Antifungal Polymeric Membranes with Chitosan Functionalized Quaternary Ammonium Salt for Drinking Water Treatment

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I would like to dedicate my thesis

to my beloved parents

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Abstract

Chitosan was functionalized to synthesize quaternized N-trimethyl chitosan (TMC) and incorporation in polyethersulfone (PES) to fabricate membranes for enhanced antifungal activity and water treatment. The TMC was synthesized from chitosan via reductive alkylation and methylation. The synthesized TMC was characterized mainly through FTIR. Three different concentrations 5, 10 and 15% (w/w) of both chitosan and TMC were taken in casting solution of PES and membranes were fabricated through phase inversion method. The effects of concentration of chitosan and TMC on the properties of functionalized PES membranes were investigated including morphology, water retention, water surface wettability, permeation flux and most importantly antifungal characteristics. The membrane with the lowest concentration of TMC 5% (w/w) resulted in the larger average pore size than the PES membrane with no chitosan or TMC. The contact angle was reduced from 89.67º to 56.78º by increasing concentration of TMC to 15% (w/w). The resultant membranes exhibited improved water hydrophilicity, permeability and inhibition against fungal species. The functionalized membranes had shown noticeable antifungal activity against *Aspergillus niger* in the case of 15% TMC with 72% antifungal activity and 15% chitosan with 62.5% antifungal activity against *Fusarium solani.*

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List of Abbreviations

CS Chitosan

TMC Trimethyl chitosan

PES Polyethersulfone

WHO World health organization

SEM Scanning electron microscopy

FTIR Fourier transform infrared

XRF X ray fluorescence

nm Nanometer

Cº Celsius

ml Milli litre

KBr Potassium bromide

CNTs Carbon nanotubes

hr Hour

MH0 Membrane code for polyethersulfone

MH1 Membrane code for polyethersulfone having 5% TMC

MH2 Membrane code for polyethersulfone having 10 % TMC

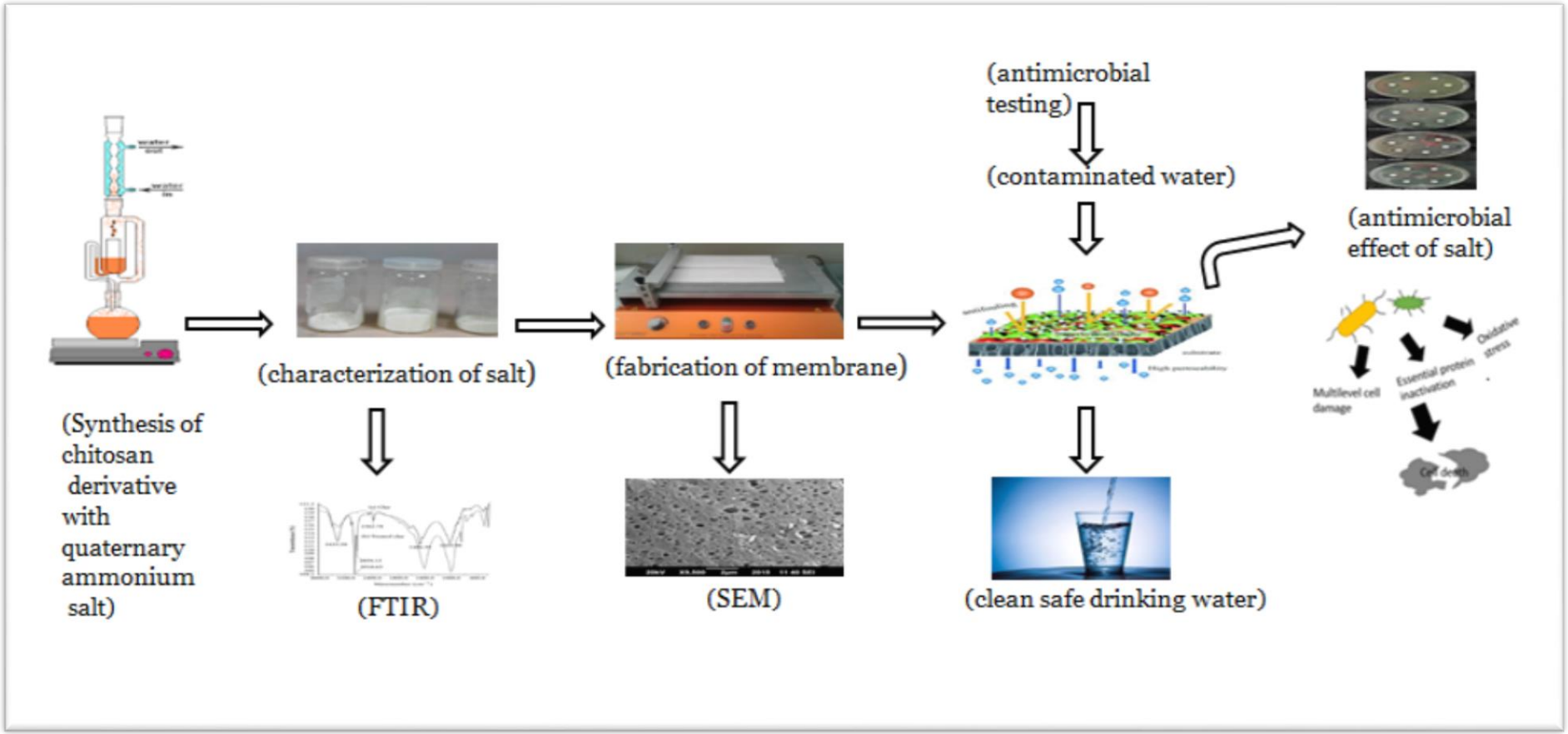
MH3 Membrane code for polyethersulfone having 15% TMC

MH4 Membrane code for polyethersulfone having 5% chitosan

MH5 Membrane code for polyethersulfone having 10% chitosan

MH6 Membrane code for polyethersulfone having 15% chitosan

ANTI-FUNGAL N-TRIMETHYL CHITOSAN INCORPORATED POLYMERIC MEMBRANE FOR WATER DISINFECTION



Chapter 1. Introduction

1.1 Background:

H_2O, two parts hydrogen and one part oxygen, this substance is known as “Water”. It is one the most important component on earth. This transparent fluid covers approximately 71% of Earth’s surface but only 3% of it is fresh water. (*"CIA – The world fact book",Central Intelligence Agency). Fresh and clean water that is foundation of life is only 0.01%. Clean and safe drinking water is scarce. If there is no water, there will be no life. Clean drinking water is basic need of humans and other living creatures, but still it is not in access to more than a billion people in world. According to United Nation Organization (UNO) survey report, world population is increasing rapidly while consumable water is decreasing [1]. Nowadays, major threat to human health is contaminated water. Pathogenic agents that cause infectious diseases such as cholera, typhoid, and hepatitis A etc. are bacteria, fungi, protozoa, viruses, and helminths (worms) that are present in contaminated drinking water. According to world health organization (WHO) every year 1,600,000 people die from diseases related to digestive system due to lack of access to clean and safe drinking water.

1.2 Pakistan and Water Pollution:

Water pollution in Pakistan, is one of the major challenges to public health. Quality of safe and clean drinking water falls under acceptable range. Pakistan’s drinking water quality lies 80 among 122 countries. Water present on surface and ground, both are infected with toxic metals, pesticides, coliforms and pathogenic microbes. The factors that are set by WHO for clean and safe drinking water are often violated in Pakistan. Due to increasing industrialization and urbanization, water pollution is increasing day by day. The main causes of public health problems are pathogenic microbes and chemical pollutants. Pakistan Council of Research in Water Resources reported that about 2 lakhs children die each year due to pathological diseases alone. Survey of leadership for Environment and Development survey reported that almost 52 nations

covering half of the world's population will face a intense clean water shortage by the year 2025.

1.3 Pathogens Present in Drinking Water:

In oldest and widest sense a pathogen is anything that causes a disease. Bacteria, viruses, fungi, prions and other parasites are included in pathogens which are responsible for contamination of drinking water. 2000 types of fungi are present on earth out which 600 cause diseases. Most infections are triggered by genera *Cryptococcus, Trichophyton, Fusarium, Aspergillus and Candida*. These all types of fungi are present in drinking water.

1.4 Emergence of Polymeric Membranes:

Membrane is a barrier that selectively allows certain things to permit but stops other. These things may be small particles, ions or molecules. It is basically a barrier between two phases [2]. There are different kinds of membranes such as biological membranes, nuclear membranes, and synthetic membranes [3]. The concept of membranes was known from eighteen century. Membrane technology plays an important role in life sustaining processes. Separation can be performed at low temperature with low energy consumption as compared to other separation processes.
Polymeric membranes have common problem of high hydrophobicity, low flux, fouling and low mechanical strength that reduce the performance of the membranes. Researchers are trying to remove these problems and improve the performance of membranes [2].

1.5 Research Framework:

PHASE-I

In the first phase, quaternary ammonium salt of chitosan (N -trimethyl chitosan) was synthesized using substitution reaction. The salt was characterized using FTIR. Antifungal tests were performed to check the activity of salt against Aspergillus niger and Fusarium solani.

PHASE –II

Polymeric membranes were fabricated after preparation and characterization of salt. Membranes were prepared through phase inversion method by mixing salt in its solution at three different concentrations. After fabrication of membranes, they were characterized by scanning electron microscope, optical profilometry, water retention and permeance flux.

Phase-III

In phase III, antifungal activity of functionalized membranes was inspected against Fusarium solani and Aspergillus Niger.

