International Journal of ChemTech Research

CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.3, pp 1335-1342, 2014-2015

ICONN 2015 [4th - 6th Feb 2015]
International Conference on Nanoscience and Nanotechnology-2015
SRM University, Chennai, India

Fabrication and characterization of Ag-PSf nanocomposite membrane towards microbial filtration of contaminated water

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Abstract : Polysulfone (PSf) and silver immobilized polysulfone (Ag-PSf) membranes were fabricated using a simple phase inversion technique along with *in situ* synthesis process. The membranes were characterized by field emission scanning electron microscopy (FESEM) to estimate the pore size and to confirm the uniform distribution of the silver nanoparticles across the membrane. The nanoparticle was characterized by UV-visible spectroscopy and the size of the nanoparticle was found to be in the range of 10 – 30 nm. Further, the use of these Ag-PSf membranes for treatment of water containing micro-organisms especially bacterias such as *E.coli*was studied and discussed. Also, the filtration efficiency was studied with emphasis on problems associated with biofilm growth and use of Ag-PSf membranes for the prevention of the same. Further, the retentivity of silver nanoparticles in the membrane was thoroughly studied using inductive coupled plasma mass spectroscopy with an intention to provide a comprehensive report on the suitability of use of these Ag-PSf membranes in water filtration. The results show that the membrane has good silver retention indicating that very little of the silver, if any gets into the treated water.

Key words: Composite membranes; Micro-organisms; Polysulfone; Silver nanoparticles.

Introduction

Contaminated drinking water is a worldwide crisis and the situation is getting worse every day.A recent investigation by World Health Organization (WHO) shows that 80% of the infectious diseases are water-borne; and that more than 5 million people die every year because of contaminated drinking water[1]. The main indication of water contamination is the presence of pathogens such as bacteria, virus and protozoa.

The use of membrane technology in water treatment has become increasingly popular choice for treating microorganisms, particulates and organic materials that contaminate water. The new generation nanocomposite membranes offer several advantages over traditional membranes such as being more chemically robust, having longer life, providing a greater rejection of dissolved salts and organics while being able to operate at much lower pressures. Despite being very efficient against pathogens, nanocomposite membranes do not have the ability to neutralize them as they typically work by the process of size mediated filtering. This results in the bacteria accumulating on the surface of the membranes over time leading to the formation of a biofilm[2] and thereby resulting in membrane fouling and performance degradation. One simple technique to overcome this drawback is to ensure that the fabricated membranes are antimicrobial in nature which would prevent the formation of the biofilm and hence significantly increase membrane performance and its lifetime. The antimicrobial effects of metallic silver (Ag) and its compounds have been known since ancient times[3] and it has also been demonstrated that, in low concentrations, silver is non-toxic to human cells[4]. Further, it has been proved that silver nanoparticles have wide environmental applications especially in water treatment due to its high surface area and high release of silver ions from the surface of silver nanoparticles even at very low concentration. It has also been noted that the activity of the silver nanoparticles depends upon its size and shape. Hence, a nanocomposite membrane immobilized with stable silver nanoparticles should be very effective in filtering microbes from contaminated water.

Various polymer based membranes like cellulose acetate[5-6], polysulfone[7-8], polyethersulfone[9], polyamide[10], polypipearazineamide, polydimethylsiloxane, polyacrylonitrile[11] etc. have been fabricated towards the development of highly efficient membranes. Among these, polysulfone based membranes are widely used for water treatment due to its wide pH tolerance, wide temperature limit, creep resistance, dimensional stability, increased flow rate and better trapping ability[16]. Silver nanoparticles have been incorporated in many polymer membranes depending upon their applications[12-14]. Many immobilization techniques like physical coating (*ex-situ*) and *in-situ* methods are available to immobilize the nanoparticles onto the support matrix. *Ex-situ* immobilization of the silver nanoparticles in the polysulfone membrane showed that there was significant depletion of the silver nanoparticles after continuous treatment of the water through the membrane[15-17]. The *in-situ* synthesis technique of nanoparticles has many advantages, the most important being the greatly reduced depletion of the nanoparticles from the membrane matrix[18-19]. Hence in our work silver nanoparticles were *in-situ* synthesized in the polysulfone matrix.

