan) and the gold coated samples scanned using SEM (JEOL, model JFC-1100E, Japan). The average fiber diameter was determined using image analysis software and at least 10 randomly selected fiber segments.

2.5. Characterization of Ag nanoparticles

Silver nanoparticles that were produced in this way as colloidal solution were observed and investigated. The Electron Micrographs were taken using a Transmission electron microscope (JEM-100 CX Joel), at the Electron Microscope Unit, Faculty of Science, Alexandria University.

2.6. Filter Media Characterization

Mercury Poroimeter (Micromeritics 9310, USA) was used to measure the porosity and pore diameter of the produced nanofiberous meshes to investigate the filtration efficiency of the nanofibers. (Metallurgical Development Research Center, Tabbin, Helwan, Egypt).

2.6 Antibacterial characterization: Microorganisms

The bacteria, used in this study, were **Escherichia coli**. E coli are a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). (Courtesy of the Department of Microbiology, Medical Research Institute, Alexandria University).

2.6.1 Qualitative assessment

Mueller Hinton agar was applied on the disposable plate, then one colony of E.coli bacteria is added all over the plate, then the samples were placed in the disposable plate in such a way that the bacteria covers all the nonwoven surface. Then the samples are placed in the incubator for 24 hours.

2.6.2 Quantitative assessment

One colony (10^8) of the E.coli has been diluted in 1 mL of TSB. From the previous TSB 10 µL was taken to be further diluted in 1 mL of TSB to give (10^6) E.coli. other dilution was made by taking another 10 µL and diluted in 1 mL in TSB to give (10^4) colonies. The last dilution would give 100 colonies to be mixed in 500 µL and then divided into 250 µL to be used as a control sample and 100 µL will be completed with 150 of the nano silver colloid. A drop of each sample was placed in a petri dish coated with MH Agar. The control sample and the silver nanoparticles samples will be placed in the incubator for 1 hr, 2 hrs and 3 hrs and the bacterial growth will be measured after each hour.