1.6 Aim and Objectives:

Following are the main aims and objective of research work:

- Synthesis and characterization of quaternary ammonium salt of chitosan.
- Fabrication of PES membranes functionalized with chitosan and quaternary ammonium salt of chitosan.
- Characterization of membranes.
- Antifungal testing of membranes.
- Aim of fabrication of functionalized membrane is to increase
    1. Permeability flux
    2. Hydrophilicity

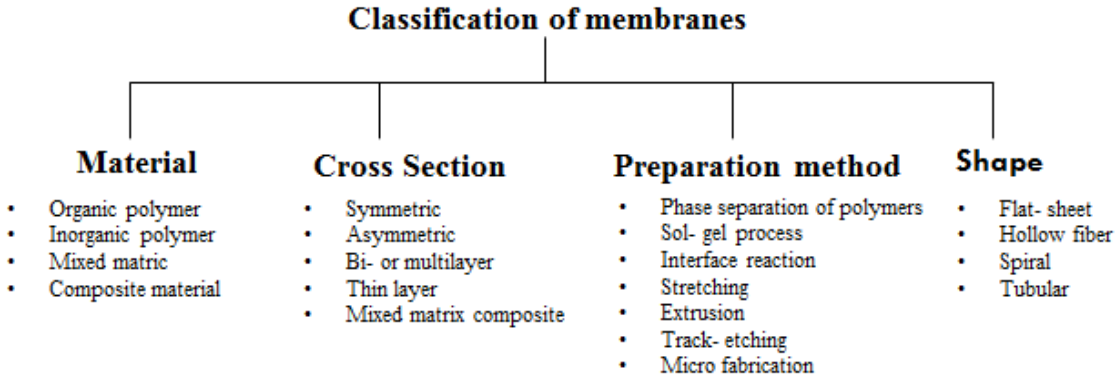
Chapter 2. Review of Literature

2.1 Current Ways of Water Disinfection

Biologically contaminated water contains bacteria, fungi, viruses and other pathogens. Filtration, boiling and chemical treatment are used to kill or remove microorganisms [4]. Water is chemically treated by chlorine, chloramines and ozone. But unfortunately after treating water with these disinfectants toxic byproducts are formed that are carcinogenic in nature. Maximum amount of chlorine is used to inhibit the growth of microorganisms. 0.4, 0.8, 4.0 mg/L concentrations of residual chlorine disinfectants are allowed by US Environmental Protection Agency (USEPA). If the concentration is increased from this level, it will cause eye and nose irritation, stomach cramps and may disturb the nervous system of young children. Another way that are used now a days is aqua filters, their efficiency is good and produce less toxins compared to other technologies but with the passage of time they are blocked by bacteria, fungi and viruses.

2.2 Significance of Membrane technology in Water Disinfection

Membrane technology plays an important in life sustaining processes. Membrane is basically a selective barrier between two phases. Separation can be done isothermally at low temperature. It is also a low energy consumption process.[2] It has also many advantages such as absolute barrier to pathogens and particles, stationary parts, compact modular construction, stable water filtration characteristics, low chemical sludge effluent, and small system layout [5, 6]. Water treatment membranes have received augmented attention they are more efficient at removing the particles, microorganisms, and turbidity present in waste water [7]. The use of membranes has increased exponentially. Considering the large collection of membranes suited for methodological applications, they are classified on the basis of material, preparation method, cross section and shape of the membrane [2].



Various membrane materials have been used, not all them yield satisfactory performences. Various membrane materials such as polysulfone (PS)[8-10], cellulose acet-ate (CA)[11-14], polyether sulfone (PES)[14, 15], polyvinyldene flouride (PVDF)[16-19], poly vinyl alcohol (PVA) [20-23], polyamide (PI)[24-26] and polyacrylo-nitrile (PAN)[27, 28] have been used for treatment of drinking water.

2.3 Polyether Sulfone (PES) Membranes :

Polyether sulfone (PES) is an organic polymeric material which is mostly use for membrane fabrication. It is commercially available polymer. It is transparent in colour [29]. It has high dimensional stability and resistance to boiling water, steam and mineral acids. Other useful properties include inherent flame resistance, creep resistance and no toxicity. It is ideal with for making asymmetric membranes with different pore size and surfaces [29-31].

Polyether sulfone is named after its two characteristic functional groups, ether group and sulfone group in its backbone. PES polymers have flexible chains and high glass transition temperature because of alternative flexible ether linkages and stiff sulfone group.

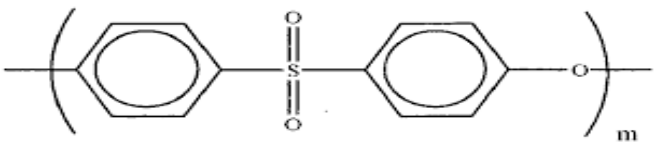


Figure1. Structural formula of Polyethersulfone

Compared with other polymeric materials used in membrane fabrication, PES has low hydrophilicity. Substantial research has been done to improve surface properties of polyether sulfone membranes [5, 32].

One of the most fundamental techniques used to study the membrane hydrophilicity/hydrophobicity is contact angle analysis. Porosity, roughness, and pore size distribution are the main factors which affect the values of contact angle. More the contact of drop of water with surface more will be the hydrophilicity. Larger pore size of the membrane results in low contact angle measurements. Whereas, by decreasing surface roughness, contact angle values also decrease. [33]

By increasing membrane hydrophilicity, fouling of membrane decreases. Water is strongly attracted to water. Water molecule layer on surface of membrane help other molecules to adhere easily to membrane. Once hydrogen bond is formed between two layers, it will be tough to break apart [34].

For improving membrane performance, usually membrane structure is controlled. The membrane structure is not controlled easily. The type of polymer, the solvent and the non- solvent, temperature, and the composition of coagulation bath determine the performance of the membrane and its structure. Membranes structure can be functionalized by coating, self-assembly, plasma treatment and chemical grafting [35].

Organic or inorganic additives are added to improve the surface properties of membranes. Blending or additive technique improve the pore size, hydrophilicity, surface charge, and the surface roughness.

To improve the properties of polyether sulfone membranes different types of organic and inorganic materials have been used. Many parameters including the polyether sulfone and additive conc., the miscibility characteristics, solvent type, the compatibility properties and molecular weight of polymeric material affect the performance of membranes [35].

In polymeric membranes different types of inorganic materials are used such as TiO_2, Al_2O_3, ZrO_2, SiO_2 and Ag. The most common method used for preparation of TiO_2/ PES nanocomposites is self-assembly of nanoparticles on membrane surfaces. Ag as an antibacterial material is added into PES membrane matrix. But leaching of particles causes bio-fouling of membranes. In water, Ag particles cause toxicity, so it is not preferred in drinking water treatment membranes.

PES membranes are frequently used for drinking water treatment. Functionalization of these membranes improves its different properties. Chitosan and chitosan derivatives with quaternary ammonium salt are added in membrane to improve its antimicrobial properties.

2.4 Chitosan:

Chitosan is a natural polymer. After cellulose, it the second most abundant polymer on earth[36]. It has groups of fully or partially deacetylated chitin compound. Chitin is obtained from shrimp and carbshell. Chitin and chitosan are commercially fascinating compounds because they have high nitrogen content approximately 6.89% compared to cellulose substituted synthetically (1.28%). Natural polymers such as dextrose, cellulose, chitosan and its derivatives have more biodegradability and biocompatibility than those of most of the synthetic polymers. However, these natural polymers have limited reactivity and processabilty [37].

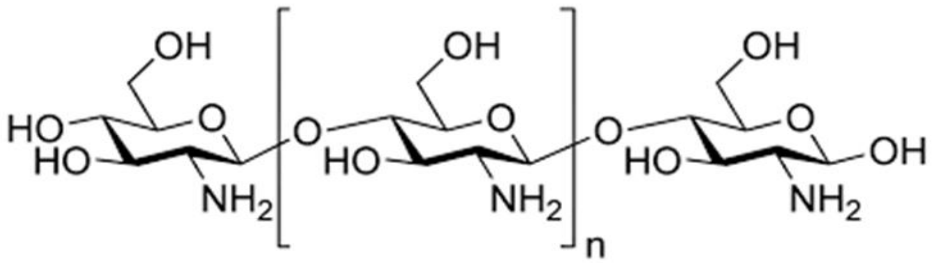


Figure2. Structural formula of chitosan

Chitosan contains more than 5000 glucosamine units. It is polycationic polymer with specific properties and structure [38]. It is soluble in some organic acids such as formic acid, acetic acid, succinic acid, malic acid and lactic acid but mostly insoluble in many solvents. Because of its high viscosity, tendency to coagulate at high pH and insolubility in water, its use is still limited. Many efforts have been done to improve its solubility in water by its chemical functionalization [39].

Molecular weight and degree of deacetylation are the important characteristic of chitosan that is considered during its functionalization.. The solution's viscosity is affected by molecular weight, degree of deacetylation, pH, ionic strength, concentration and temperature. Generally, viscosity is decreased by increasing temperature. The effect of pH on viscosity depends on specific acid used. Chitosan is soluble in organic acid when pH is less than 6.0 and insoluble in alkaline media, water, inorganic solvents.

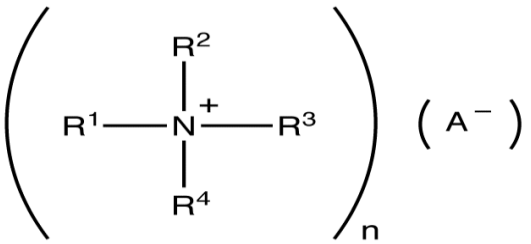
Chitosan exhibits numerous promising biological applications including antimicrobial activity, biocompatibility, biodegradability and hemostatic activity. Applications of

chitosan to the field of chemical engineering, food, pharmaceuticals, environmental protection and nutrition have gained a lot of attention in recent years. Applications in the environmental industry include its use as coagulation agent for the recovery of polysaccharides, fatty acids, proteins, phospholipids and bile acids from food processing waste [40]. It also used as chelating agent to absorb heavy metals from industrial waste water. It is also attractive for the removal of organic compounds and colors from waste water than typical absorbents such as activated carbon [41]. Chitosan also exhibits antibacterial properties. In the past times it was used in coatings and as a spray on fresh strawberries and tomatoes to increase their short life. Researchers are also trying to use it for plant protection. A possible application of chitosan as a bactericidal agent in waste water treatment was examined recently [42].

