CHAPTER 5

TESTING THE EFFICIENCY OF THE MEMBRANE TOWARDS MICROBIAL FILTRATION

5.1 INTRODUCTION

Nanoparticle enhanced membrane filtration is a hybrid membrane process which is used to target the contaminants using selective nanoparticles [251-255]. Incorporation of the nanoparticles within the membrane structure enhances the mechanical and separation properties and there might be increase in surface area also due to the introduction of the nanoparticles [256-259]. The increasing availability and diversity of nanoparticles with unique physico-chemical properties has opened up new possibilities for the nanocomposite membranes [260-274]. In particular, silver nanoparticles show promise as nanoscale fillers that can controllably release the bactericidal silver ions into the membrane feed stream and thus prevent the formation of biofilm on the surface of the membrane and also reduce the intra pore biofouling [275-279]. Improvement of the mechanical properties of the polymeric membranes via the nanoparticle incorporation into the polymeric membranes has earned considerable attention in the membrane field [280, 46, 48].

Besides the advantages described above one major problem faced by the membrane industry is membrane fouling. The passage of the water through the membrane material can decrease with time which is described as “fouling” [281, 227]. Fouling occurs due to the blocking of pores by microbial, organic and inorganic contaminants. Biofouling occurs due to the colonization of the bacteria’s as biofilm on the surface of the membrane thereby decreasing the life time of the membrane [282-285]. In full-scale membrane water treatment plants, chemical cleaning and UV treatments were typically performed if the productivity declines by 10-15 percent. The purpose of the cleaning is to remove foulants and restore
productivity [11, 286-289]. But recent advances in nano-scale research and membrane technology promise solutions to many contemporary water quality issues by modifying the surface of the membranes towards various applications [290-296].

The antimicrobial effects of metallic silver (Ag) and its compounds have been known since ancient times [297] and it has also been demonstrated that, in low concentrations, silver is non-toxic to human cells [298]. Further, it has recently been proved that nanoscale silver particles 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.

Total and faecal coliform bacteria are the indicators of bacterial contamination of water. Coliform bacteria (for e.g. Escherichia coli) are commonly used bacterial indicator for faecal contamination of water. The other microorganisms sometimes found in surface waters which can cause human health problems include Escherichia coli, Salmonella spp, Vibrio cholerae, Shigella spp, Burkholderia pseudomallei, Cryptosporidium parvum, Giardia lamblia, Salmonella, Novovirus and other viruses, parasitic worms (helminths). Water borne diseases may take over by different routes of transmissions like, intake of water contaminated with human or animal faeces which leads to cholera, typhoid and other diarrheal diseases. It can also be caused by the presence of parasites in the contaminated drinking water.

Among the three different concentrations (9%, 12%, 18%) taken for testing, 12% membranes (PSf and Ag-PSf) were found to have good water permeating and sulfate rejecting properties when compared with the other membranes operated at low pressure. Hence the 12% membranes were investigated for their performance towards the removal of bacterial contaminants of interest. The three bacterial species used in this study for testing were E.coli, Salmonella typhi and Klebsiella.
5.2 ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES

5.2.1 Test with pathogenic Escherichia coli:

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. The filtration unit shown in fig. 5.1 (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 \textit{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.5.1 (b) a polysulfone (PSf) control membrane and an Ag-PSf nanocomposite membrane. The effect of silver nanoparticles on \textit{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°C for 24 hours. Fig. 5.2 (a) exhibits the presence of countable colonies of *E.coli* in the water sample that was used for the filtration. Fig.5.2 (b) and (c) shows the petri plates incubated with the water filtered through the PSf membrane and the Ag-PSf membrane respectively. 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 (as seen from the FESEM results & BET analysis), 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 [299] 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. Membrane fouling is usually the result of formation of a biofilm on the membrane surface due to the accumulation of the bacteria on the surface and typically leads to degradation of water flux and membrane performance. It is believed that the presence of uniformly dispersed silver nanoparticles in the Ag-PSf membrane would help in mitigating the issue by killing the bacteria which comes in contact with the membrane surface thereby preventing the formation of a biofilm. It also prevents the growth of microbes on the surface and avoid pore blocking in the membranes thereby increasing the flux.

Hence to assess the biofouling potential of the membranes, the PSf and Ag-PSf membranes were investigated against \textit{E. coli} using the 12\% membranes. The membranes used for filtration were also tested for any possible growth of the bacteria.
on the surface of the membranes, i.e. biofilm formation, by inoculating them on separate culture plates and incubating the same for 24 hours. This was done to give us an indication about the membrane fouling process.