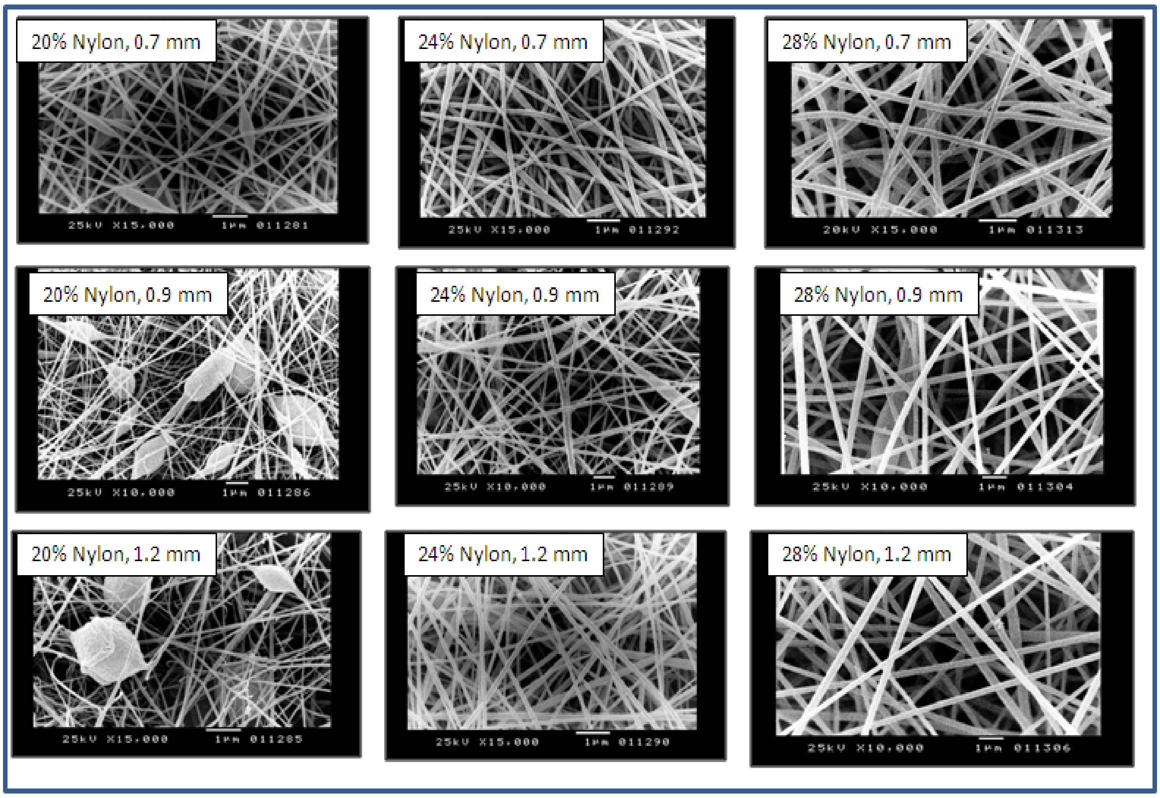

Fig.3 SEM micrographs showing the effect of Nylon content and spinneret diameter on the morphology and diameter of nanofibers.

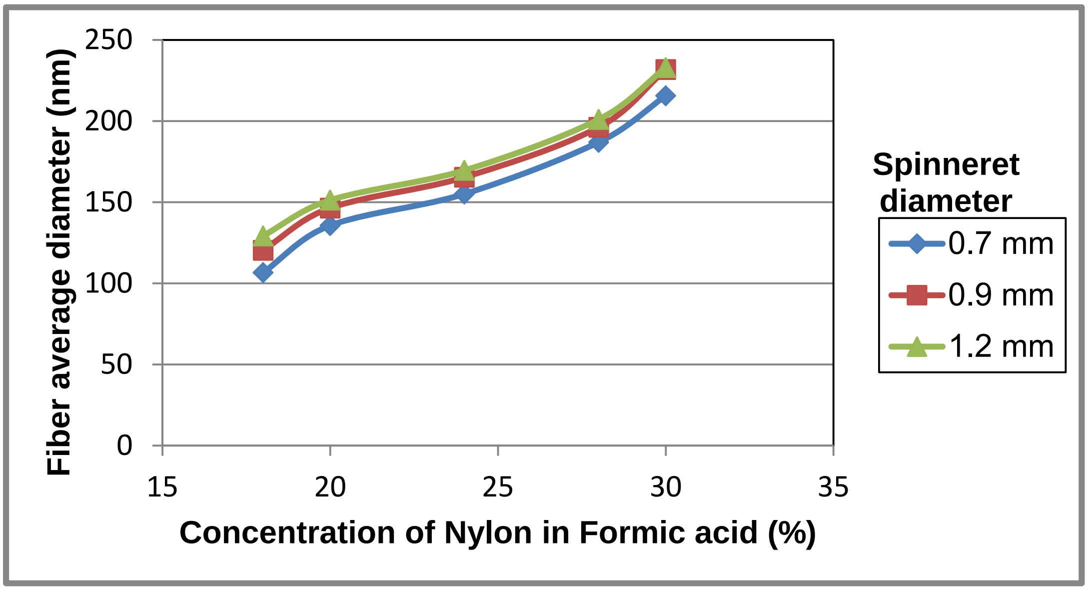

Fig.4 The effect of Polymer concentration and spinneret diameter on the diameter of nanofibers.