2.5 Chitosan Derivatives with Quaternary Ammonium Salt of Chitosan:

In broad-spectrum, chitosan exhibit its antimicrobial action only in acidic medium below 6.0 due to very poor solubility. Chitosan is chemically functionalized by reacting it with different chemicals at different pH and temperatures [43]. A positive charge is introduced in chitosan derivatives by quaternization of amino group. Positive charge enhances the antimicrobial activity [44].

In Chemistry, Quaternary ammonium salt is an ionic compound consisting of positive charged nitrogen surrounded by four substituents (hydrogen or alkyl group or aryl group).



(Where "A^{-}" is anion group and "N^{+}" is positive charge.)

Under a heterogeneous condition, the permanent positive charge in the form of quaternary ammonium group was appeared on the surface of chitosan particles. It is done by reductive N- alkylation or direct methylation using aldehydes (RCHO) and by methylation with methyl iodide (CH_3I). This reaction can be done simply in the absence of tedious purification process that is usually mandatory for homogeneous

quaternization. This type of process for enhancing antimicrobial activity particularly in neutral pH environment has been previously reported.

The functionalization of chitosan is done by nucleophilic substitution at C2 position with sodium iodide and iodomethane. This reaction depends predominantly on the reaction time, the reaction steps and concentration of sodium hydroxide. The higher the number of steps and reaction time, greater will be the degree of quaternization. The strongly alkaline media promotes the methylation of OH group present at C6 and C3 positions. It also allows cleavage of glycosidic linkages [45].

2.6 Fungicidal applications of chitosan and quaternary ammonium salt of chitosan:

Chitosan's antifungal activity is affected by inherent factors such as chitosan type,the host, degree of deacetylation, the chemical composition, the nutrient composition of broth or substrate, the natural nutrient consistency and the environmental conditions. The chitosan antimicrobial activity is more affective on fungi as compared to bacteria.

Chitosan has shown its antifungal activity against *Piricularia oryzar, Botrytis cinerea, Drechstera sorokiana, Fusarium oxysporum, Micronectriella nivalis, Rhizoctonia solani,* and *Trichophyton equinum* [46]. The lowest inhibitory concentrations reported were 0.0018% to 1.0% for particular target organism range. The MIC is affected by many factors such as presence or absence of proteins and lipids, the polymerization of chitosan and pH of the growth media.

Chitosan also inhibit the soil borne pathogenic fungi *Fusarium solani and Colletotrichum lindemuthianum.*

Quaternary ammonium salt of chitosan also shows antifungal activity. The salt shows its antifungal activity when it comes in contact with cell wall of fungi. Positive charge present on salt interacts with the cell wall of fungi that contain negative charge, lead to the release of intracellular electrolytes

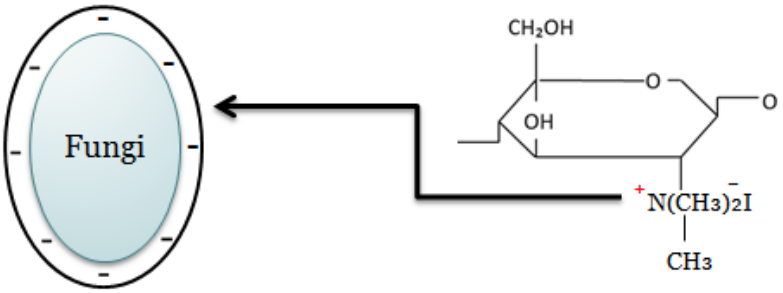


Figure3. Mode of action of quaternized N - Trimethyl chitosan on fungus

By increasing the degree of quaternization, the antifungal activity of functionalized chitosan is improved. By adding the number of N^+ sites, interaction with cell wall and solubility will increase, which eventually increase the antifungal activity compared to chitosan.

The antifungal activity enhance with the increase in the degree of quaternization, molecular weight and hydrophobic group having substituted aromatic groups. Functionalized chitosan activity also depends on the type of fungus and the chemical structure of chitosan derivative [47].

2.7 Phase Inversion (Phase Separation) of Polymers:

Phase inversion is the most commonly used membrane fabrication method. A wide range of morphology from dense to open structure is obtained. For this reason, this method is mostly preferred over other methods, since it provides control over the structure, morphology of polymer and its permeability.

A polymer is dissolved in its favorable solvent and then casted over a proper substrate. It is then immersed in coagulation bath having non-solvent. Precipitation takes place due to exchange between non-solvent and solvent. Pores are formed due to this precipitation process. It is then dried in air or vacuum. The combination of mass transfer and phase separation affects the membrane surface, porosity and morphology.

Chapter 3. Materials and Characterization Techniques

3.1 Materials and Methods

All the chemicals used in this experiment were of analytical grade. Chitosan (degree of deacetylation 94%), formaldehyde, sodium borohydride (NaBH4), N-methyl-2-pyrrolidione (NMP), acetone and sodium hydroxide (NaOH) were purchased from Sigma Aldrich Chemical Co. Polyether sulfone (PES) was purchased from Ultrasone Germany. Polyester support, methyl iodide (CH3I), sodium iodide (NaI), and nutrient agar were purchased from Merck. Fungal strains *Fusarium solani* and *Aspergillus niger* were obtained from clinical isolates.

3.2 Characterization of N-trimethyl Chitosan:

Different characterization techniques which were used to characterize the N-trimethyl chitosan are described below:

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR) of Chitosan and N-trimethyl chitosan:

FT-IR Perkin Elmer Spectrometer was used to confirm the functionalization of chitosan. The spectrum was recorded by Perkin Elmer instrument. The spectrum was observed by Perkin Elmer instrument. Little amount approximately 30mg of chitosan and N-trimethyl chitosan was added separately with KBr for characterization.

3.2.2 Antifungal Testing of Chitosan and N-trimethyl Chitosan (TMC):

Antifungal activity of N-trimethyl chitosan was studied against *Aspergillus niger* and *Fusarium solani* through well diffusion method. Chitosan and TMC were taken and dissolved separately in 1% acetic acid solution and three different concentrations of each were prepared “5% , “10%” and “15%” respectively. Potato dextrose media was used as nutrient media. Dissolve 39g of potato dextrose agar in

one liter of distilled water. Boil it until it dissolve completely. Sterilize it by autoclaving at 121°C for 15 minutes. Shake it well before pouring it into the plates. 10 µl was poured into the well. Antifungal drug Amphotericin B was used as a positive control while 1% acetic acid solution was chosen as negative control. Testing was done in triplicates and average of readings was calculated.

3.3 Membrane Characterization:

Fabricated mixed matrix membranes were characterized by following ways:

1. Scanning electron microscopy (SEM)
2. Contact angle measurement
3. Wettability
4. Optical profilometry
5. Permeation Flux
6. Antifungal testing of membranes

3.3.1 Scanning Electron Microscopy (SEM):

Scanning.electron.microscopy (SEM). Joel JSM 6490A.was used to check the cross section, morphology, pore size and topography of membrane. Sample was prepared for SEM. It was cut into 25cm² pieces, sticked on a steel stud by using carbon tape, gold coated and then analyzed.

Porosity is the measure of voids (pores) spaces in a material. Average pore size of each membrane was calculated by Scanning Electron Microscopy (SEM). Most the membrane characteristics depend mainly on the pore size of the membrane.

3.3.2 Contact Angle Analysis:

Contact angle is one of the important factors used to determine the hydrophilicity of membrane. More the contact of water drop with the surface of membrane, greater will be the hydrophilicity. So, hydrophilic/hydrophobic nature of membranes is analyzed by using contact angle. 10µl of distilled water is dropped on the surface of membrane. Images were captured through camera and an average five different measurements are taken to calculate the contact angle. Experimental error is

removed by determining contact angle at different positions on the membrane and taking its average.

3.3.3 Water Retention Test:

This method is used to determine the moisture content absorbed by the membrane. Water retaining capacity of each membrane was investigated by

1. Soaking each membrane in water for 24 hr.
2. Measure the wet weight of the membrane.
3. Then membrane was dried in vacuum oven for 12 hr. and its dry weight was calculated.
4. Water content absorbed by each membrane was calculated using this formula

[(wet weight - dry weight) / wet weight] × 100.

3.3.4 Permeation Flux:

The membrane permeation flux is defined as volume of the fluid flowing through the membrane per unit area per unit time. Its unit is $m^3/m^2.sec$. Membrane flux test was performed using vacuum filtrate assembly at constant pressure of 40cmHg and 60cmHg at room temperature. Area of membrane was 0.025m.

Flux = flow rate/area×time

Flow rate = initial volume – final volume

3.3.5 Antifungal Testing of Membrane:

Hemocytometer is a device used to find the number of spores or cells. A solution of *Fusarium solani* and *Aspergillus niger* was prepared in 0.01% tween to make better dispersion of fungal spores because they were hydrophobic in nature. This solution was passed through each membrane fixed in the Millipore vacuum filtrate assembly. Spores/ ml were counted using a

hemocytometer. Testing was done in triplicates and average of readings was calculated.