![Fig. 5.3(a) Culture plate inoculated with PSf membrane used for filtration, (b) Culture plate inoculated with Ag-PSf membrane used for filtration]

The susceptibility to membrane fouling of both the membranes was established by inoculating them in the nutrient broth as mentioned before. The results are shown in fig.5.3 (a) and (b). It can be seen that the control membranes have several colonies of *E.coli*. formed on its surface whereas no colony can be seen on the Ag-PSf membrane. Even though Ag-coated PSf membranes have filtered the bacteria, while staying on the membrane surface they can produce the poisonous by-product called bacterial toxins which can freely enter the filtered water. Hence the immobilized silver nanoparticles were successful in reducing the formation of the biofilm (susceptibility to fouling) and thereby help to enhance the lifetime of the membrane. This in turn confirms that the Ag-PSf membrane is the better membrane for water filtration as compared to the control.

5.2.2 Test with *salmonella typhi and klebsiella pneumoniae*:

The experiments were repeated with different bacterial strains of *salmonella typhi* and *klebsiella pneumoniae* following the procedure explained in section 5.2.1. The contaminated water loaded with *salmonella typhi* was filtered through the Ag-
PSf membrane. The unfiltered and filtered water were inoculated in the nutrient agar and the results were shown below in figures 5.4 a&b respectively.

Similarly the water with *klebsiella pneumonae* was also tested against the Ag-PSf membranes and the effect of removal of bacteria is shown in the figures 5.5 a&b. After doing the above experiments and from the figures shown, Ag-PSf membranes were more efficient in removing microbes and against the formation of biofilm.

![Fig.5.4](image1.png)  
**Fig. 5.4 (a)** Culture plate inoculated with unfiltered water showing the presence of *salmonella typhi*  **(b)** Culture plate inoculated with filtered water showing nil bacterial colonies

![Fig.5.5](image2.png)  
**Fig. 5.5 (a)** Culture plate inoculated with unfiltered water showing the presence of *klebsiella pneumonae*  **(b)** Culture plate inoculated with filtered water showing nil bacterial colonies
5.3 ENVIRONMENTAL WATER SAMPLE

Though these results are a strong statement in favor of the bacterial filtration efficiency and antibacterial nature of the Ag-PSf membranes, it is very important to test the membrane performance when used to filter water containing different kinds of microbes of different sizes as is typical of an environmental contaminated water source. This is essential to prove the viability of the Ag-PSf membrane functioning as a filtration system in pilot studies. Hence, an environmental water sample was collected from a lake in Potheri Village, Tamil Nadu, India which has a major source of contamination as it is located near the national highways. The culture plate was inoculated using the unfiltered lake water and incubated. The culture plate fig.5.7 (a) shows the presence of major contamination in the form of fecal coliform (E.coli), confirmed using gas production and fermentation of sugar using biochemical (IMViC)[300] tests.

Biochemical tests are used to find the metabolic activity of the bacteria using IMViC test. IMViC test are employed to find the group of microbes in which E.coli is considered as the indicator of the fecal contamination. IMViC stands for indole, methyl red, Voges-Proskauer and citrate. Indole test determines the production of indole from tryptophan, methyl red determines whether or not bacteria produces acids, Vogus-Proskauer used to find whether bacteria produces acetoin during fermentation. E.coli produces acid hence there will be reduction of the pH. Citrate test detects the ability of the bacteria to use citrate as carbon source of energy. The presence of E.coli gives +++- results on the IMViC test.

![Biochemical tests explaining the positive (left tubes) and negative tests (right tubes) for (a) Indole test (b) Methyl red (c) Voges-Proskauer (d) Citrate test](source: Jackie Reynolds, Richland College, BIOL 2421)
In Fig. 5.7 (b), the biochemical tests of the unfiltered water was shown which gave the change in colour as shown in fig. 5.6, in which the +++- result confirms the presence of *E.coli* in the sample. The viability and motility of the bacteria on the membrane surface was tested using Mannitol Motility Medium (MMM) test to establish the anti-fouling nature of the Ag nanoparticles. Fig.5.7 (c) shows the presence of fermentation through the change in color, in which the acid changed the colour from brown to yellow and the gas is collected in the Durhan’s tube which confirms the presence of *E.coli*.