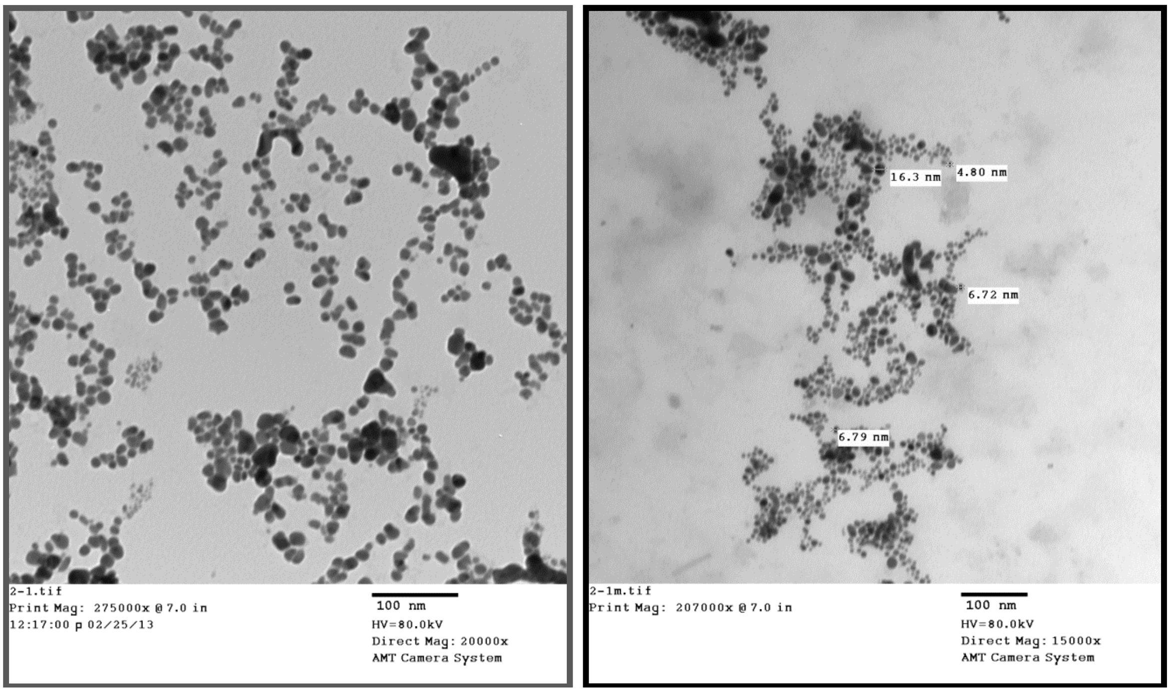

Fig.5 TEM micrographs of Silver nanoparticles.

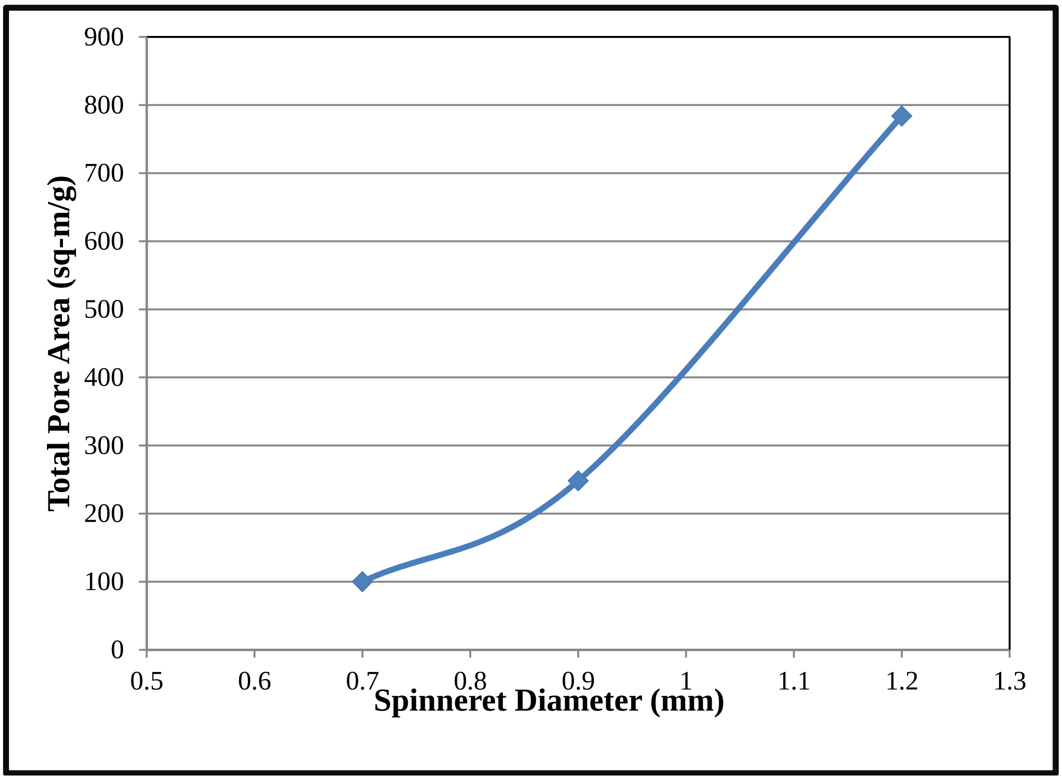

Fig.6 The effect of spinneret diameter on the total pore area of nanofibrous mesh.