Chapter 4. Experimental Work

4.1 Synthesis of Chitosan Derivative with Quaternary Ammonium Salt:

Chitosan derivative was synthesized via Schiff's base intermediates .To produce quaternary ammonium salt, chitosan was chemically modified. Alkyl group was introduced into chitosan's amine group. Reaction was done in two steps. In first step, N methyl chitosan was synthesized. 700mg of chitosan was suspended into 70ml of 1% (v/v) acetic acid. 1.5ml of formaldehyde solution (37%) was mixed in it. The solution was agitated for 1 hour at ambient temperature. pH of the solution was maintained to 4.5 by adding 1M NaOH solution. NaBH4 (2ml) was then added, it was agitated for 1.5 hours. Methyl chitosan precipitates were attained by adding 1M NaOH solution (pH 10). The precipitates were washed away with distilled water and pH was adjusted to 7.

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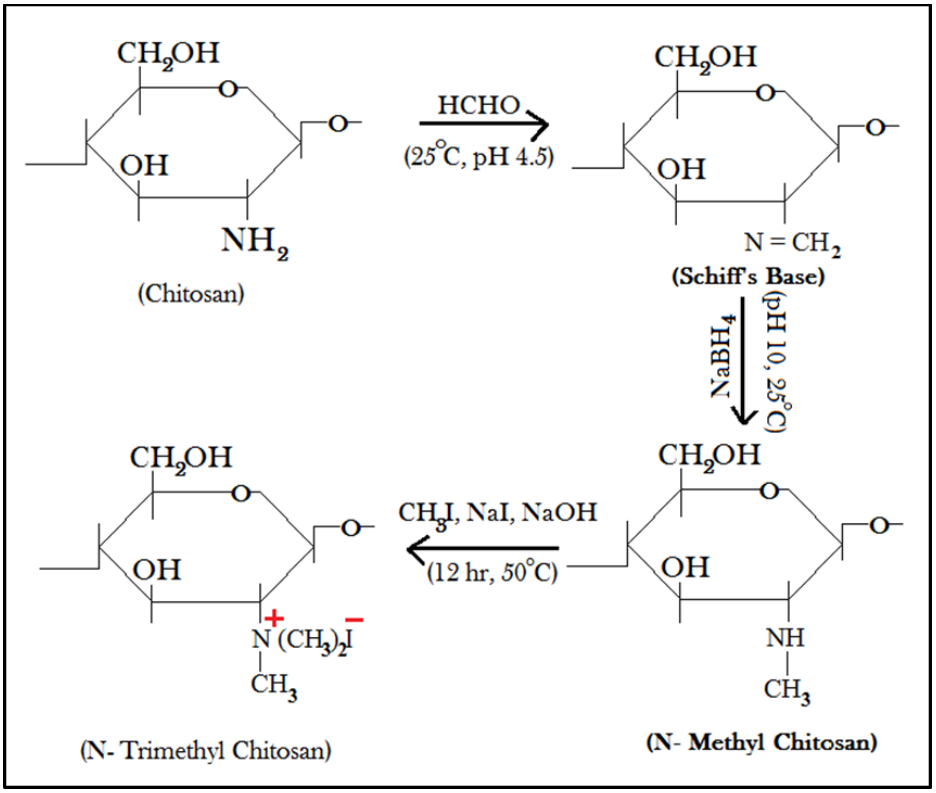


Figure4. Preparation route of quaternized N-trimethyl chitosan from pure chitosan

After filtration, unreacted reagents were eliminated by Soxhlet extraction using ethanol and diethyl ether (1:1 v/v) for 3-4 days. The resulting product, N-trimethyl chitosan, was dried in vacuum oven for 48 hours at 40 °C

Second step lead to the formation of quaternized trimethyl chitosan. Methyl chitosan (200mg) was suspended in 10ml of N-methyl pyrrolidone (NMP) for 12 hours at room temperature. Then 2ml NaOH solution of 30% w/v solution, 600mg sodium iodide and 4ml methyl iodide were added. Reaction was started by vigorous agitation for 6 hours at 60°C. At the end, acetone was added and the precipitates of quaternized N-trimethyl chitosan were obtained. Rotary evaporation and oven drying was done to obtained dry powder [48].

4.2 Membrane Fabrication:

Phase Inversion method was used to fabricate the membranes. Custom design tray and thin film applicator was used to fabricate the membranes. Different concentration of chitosan and trimethyl chitosan (5%, 10%, & 15%) were embedded in PES membranes. Polyester sheet was used as support material.

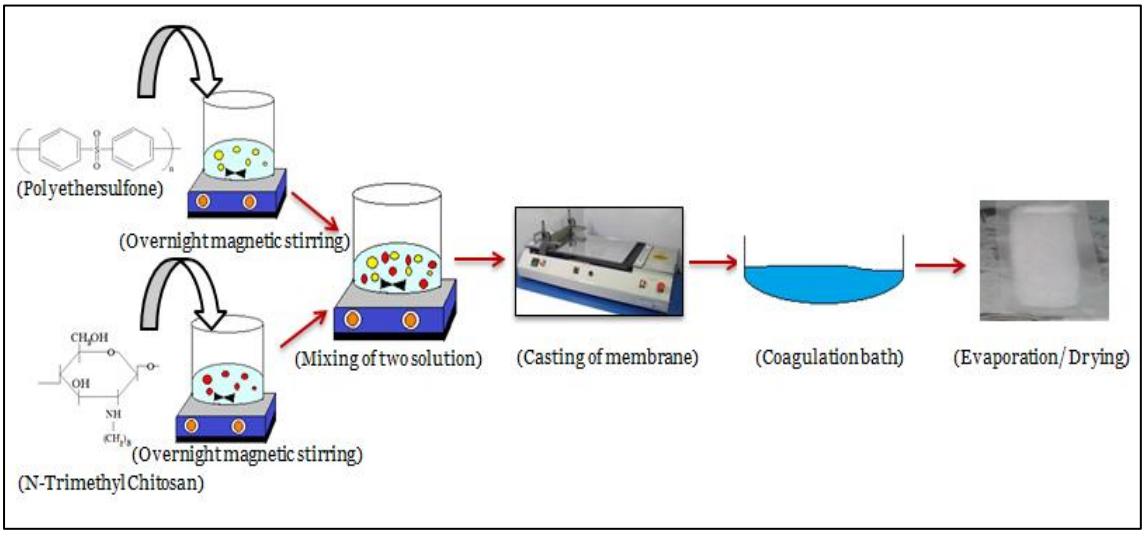


Figure5. Schematic diagram of membrane fabrication.

4.2.1 Pure PES Membrane (MH0):

2g of PES was dissolved in 12 grams of NMP. Solution was stirred for 24h. Solution was cast on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.2 5% Trimethyl Chitosan + PES Membrane (MH1):

2g of PES was dissolved in 9g of NMP and 0.1g of trimethyl chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.3 10% Trimethyl Chitosan + PES Membrane (MH2):

2g of PES was dissolved in 9g of NMP and 0.2g of trimethyl chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.4 15% Trimethyl Chitosan + PES Membrane (MH3):

2g of PES was dissolved in 9g of NMP and 0.3g of trimethyl chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.5 5% Chitosan + PES Membrane (MH4):

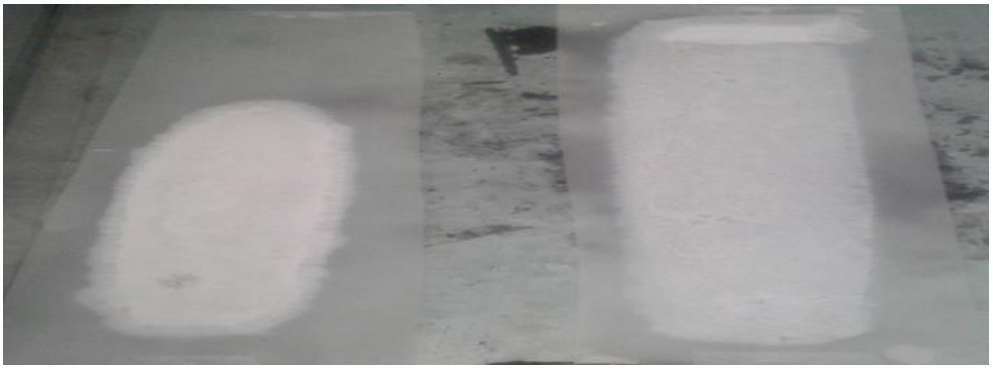
2g of PES was dissolved in 9g of NMP and 0.1g of chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.6 10% Chitosan + PES Membrane (MH5):

2g of PES was dissolved in 9g of NMP and 0.2g of chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.

4.2.7 15% Chitosan + PES Membrane (MH6):

2g of PES was dissolved in 9g of NMP and 0.3g of chitosan was dissolved in 3g of NMP. Stirred two solutions for 2h separately and sonicated for 15 minutes. Mixed two solutions again and sonicated for 15 minutes. Solution was casted on polyester support with the help of film applicator. Dipped this coated support in distilled water coagulation bath for 10 minutes. Dried this film in ambient temperature.



Figures6. Top: fabricated membrane, bottom: Electromotor thin film applicator

Chapter 5. Results and Discussion

5.1 Fourier Transform Infrared Spectroscopy (FTIR) of chitosan and N-trimethyl chitosan:

Figure 7 shows the FTIR spectra of chitosan and N-trimethyl chitosan. Chitosan and polysaccharides show very intense peaks in 1000-1200 cm^{-1} because of C-O vibrations. Chitosan shows two characteristic peaks around 3300 and 1093 cm^{-1}. At 3300 cm^{-1} two short pointed absorbance peaks are present that is due to presence of primary amine in chitosan. At 1093 cm^{-1}, a sharp characteristic peak is also present that is due to C-O stretching. Characteristic peak of amine NH_2 vibration deformation peak of chitosan appeared at 1560 cm^{-1}. Double bond reduction form N-methyl chitosan. Methyl iodide help in quaternization of N- methyl chitosan [51]. After the functionalization of chitosan, this characteristic disappeared in TMC spectrum because of the deformation of chitosan at C-2 due to formation of TMC. A characteristic peak in TMC spectrum appeared at 1450 cm^{-1} due to formation of CH_3 (bend) [30, 31].