As far as the Ag-PSf based membrane filtration is concerned the three major issues are the broad spectrum antimicrobial nature of the membrane, the membrane performance degradation due to the fouling process and the induced toxicity of the filtrate due to the presence of silver nanoparticles in the same, if any. In this paper, we report the fabrication of the silver immobilized nanocomposite membrane (Ag-PSf) using the *in situ* synthesis process and the characterization of the physical properties and the antimicrobial efficiency of the nanocomposite membrane. Also, the filtration efficiency with respect to the biofilm growth and prevention of the same and the retentivity of silver in the membrane has been thoroughly studied with an intention to provide a comprehensive report on the suitability of use of Ag-PSf membranes in water filtration.

Experimental

Materials

All the chemicals used in the experiment were obtained from commercial sources as guaranteed analytical grade and used without further purification. Silver nitrate was procured from Rankem, New Delhi. Sodium borohydride and Polyvinylpyrrolidone procured from SRL, Mumbai and Loba Chemi, Mumbai respectively. Polysulfone Resin pellets 75000 (GPC) and 1-methyl-2-pyrrolidone were procured from Acros Organics, Belgium. Absolute alcohol was procured from Hyman, England.

Fabrication and characterization of Ag-PSf membrane

The nanoporous polysulfone support membrane was prepared using the phase inversion technique. 12% polysulfone by weight was dissolved in 1-methyl-2-pyrrolidone at 60°C in completely anhydrous condition till it forms a viscous, homogeneous solution[20-23]. To this homogeneous solution, polyvinylpyrrolidone 10%, the pore-former was added with immense care to prevent the enlargement of the macrovoids (which results in significant increase in the permeability of the membrane) in the membrane[24]. To this colorless homogeneous mixture 0.1% of silver nitrate was added which gave a brownish yellow solution. This mixture is kept undisturbed to eliminate air bubbles which may cause voids during membrane casting process. Then the casting solution was cast on a glass plate with a thickness of ~150µm at 25°C using a casting knife (Elcometer Model 3570). This thin layer of cast solution was then precipitated by immersing for 5 minutes in water containing 0.2% sodium borohydride which acts as reducing agent for the formation of silver nanoparticles. The membrane was then immersed in deionized water at room temperature for 24 hrs to completely remove the pore-former and excess solvent. The membranes were air dried and stored before testing for structure and performance. A polysulfone membrane without silver immobilization was also fabricated for use as a control.

Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) observations of the membranes were carried out using a FEI's, Quanta 200 FE-SEM and an EDAX's Genesis EDS respectively. The characterization of the silver nanoparticles using UV-visible spectroscopy was done by extracting the yellow colored Ag-PSf membrane in Dimethylformamide (DMF) solution and characterizing the same. This was done using a Perkin Elmer Ultraviolet-Visible Spectrometer.

Antibacterial nature of the silver nanoparticles

The effect of silver nanoparticles on *E.coli,was* investigated by culturing the organisms on LB agar plates.

Retentivity of silver in the Ag-PSf membrane

Leaching of silver from the membrane was studied using Perkin Elmer's Nexion 300 inductive 1coupled plasma mass spectrometer (ICPMS).

Results and Discussion

Characterization of Ag-PSf membrane and silver nanoparticles

FESEM images were taken on the surface of the membrane samples to examine the morphology of the membranes formed.

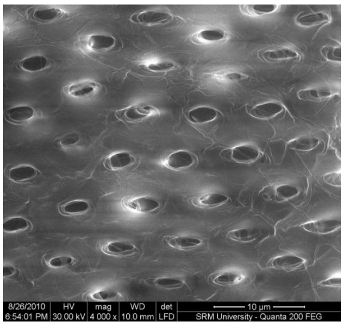

Fig.1 FESEM image of the Ag immobilized PSF membrane

Fig.1 shows the FESEM image of the polysulfone membrane. The image shows the formation of asymmetric and fully developed pores. The pores in the membranes are found to be in the range 100–200nm. The figure also show the formation of elongated and uniform sized pores in which the pores are not spherical in shape and might be due to formation of macrovoids. The formation of macrovoids are due to the rapid exchange or penetration of the non-solvent into the weak points of the membrane surface and and the skin layer at the top of the membrane can hinder the nonsolvent entering into the sublayer in order to create many nuclei thereby forming macrovoids and more void structures. The formation of uniform pores might be due to the formation of uniform nucleation and growth. These interconnected pores also include the macrovoids which extend through most of the substructures. Macrovoids are most commonly found in membranes prepared by immersion precipitation method (phase inversion), because of the thermodynamic instability and low rheological barrier.