3. RESULTS & DISCUSSION

3.1. Development and characterization of plain and silver-loaded Nylon nano-fibers

3.1.1. Morphology by SEM

Plain electrospun fibers obtained had a smooth surface and showed a relatively broad diameter distribution. Their mean diameters ranged from 150 nm to 250 nm.

Fig. 3 shows the effect of Nylon content (20%, 24%, and 28%) on the morphology of plain Nylon nanofibers. As demonstrated previously (Deitzel, 2001), increasing Nylon content resulted in progressively reduced beading, increased fiber diameter, and reduced diameter distribution. These results are illustrated in Figure 4 as well as reducing the spinneret tip diameter would decrease the electrospun nanofibers. The 24% polymer content was chosen for face mask fabrication as it represents the optimum parameters of smooth & uniform size of nanofibers.

3.1.2 Morphology by TEM

Nano-silver colloids have been investigated by means of TEM The morphology of the nanoparticles formed was recorded as smooth and spherical in shape. The average diameter was measured to be 16 nm. It was also found that the silver nanoparticles produced were generally uniform in size and shape as illustrated in the images of figure 3 with min diameter of 5nm and maximum diameter of 20nm .

3.2 Filter Media Characterization

Figure. 6 illustrates the effect of spinneret diameter for the different polymer concentration of 24% on the total pore area of the fabricated nanofibrous filter material. It is obvious that the total pore area increases with the increase in the spinneret diameter due to the increase in the fiber diameter which captures more voids leading to higher pore area.

Figure 7 shows that the average pore diameter tends to increase with the higher polymer concentration due to the increase in nanofibers diameter. The average pore diameter was in the range of 100nm to 200 nm which place the nanofibers filter within the high efficiency particle air filters.

Figure.8 presents the relation between the polymer concentration with the nano-fibrous mesh porosity. It illustrates that the porosity diminshes with the increase of concentration. It is evidant that the fiber diameter decreases with the decrease of polymer concentration, but due to the 3-D interconnected net work structure of the nanofibrou mesh, the porosity of the nanofibers mesh relatively gets higher with the nanofibers scale despite the small pore diameter of the nonwoven mesh. That is why nanofibrous nonwoven is an ideal candidate for face mask application.

The cumulative Intrusion was measured for the samples of concentration 24 % at different spinneret diameters 0.7mm, 0.9mm and 1.2mm as shown in figure.9.

From figure. 9 it is concluded that the intrusion of mercury enlarged rapidly till it becomes constant at the nano pore size for differet spinneret diameters. These curves agree with those comparing with concentration as both concentration and spinneret diameter affect the nanofiber diameter. So, the finer is the nanofibers the smaller is the air voids and less amount of the accumlated mercurey retained by the tested sample.