![Fig.5.7](image)

**Fig.5.7 (a) Culture plate inoculated with Potheri lake water (b) Biochemical test of unfiltered Lake (c) Fermentation test**

The lake water was then filtered through the Ag-PSf membrane and the filtrate was inoculated on a culture plate. Fig 5.8 (a) clearly shows that there were no colonies of coliform and the filtrate is free from any contamination. Further, biochemical (IMViC) tests shown in fig.5.8 (b) indicate the absence of gas production and fermentation of sugar in filtrate in which the change in colour can be
compared with the images shown in fig. 5.6 which has shown the ++++ colours for the absence of *E.coli*, and confirms that the filtrate is free from fecal coliform. In fig. 5.8 (c) the dark brown solution, which is the characteristic colour of the MMM in the test tube, shows the absence of fermentation and no growth of the coliform which confirms the non-viability of *E.coli* due to the interaction with Ag nanoparticles.

![Fig.5.8 (a) Culture plate inoculated with filtered lake water (b) Biochemical test of filtered lake water (c) Fermentation test](image)

5.4 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 [301, 302]. 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 [303-305]. 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 [306, 307]. Though the mechanism of the antimicrobial nature of the Ag nanoparticle is not clearly understood, some studies showed that 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 [90, 91, 308]. 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 [309-311].

5.5 TESTS FOR THE PRESENCE OF Ag IN THE FILTERED WATER

5.5.1 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) as discussed under section 2.5.9. 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 5.1. 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.

Table. 5.1 ICPMS data of filtered water

<table>
<thead>
<tr>
<th>Time</th>
<th>Test</th>
<th>Protocols</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>Silver as Ag</td>
<td>ICPMS</td>
<td>BDL (DL: 0.005mg/l)</td>
</tr>
<tr>
<td>24 hrs</td>
<td>Silver as Ag</td>
<td>ICPMS</td>
<td>BDL (DL: 0.005mg/l)</td>
</tr>
<tr>
<td>48hrs</td>
<td>Silver as Ag</td>
<td>ICPMS</td>
<td>BDL (DL: 0.005mg/l)</td>
</tr>
</tbody>
</table>

5.5.2 XPS Analysis

The amount of silver present on the membrane before and after the filtration was further confirmed by an method using X-ray Photoelectron Spectroscopy (XPS). The measurements were carried out using Versa Probe’s XPS on the Ag-PSf membranes which are used for the filtration. Typical scans were acquired on the surface of the filters using a 100W X-ray beam and the survey scans of membranes used for filtering water for 0 hrs 24 hrs and 48hrs are shown in figures 5.9 a,b &c. which shows the presence of carbon, hydrogen, oxygen and sulfur as expected and small quantities of silver (0.1%). The relative atomic concentration of the Ag3d was consistently at 0.1% even after the continuous treatment of 48hrs as shown in table 5.2.

The results obtained from the depth profiles of the Ag-PSf membrane were used along with the ICPMS results to draw conclusions on the leaching of silver from the membranes. Though, the 0.1% relative atomic concentration is close to the detection limit of the XPS system, these results can be interpreted as supporting evidence to the ICPMS results which indicate that the quantity of silver being leached from the Ag-PSF membrane is quite negligible.
Fig. 5.9 XPS survey scans on membranes at (a) 0hr (b) 24hrs (c) 48hrs
Table 5.2 XPS data showing the relative atomic concentration

<table>
<thead>
<tr>
<th></th>
<th>C1s</th>
<th>O1s</th>
<th>N1s</th>
<th>S2p</th>
<th>Ca2p</th>
<th>Cl2p</th>
<th>Si2p</th>
<th>Na1s</th>
<th>Ag3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>81.2</td>
<td>12.7</td>
<td>2.4</td>
<td>1.4</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>24 hrs</td>
<td>78.8</td>
<td>14.5</td>
<td>2.7</td>
<td>1.6</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>48 hrs</td>
<td>81.2</td>
<td>12.7</td>
<td>2.4</td>
<td>1.4</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

5.6 CONCLUSION

PSf and Ag-PSf membranes for the treatment of water containing micro-organisms especially bacteria such as *E.coli*, *salmonella typhi* and *klebsiella pneumonae* were studied and it was found that the PSf and Ag-PSf membrane were good at filtering out microbes. Also, the presence of silver killed the bacteria and thus prevented membrane fouling due to the formation of a biofilm on the membrane surface over time as compared with polysulfone membranes without silver nanoparticles. Also, the filtration efficiency with respect to a broad spectrum microbial sample was studied and it was found that the membrane performs equally well for such environmental samples. Further, the retentivity of silver nanoparticles in the membrane was studied using inductive coupled plasma mass spectroscopy and X-ray photoelectron 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 Ag-PSf membranes are excellent systems for microbial filtration of contaminated water.