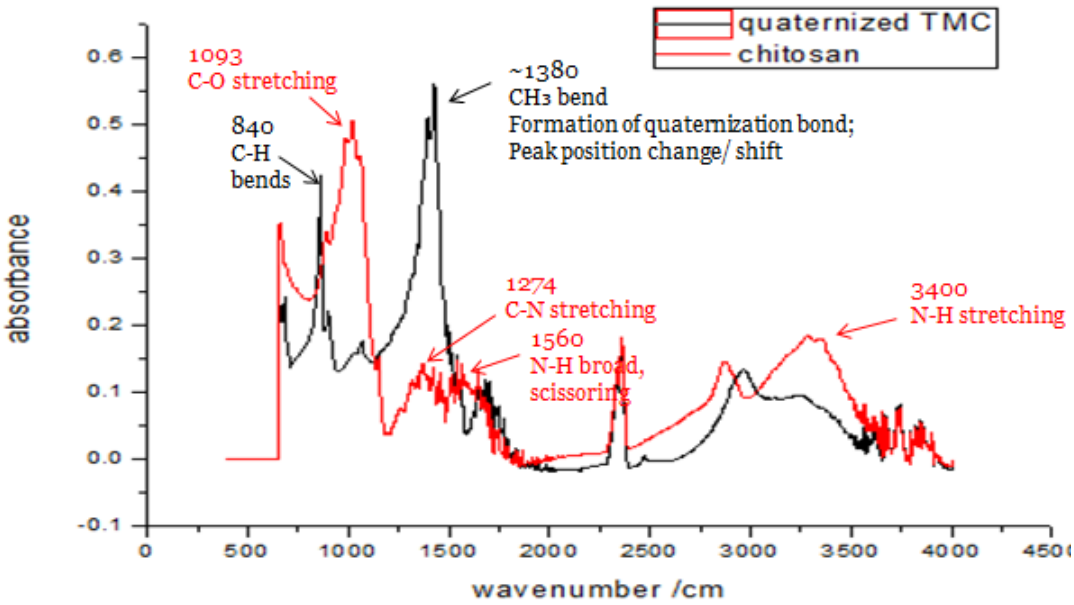


Figure7. FTIR analysis of pure chitosan and quaternized N- trimethyl chitosan

5.2 Antifungal Testing of Chitosan and N-trimethyl Chitosan:

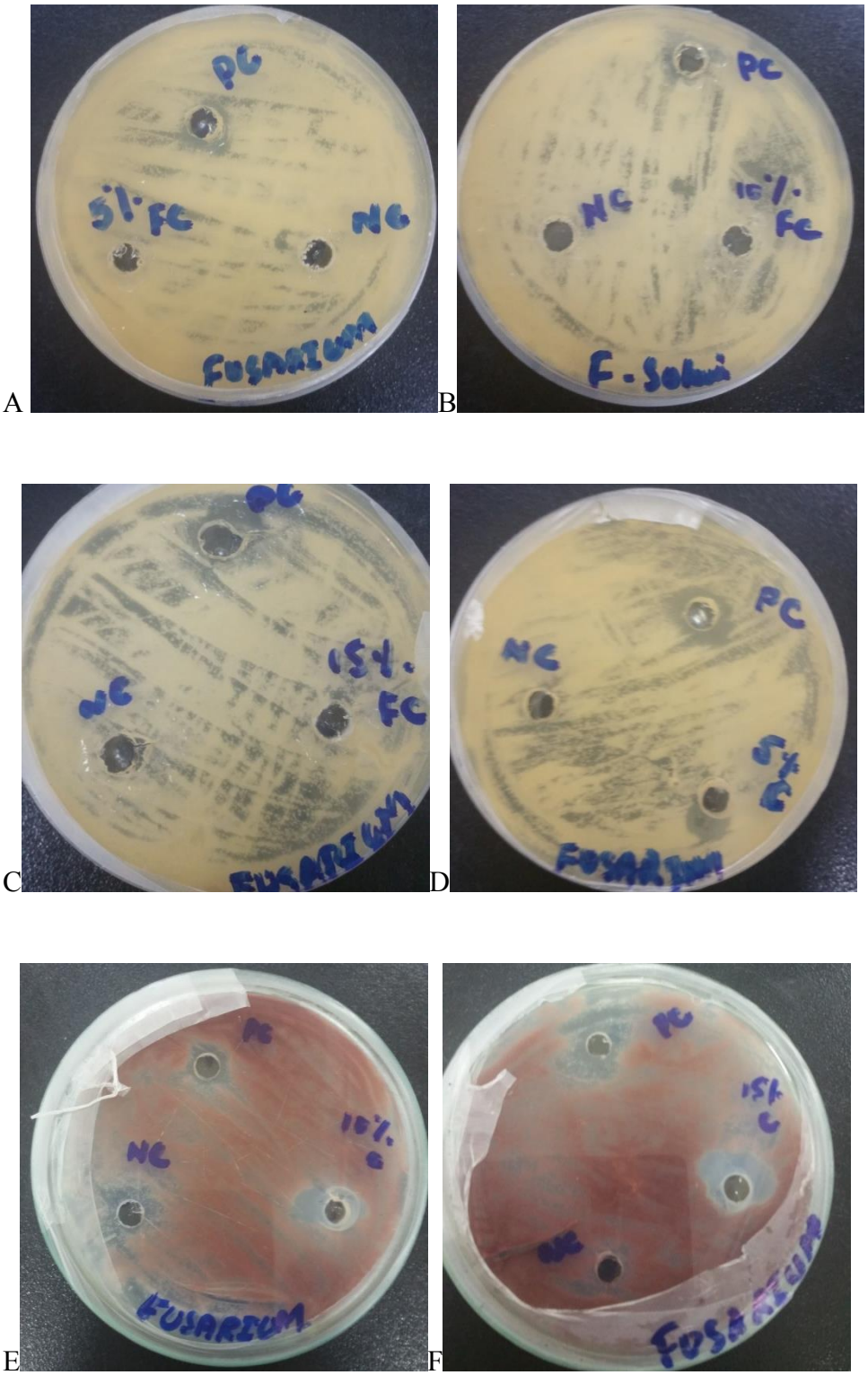


Figure8. Antifungal activity of 5% chitosan (A), 10% chitosan(B), 15% chitosan(C), 5% TMC (D), 10% TMC (E) and 15% TMC (F) against *Fusarium solani.*

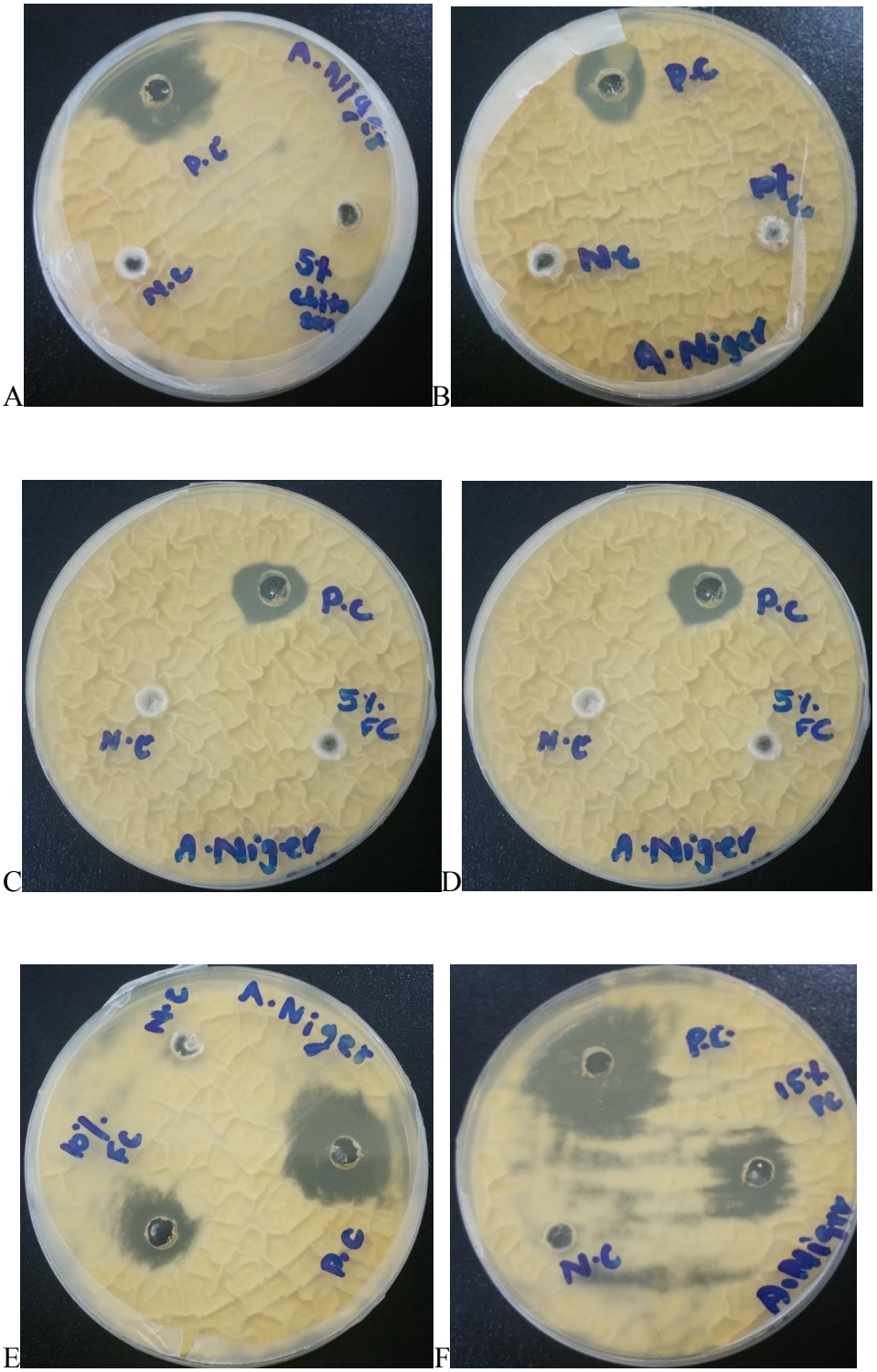


Figure 9. Antifungal activity of 5% chitosan (A), 10% chitosan(B), 15% chitosan(C), 5% TMC (D), 10% TMC (E) and 15% TMC (F) against *Aspergillus niger*