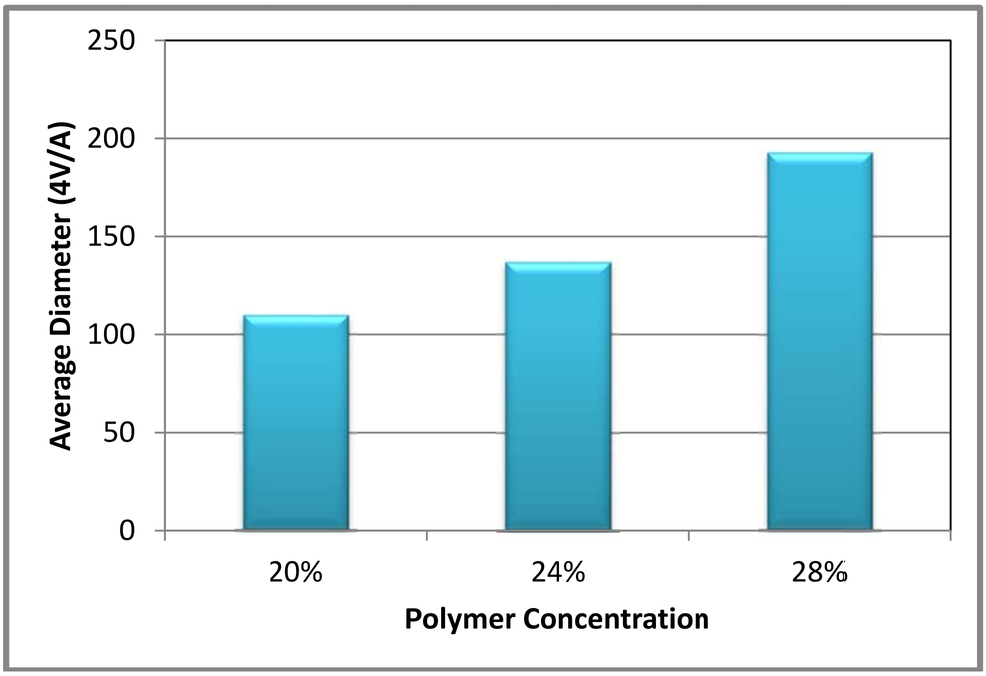

Fig. 7 The effect of polymer content on the average pore diameter

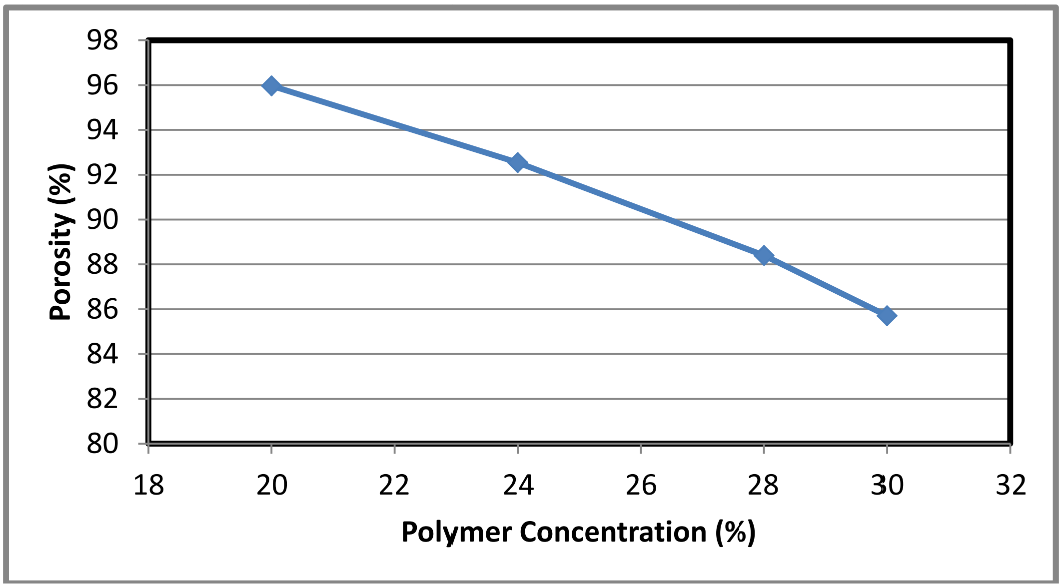

Fig. 8 The effect of polymer content on mesh porosity

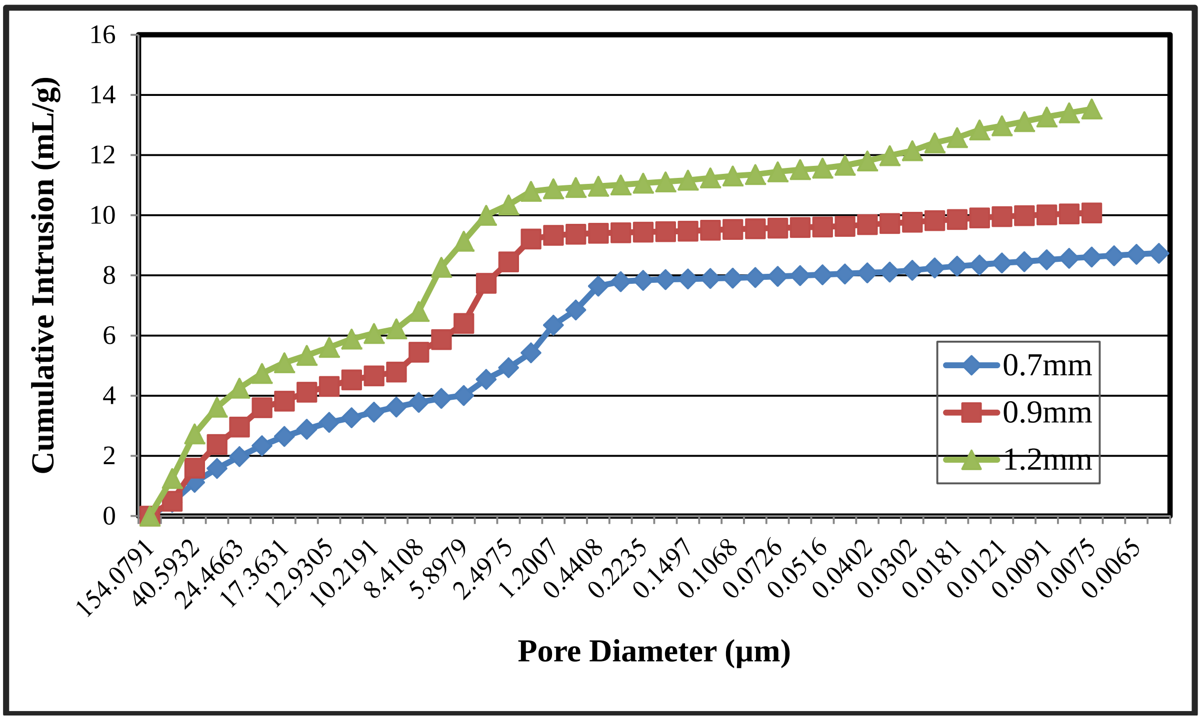

Fig. 9 The effect of spinneret diameter along the pore diameter and filtration effeciency

3.3. Interaction of nanosilver-loaded Nylon nanofibers with E-coli bacteria

3.3.1 Qualitative assessment method of silver nanoparticles antibacterial effect

Two different thicknesses of nanofibers were used (thin ~ 1mm and thick ~ 2mm) (figure 10). It was found that for the thin layer. silver nano-particles have no effect on the growth of the bacteria while for the thick layer, that the growth of bacteria has decreased & the decreasing of growth of the bacteria was effected by the time of soaking, as the time of soaking increased, the nanosilver loaded mesh hinders the growth of bacteria. This is explained due to the more surface area of the thick nanolayer that captures more Ag nano-particles and hence better antibacterial effect.

3.3.2 Quantitative assessment method of silver nanoparticles antibacterial effect

By counting the bacteria colonies per drop, the antibacterial effect of nanosilver can be observed. As shown in Fig. 11 the presence of nanosilver reduces the growth of bacteria as clear in the right hand side of the Petri dish compared to the left hand side. The effect of nanosilver has been increased as demonstrated in Fig. 12. It is evident that after one and two hours the bacteria colonies have been completely vanished under the antibacterial effect of nanosilver. The 3 colonies of bacteria formed after 3 hours can be controlled by increasing the concentration of the nanosilver. In further study the time of usage of the antibacterial face mask will be calculated according to the nanosilver concentration and the disposable time recommended at hospitals.