Figs. 2 (a&b) shows the FESEM image of the Ag-PSf membrane and the EDS graph respectively.

Figs. 2 (a&b) shows the FESEM image and the EDS graph of the Ag-PSf membrane respectively. The macrovoid surface formed in PSf membranes were suppressed by the formation of spherical shaped pores and hence slight decrease in the pore size due to the change in shape. Other than this, there was no observed change on the surface of the silver immobilized membranes. EDS measurement was also taken on the Ag-PSf membranes to identify and confirm the presence and composition of silver nanoparticles on the surface of the membrane. EDS mapping indicates the uniform distribution of the Ag nanoparticles across the surface of the membrane.

As mentioned before, the yellow colored Ag-PSf membrane was extracted in Dimethylformamide (DMF) solution and analyzed using UV-Vis spectroscopy. For the UV-Vis studies, DMF solution was used as the reference. The results for the same is shown in figure 3, indicating that the suspension of extracted Ag nanoparticles shows strong absorption band (plasmon excitation) in visible region[25], at about ~420nm.

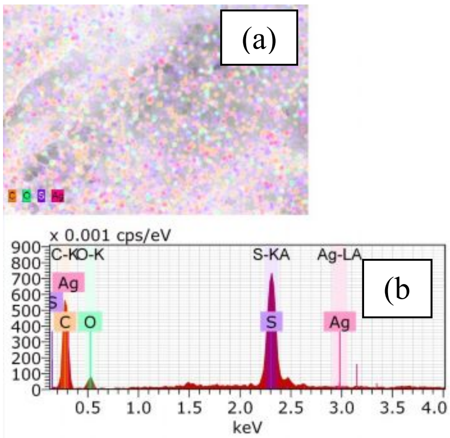

Fig.2 (a) FESEM of the Ag-PSf membrane, (b) EDS graph of the Ag-PSf

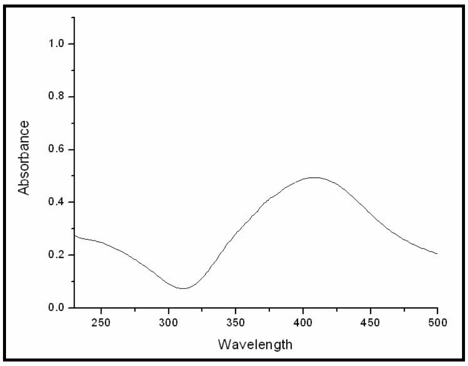

Fig.3. UV-Vis absorption spectrum of Ag nanoparticles

This shows the presence of roughly spherical silver nanoparticles as the position of the plasmon absorption depends upon the particle size and shape and it has been reported that the absorption spectrum of spherical silver nanoparticles lies between 420–490 nm depending upon the particle size[26].

Testing of antibacterial efficiency of the Ag-PSf membrane

The efficiency of the membrane towards the filtration of the bacteria was carried out using the PSf and Ag-PSf membranes. The growth of bacterial colonies gives the direct count of bacterial contamination in the unfiltered and filtered water.

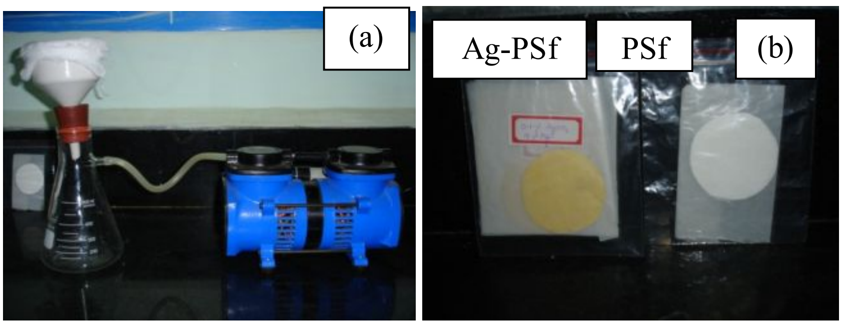

Fig.4 (a) Filtration unit, (b) PSf and Ag-PSf membranes

The filtration unit shown in fig.4 (a) was used for the filtration experiments. The filtration unit was sterilized continuously before each and every filtration process in order to avoid contamination. The water is filtered through the buckner funnel with the low pressure around 2 bar was maintained using a small pump.