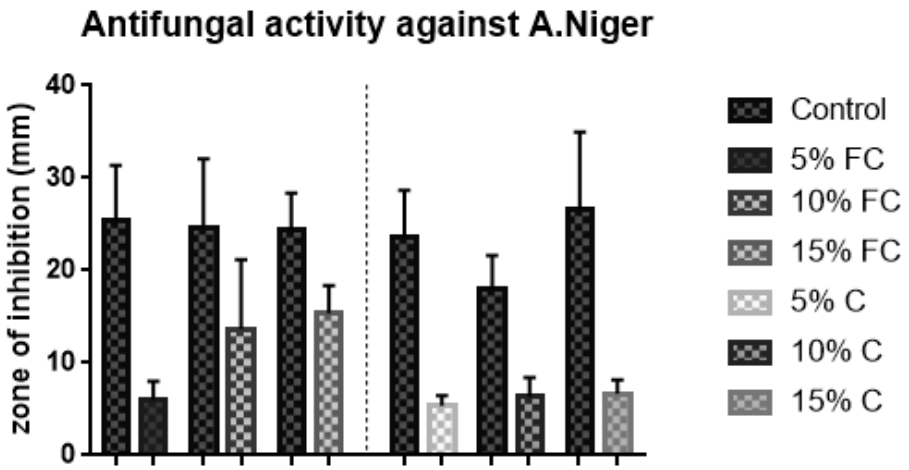


Figure10. Graph showing zone of inhibition of 5%, 10% and 15% chitosan and N-trimethyl chitosan against *Aspergillus niger*

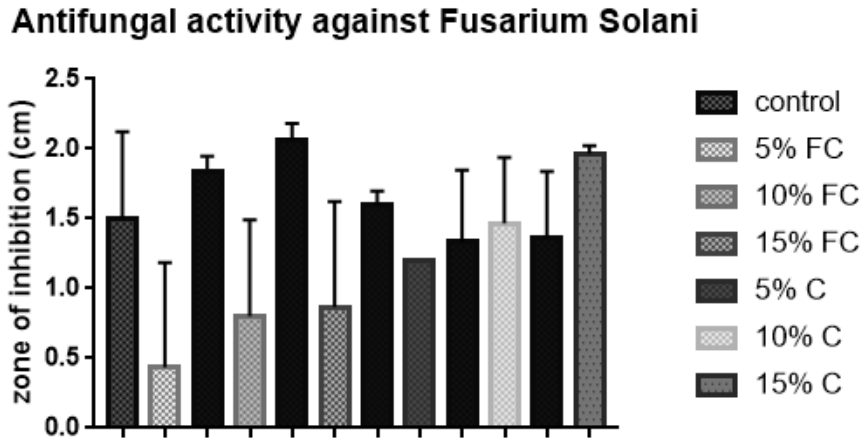


Figure11. Graph showing zone of inhibition of 5%, 10% and 15% chitosan and N-trimethyl chitosan against *Fusarium solani*

Antifungal testing of chitosan and trimethyl chitosan is shown in figures 9 and 10. Chitosan has given better antifungal activity against Fusarium solani as compared to trimethyl chitosan. Zone of inhibition given by 5%, 10%, and 15% chitosan was 1.2cm, 1.55cm, 1.95cm respectively whereas zone of inhibition given by 5%, 10% and 15% TMC was 0.6cm, 0.65cm and 0.7cm respectively. On the other hand, during antifungal testing against *Aspergillus niger,* N- trimethyl chitosan has shown better antifungal activity as compared to chitosan. Zone of inhibition given by 5%, 10%, and

15% chitosan was 0.53cm, 0.63cm, 0.77 cm respectively whereas zone of inhibition given by 5%, 10% and 15% TMC was 0.6cm, 1.37cm and 1.53cm respectively. In the previous studies, chitosan antifungal activity is already investigated. Chitosan show its antifungal activity against *Aspergillus niger* on a Candied Kumquat (fruit)[51]. Chitosan has also shown its antifungal activity agaist B.cinerea in strawberries and tomatoes. The fungicidal activity of chitosan agaimst R. stolonifer is due to its effect on morphology of cell wall of fungi[52,53]. Interaction between negatively charged cell wall and cationic surface of quaternized chitosan gives antigungal activity[53]. Functionalized chitosan directly supess the activity of enzymes that are responsible for fungal activity[51].

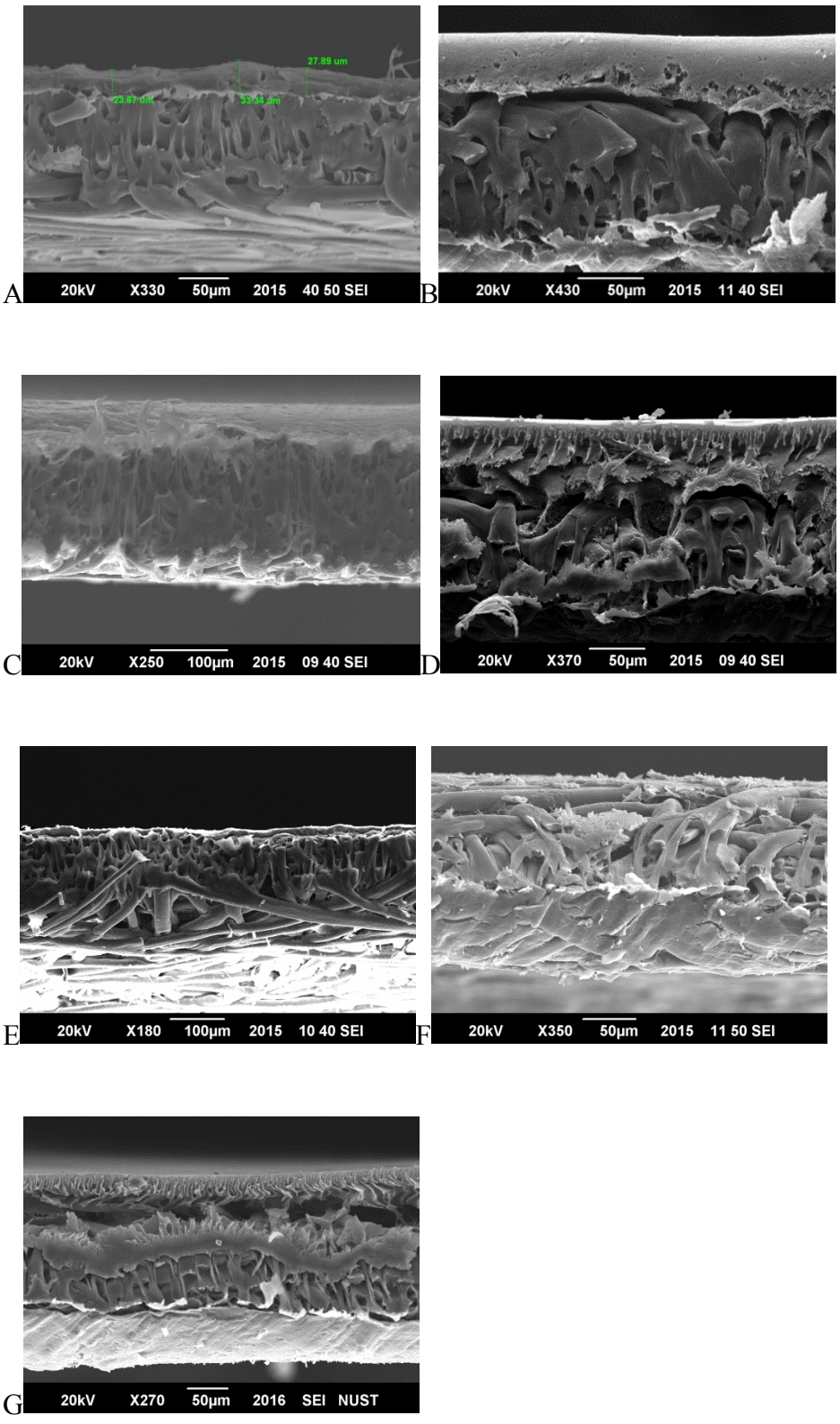
Table1. P values of chitosan and N-trimethyl chitosan against *Aspergillus niger* and *Fusarium solani*

Salt Vs control	**P value against *Aspergillus niger***	**P value against *Fusarium solani***
5% FC	0.0035 **	0.0023**
10% FC	0.0083**	0.7572
15% FC	0.0150	0.0944*
5% C	0.0062 **	0.1314
10% C	0.1444	0.0634
15% C	0.035	0.533
5% Vs. 15% FC	0.0114*	-