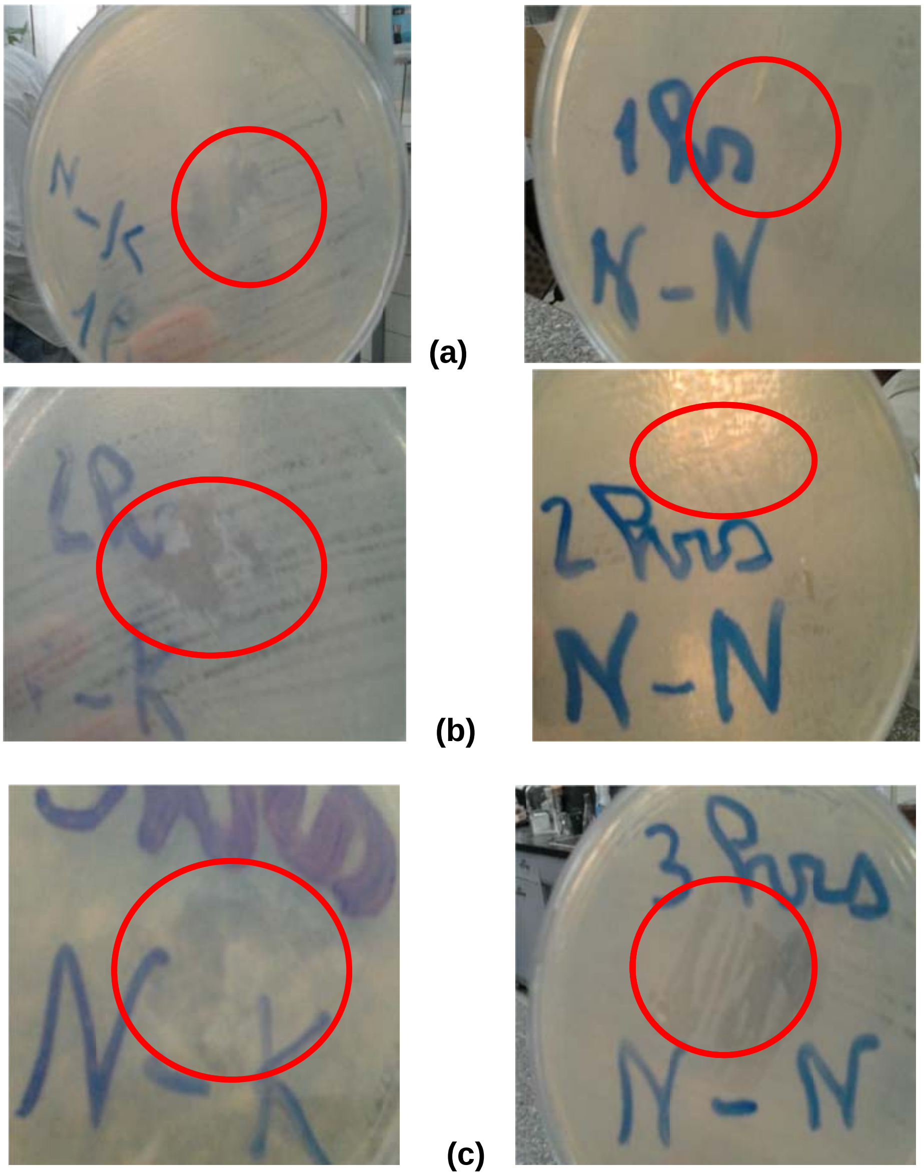

Fig.10 Images of thin & thick layers effect hindering the growth of bacteria underneath their places.

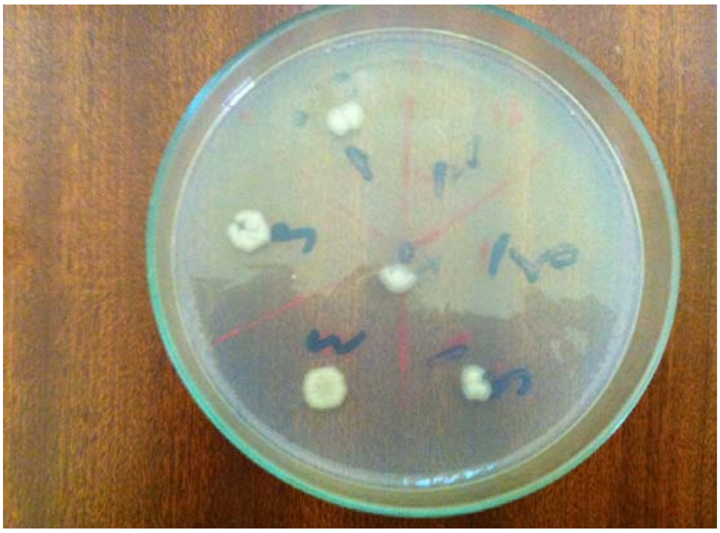

Fig. 11 The effect of nanosilver loaded nanofibers on bacteria growth (right hand side of Petri - dish)