The filtrate was collected in the flask and taken for testing periodically. The used bacterial strains were serial diluted until countable (60-80) required colonies were obtained. The dilution which gave required colonies were mixed with deionized water and used for filtration under sterile condition. For the purpose of testing, a 100ml water sample was made by adding 20µl of *E.coli* suspension to the sterilized deionized water which approximately gave a colony count of 65-70.

Two membranes were used for the water treatment, as shown in fig.4 (b) a polysulfone (PSf) control membrane and an Ag-PSf nanocomposite membrane. The effect of silver nanoparticles on *E.coli* bacteria was investigated by culturing the organisms on LB agar plates. Nutrient agar medium was used to inoculate the water samples. The bacterial loaded water was filtered through the PSf and Ag-PSf membranes in which 100µl of unfiltered water was inoculated over a culture plate before filtration and spread uniformly. Two culture plates were inoculated with 100µl water filtered using PSf and Ag-PSf membranes. All three inoculated culture plates were incubated at 37^{o}C for 24 hours. Fig. 5(a) exhibits the presence of countable colonies of *E.coli.* in the water sample that was used for the filtration. Fig.5(b) and (c) shows the petri plates incubated with the water filtered through the PSf membrane and the Ag-PSf membrane respectively.

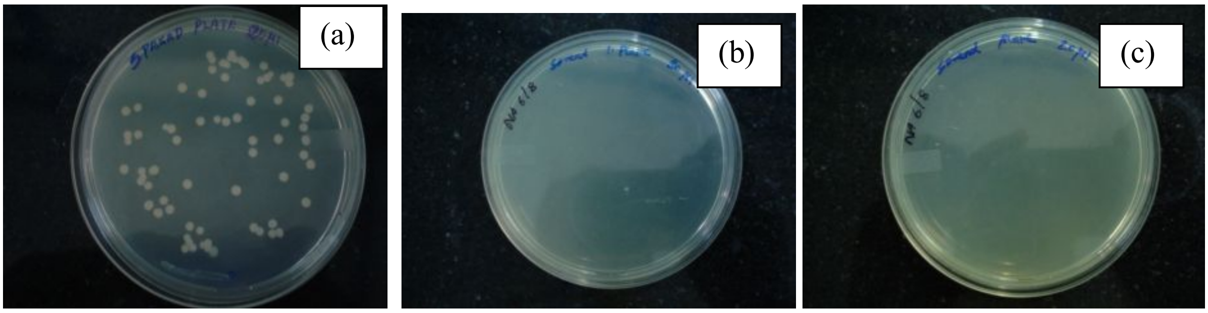

Fig.5 (a) Culture plate inoculated with unfiltered water (b) Culture plate inoculated with water filtered with PSf membrane (c) Culture plate inoculated with water filtered with Ag-PSf membrane

It can be clearly seen that the filtrates of both the membranes are free from bacteria. This is due to the fact that the process of bacterial filtration by polysulfone membrane is dominated by the size exclusion process which depends on the pore size of the membrane used. Since, both membranes have comparable pore sizes both membranes are equally good in the removal of bacteria from the water. It is known that typically the size of the bacteria is roughly around 1-3µm[27] which is significantly greater than the pore sizes, thereby enabling the membranes to easily filter the bacteria through the sieving mechanism. However, despite its obvious advantages, the size exclusion filtration process has one major drawback that the filtered bacteria are not completely terminated. Bacteria remain active on the surface of the membrane which in contact with the unfiltered water, leads to biofouling over time and after the saturation point it may permeate through the membrane causing bacterial toxins in the filtrate.

As mentioned before, one of the most important factors influencing the membrane performance is the membrane fouling process. Fig.6 a&b shows the picture of PSf and Ag-Psf membrane respectively which was used for the filtering bacterial water. Fig.6 (a) shows the presence of biofilm grown on PSf membrane and 6 (b) shows the absence of biofilm on the membrane.