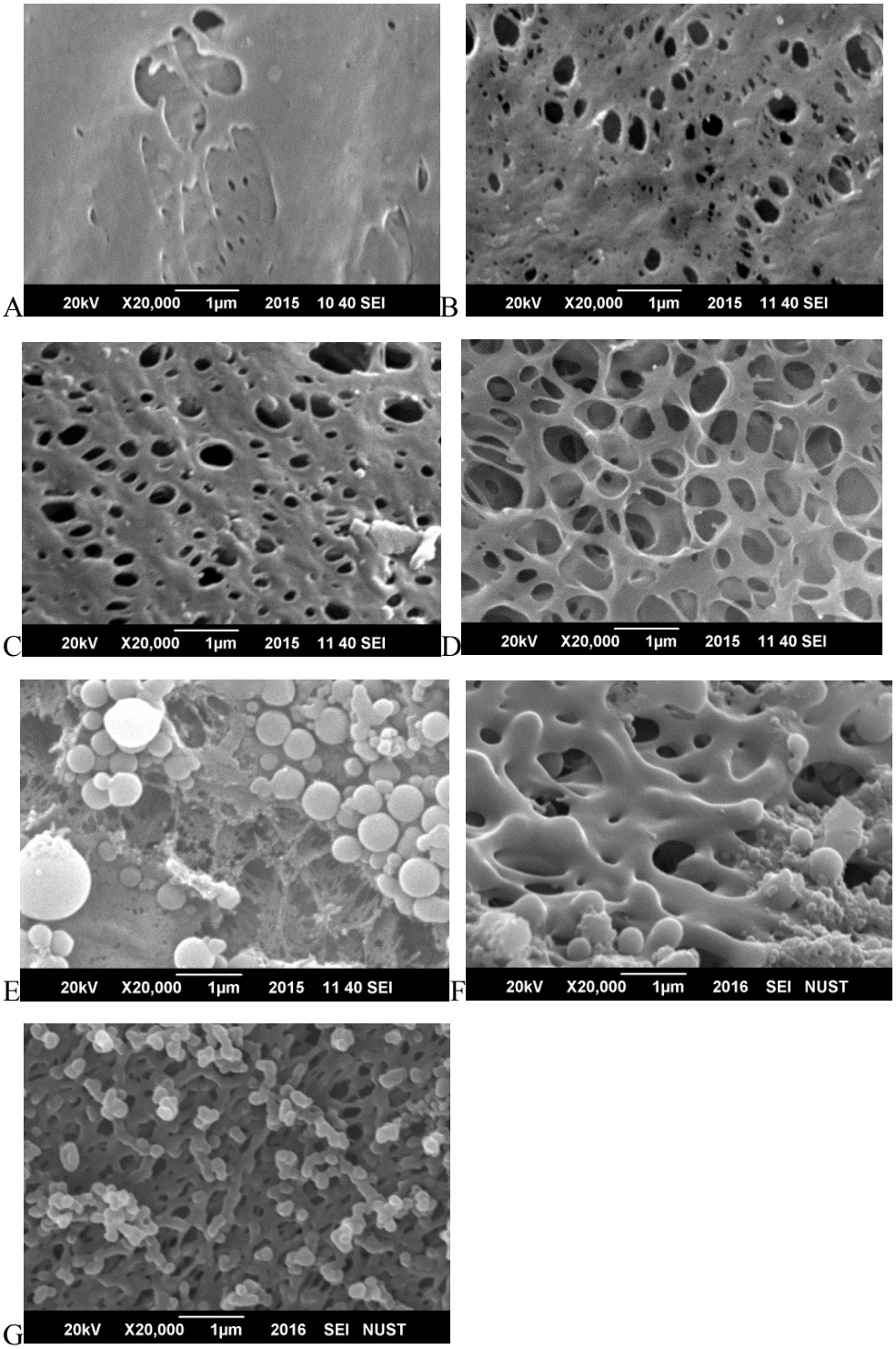
Note: Error bars tell the standard deviation while asterisks (*) shows p value. The asterisks (*), (**), (***) show significance difference ($p \leq 0.05$), ($p \leq 0.01$), ($p \leq 0.001$) respectively.

5.3 Scanning Electron Microscopy

Scanning.electron.microscope.images helps to find the morphology, cross section, and average pore size of each membrane. Surface topography and cross sectional images of all the prepared membranes are shown in labeled Figures 12 and 13. From the SEM images, it is clear that membranes have asymmetric and finger like structure which facilitates their flux. The micrographs of all the membranes clarify the remarkable porosity. The average pore size of membranes is increased by increasing concentration of chitosan and N-trimethyl chitosan. Pore size of MH0, MH1, MH2, MH3, MH4, MH5 and MH6 was 104nm, 185nm, 217nm, 258nm, 93nm, 140nm and 151nm respectively. Maximum pore size of the membrane was shown by MH3 membrane which had maximum percentage of N-trimethyl chitosan. An important conclusion is made that number of large pores that alters the hydrophobic nature of the pure polyether sulfone membrane obtained remarkably by increasing the concentration of filler (chitosan and N-trimethyl chitosan). Addition of chitosan and N-trimethyl chitosan alter membrane morphology. From this discussion, we can conclude that by increasing concentration of chitosan and N-trimethyl chitosan, morphology of membrane is affected. In the previous research it is clear that by increasing the pore size , porosity and hydrophilicity of membrane, wettability is increased and flux is also increased[34].



Figures12. SEM images of cross section of MH0 (A), MH1 (B), MH2 (C), MH3 (D), MH4 (E), MH5 (F) and MH6 (G) membranes.



Figures13. Scanning Electron micrographs of prepared membrane samples MH0 (A), MH1 (B), MH2 (C), MH3 (D), MH4 (E), MH5 (F) and MH6 (G) membranes.

5.4 Water Retention and Contact Angle Analysis

Table 2 shows the results of contact angle and moisture content of each membrane. These all parameters are required for determining the hydrophilicity/hydrophobicity of membrane. At 90º and above 90º, material is hydrophobic in nature, whereas below 90º material is hydrophilic in nature. As the angle decrease from 90º its hydrophilic nature is increase. Figure 14 show contact angle of all the membranes. The contact angle of MH0, MH1, MH2, MH3, MH4, MH5 and MH6 membrane was 89.67º, 87.62º, 67.75º, 56.78º, 85.39º, 79.90º and 68.08º respectively. Minimum contact angle is shown by MH4 membrane which has maximum percentage of N-trimethyl chitosan. Contact angle also decrease by addition of chitosan but it is greater than N-trimethyl chitosan. So, we can conclude that by addition of N-trimethyl chitosan, hydrophilic characteristic of membrane is increasing.

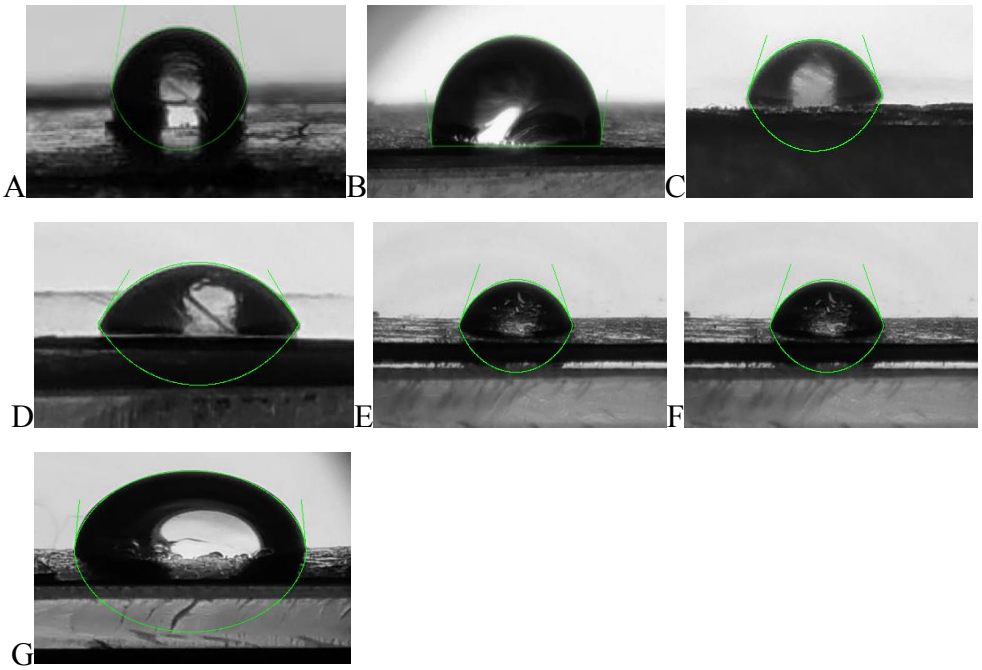


Figure14. Contact angle images of MH0 (A), MH1 (B), MH2 (C), MH3 (D), MH4(E), MH5 (F) and MH6 (G) membranes.

Water retention is a bulky property. It shows the maximum moisture content that can be absorbed by a membrane. MH0 membrane has absorbed minimum moisture content whereas, MH3 which has maximum concentration of N-trimethyl chitosan absorbed maximum moisture content.