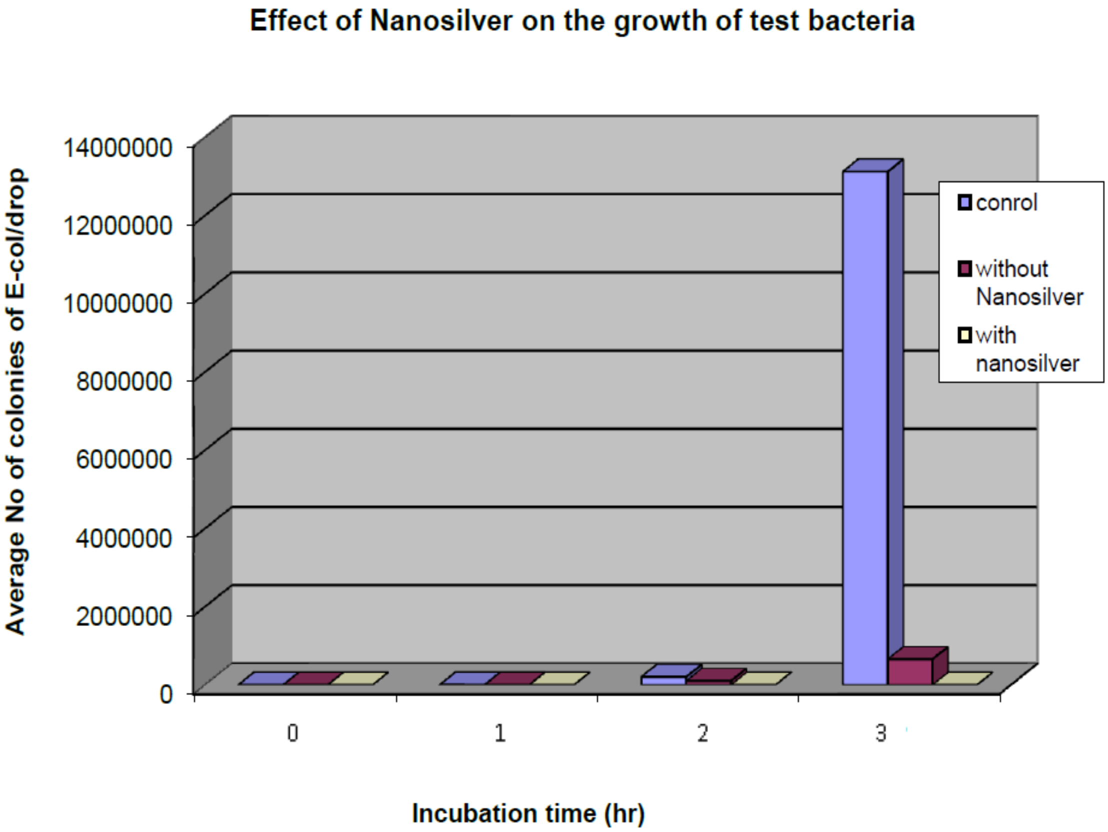

Fig.12 Effect of incubation time on bacteria growth for samples loaded with nanosilver

4. CONCLUSIONS

The electrospun Nylon 6 nanofibers have been produced successfully. The optimized conditions of electrospinning were achieved at 24% of polymer content. The chemical reduction method leads to smooth spherical Ag nano-particles with 16nm average diameter. The qualitative and quantitative microorganism assessment proved the antibacterial response of Ag nano-particles. The filtration efficiency and porosity of nanofibers introduce an ideal candidate for face mask application. The achieved antibacterial properties & higher efficiency of the nanosilver loaded nanofibers paved the road towards the fabrication of the target face mask.

ACKNOWLEDGEMENTS

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