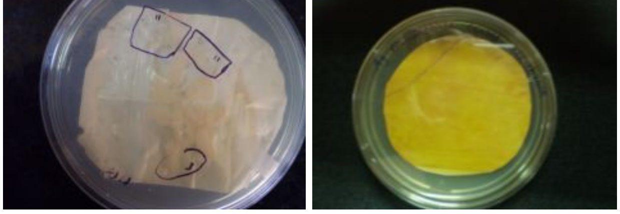

Fig.6 (a) Biofilm on PSf (b) Absence of biofilm on Ag-PSf

Mechanism of the antibacterial activity of silver nanoparticles

The antibacterial nature of the silver is attributed to its ability to release silver ions which interact with the thiol (S-H) groups in the bacteria proteins affecting the natural replication process of bacterium's DNA[28,29].

Interaction of ionic silver with thiol groups and formation of S-Ag or disulfide bonds can also affect the bacterial proteins, interrupt the electron transport chain, and dimerize the DNA[30-32]. Silver nanoparticles can damage the bacteria not only through release of silver ions but the toxicity of the silver nanoparticles might also be due to the oxidative stress on the cell surface caused by the nanoparticles thereby creating reactive oxygen species (ROS) on the surface[33,34]. Though the mechanism of the antimicrobial nature of the Ag nanoparticle is not clearly understood, some studies have shown that the silver species is quite effective because of the ability of the silver to attach/adsorb onto the cell membrane and impede or disrupt the metabolic processes, such as cellular respiration and membrane mediated transport mechanisms which in turn leads to breakdown of the bacteria by suffocation[35]. Being a surface mediated phenomenon, the large surface area of silver nanoparticles greatly enhances the speed and efficiency of its antimicrobial nature as compared to bulk silver due to the presence of a significantly higher number of active sites. The antibacterial activity has also been attributed to the electrostatic attraction between the negatively charged cell membrane and the positively charged nanoparticles[36-38].

Test for the presence of Ag in the filtered water

ICPMS Analysis

The filtration experiment was carried out to measure the amount of silver eluted from the membrane. To measure the amount of silver leached quantitatively, deionized water was filtered continuously through the silver immobilized polysulfone membrane using a cross-flow model filtration setup in which the water is circulated with the help of a gear pump and the water is collected for every 24hrs for two consecutive days. The filtered water, i.e. the permeate samples were analyzed to check the leaching of the silver from the membrane using PerkinElmer Nexion 300 inductive coupled plasma mass spectrometer (ICPMS), in which the sample is ionized in the presence of plasma by the removal of electron from the sample thereby dissociating, and the ions are detected using mass spectrometer. The results of the silver detected in the filtered water were shown in table 1.

Table 1: ICPMS data of filtered water

Time	Test	Protocols	Result
0 hr	Silver as Ag	ICPMS	BDL (DL: 0.005mg/l)
24 hrs	Silver as Ag	ICPMS	BDL (DL: 0.005mg/l)
48hrs	Silver as Ag	ICPMS	BDL (DL: 0.005mg/l)

The water samples tested at regular intervals show that the concentration of the silver in the filtrate is below the detection limit of the ICPMS. The detection limit of ICPMS used is known to be ~0.005mg/l as given in the instrument specification. The collected permeate after 48 hours, had no or less than 0.005mg/l of silver in it which is less than the EPA standard of silver in the drinking water. This confirms that the silver immobilized in the Ag-PSf membrane does not get leached significantly after 48 hours of water filtration.

Conclusion

PSf and Ag-PSf membranes were fabricated, characterized and tested with the water containing micro-organisms especially bacteria such as *E.coli.* It was found that the PSf and Ag-PSf membrane were good at filtering out microbes. Also, the presence of silver nanoparticles has prevented membrane fouling due to the non formation of a biofilm on the membrane surface over time as compared with polysulfone membranes without silver nanoparticles. Further, the retentivity of silver nanoparticles in the membrane was studied using inductive coupled plasma mass spectroscopy which showed that the membrane has a very good silver retention and that the amount of silver which gets into the water is negligible. This proves that the fabricated silver nanoparticles immobilized polysulfone composite membranes act as excellent systems for microbial filtration of contaminated water.

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