Table2. Membrane composition, %water content and average contact

Membrane code	**Membrane composition**	**%Water retention Content**	**Average Contact angle**
MH0	PES	20.62	89.67
MH1	PES+5% Chitosan	41.06	87.62
MH2	PES+10% chitosan	42.04	67.75
MH3	PES+15% chitosan	57.42	56.78
MH4	PES+5% TMC	12.74	85.39
MH5	PES+10% TMC	13.06	79.90
MH6	PES+15% TMC	26.17	68.08

5.5 Permeation Flux

Water flux through membrane was analyzed by using a conventional filtrate assembly and results are shown in Figure 15. It is cleared from the figure that increasing the content of chitosan and N-trimethyl chitosan in membrane, a drastic change in the permeation flux is observed. Permeation flux was checked at two different pressures 40cmHg and 60cmHg. At 40 cmHg, MH0 membrane had given minimum flux 720 L/m².h due to its hydrophobic nature. Whereas, the maximum value was given by MH3 which was 1400 L/m².h, containing maximum amount of N-trimethyl chitosan. Hydrophilicity was improved remarkably by increasing content of chitosan and tri-methyl chitosan. Hydrophilicity of the membrane stimulated the passage of water. Water molecules were attracted towards the membrane. At 60cmHg, the minimum value of water flux was given by MH0 membrane that was 780L/m².h; whereas, MH3 membrane has given maximum water flux 1547L/m².h. It has been already concluded from the above SEM images, contact angle, pore size, water retention test and surface roughness analysis that by increasing the concentration of chitosan and N-trimethyl chitosan, hydrophilicity is improved. The two main factors that are responsible for higher flux values are the surface hydrophilicity and pore size. The flux of pure PES membranes is very close to the PES membranes that are already reported in literature [51].

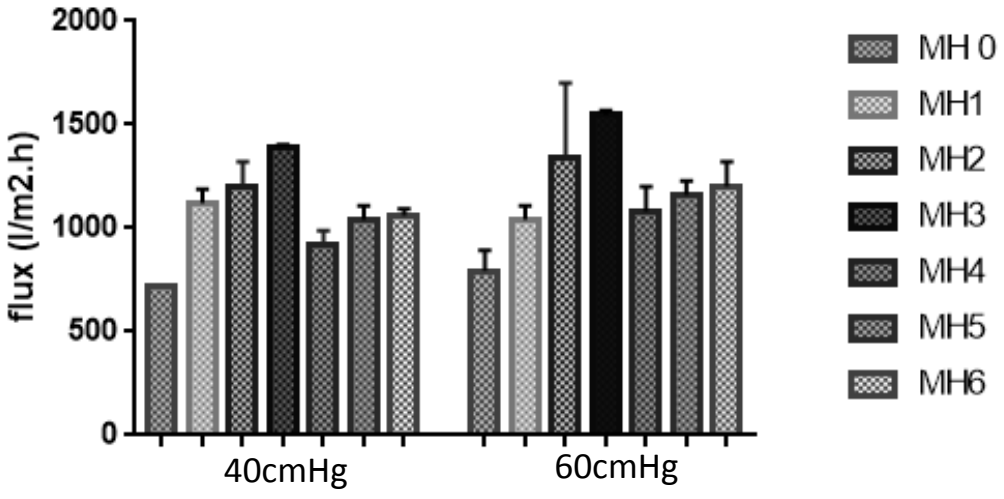


Figure15. Graph showing flux through membranes at pressure of 40 cmHg and 60mHg.

5.6 Antifungal Testing of Membrane:

To investigate the antifungal activity of each membrane, 10ml spores dilution was passed through membranes placed in the conventional vacuum filtrate assembly. By using hemocytometer spores/ ml of permeate and retentate was counted and difference between them was shown in the form of percentage.

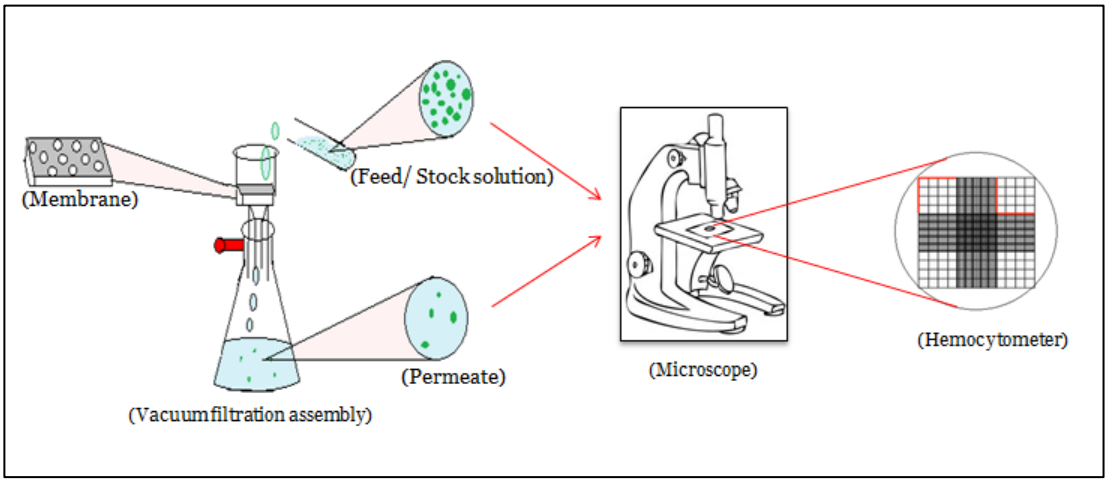


Figure16 . Scematic diagram of antifungal testing of membrane.

In case of Aspergillus niger, MH0, MH1, MH2, MH3, MH4, MH5 and MH6 reduced spores/ml 34.34%, 46.47%, 49.49%, 56.57%, 56.20%, 61.61%, 72.72% respectively. Maximum reduction was done by MH6 membrane, having maximum concentration of N- trimethyl chitosan. In case of Fusarium solani, MH0, MH1, MH2, MH3, MH4, MH5 and MH6 reduced spores/ml 7.5%, 30.84%, 40.00%, 62.5%, 13.75%, 17.5%, 25% respectively. Maximum reduction was done by MH3 membrane, having maximum concentration of chitosan. The previous research has been already revealed that chitosan had exhibit significant antifungal properties against different fungal species. This helps the broad spectrum inhibition of the fungal hyphae and destroys the spore germination [32]. Chitosan films are used in food industry due to their antifungal activity. Chitosan films lessen the intermolecular electrostatic repulsion between the chitosan molecules[51]. Figure 16 shows Graphical representation of spores/ml for all fungal strains. The standard deviation is representing by error bars.

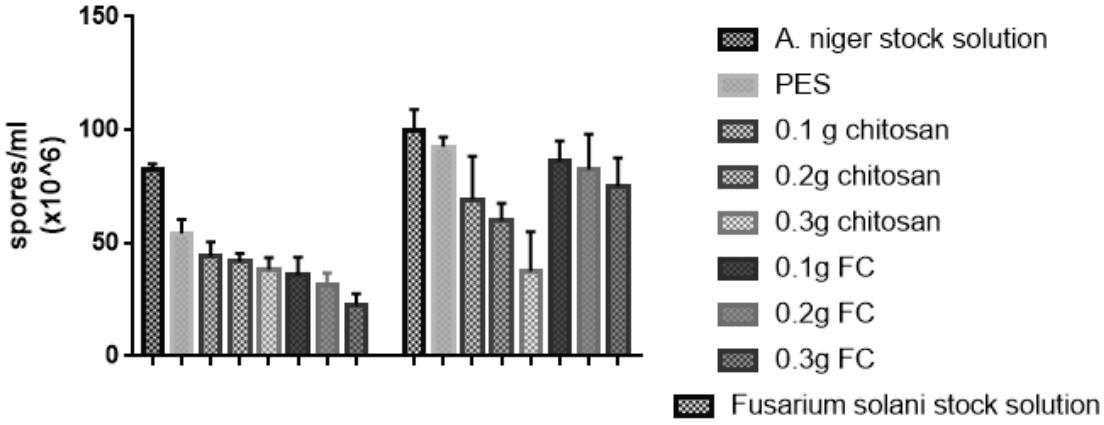


Figure17 . Graph showing reduction in spores/ml of *Aspergillus niger* solution and *Fusarium solani* solution after passing through all membranes.

Table3. Percentage reduction in spores/ml of solution after passing through membrane.

Stock solution Vs membrane filtrate	***Aspergillus niger***	***Fusarium solani***
PES	34.34% **	7.5%
0.1g C	46.47% ***	30.84%
0.2g C	49.49% ***	40.00%**
0.3g C	56.57% ***	62.5% **
0.1g FC	56.20% ***	13.75%
0.2g FC	61.61% ***	17.5%
0.3g FC	72.72% ****	25%

Note: Error bars tells the standard deviation and asterisks (*) shows p value. The asterisks (*), (**), (***) show significance difference ($p \leq 0.05$), ($p \leq 0.01$), ($p \leq 0.001$) respectively.

Chapter 6. Conclusion

Polyether sulfone (PES) membranes with different content of chitosan and N-trimethyl chitosan were successfully fabricated and behavior of each membrane for water disinfection was studied. Followings conclusions were drawn from this research study:

1) Through phase inversion method, the membranes were effectively prepared. Proceeding to membrane synthesis, N-trimethyl chitosan were synthesized from chitosan and characterized by FTIR. The analysis FTIR results confirmed the methylation and quaternization of chitosan. Antifungal test was also done to check the minimum inhibitory concentration (MIC).
2) Membrane characterization results revealed that by increasing the concentration of chitosan and N-trimethyl, a remarkable effect on membrane porosity, hydrophilicity, contact angle and permeance flux was observed. In addition, the membrane morphology was also changed by incorporating chitosan and N-trimethyl chitosan in it.
3) Antifungal testing of membranes was done and the reduction in spores/ml count was observed in all membranes with chitosan and N-trimethyl chitosan but the maximum inhibition was observed in MH3 containing 15% TMC against *Aspergillus niger* and MH6 containing 15% chitosan against *Fusarium solani*.

6.1 FUTURE RECOMMENDATIONS

- By adjusting the proper ratio of polymer and N-trimethyl chitosan, water purification can be further enhanced.
- Doping of membrane material with other material can also be examined.
- Blending of various hydrophilic polymers can be used for fabrication of membranes that can further enhance their properties.

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