

SYNTHESIS OF NOVEL MATERIALS FOR HIGH
PERFORMANCE POLYMERIC MEMBRANE APPLICATIONS

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APPLICATIONS**

submitted by **SADIA TUL MUNNA** in partial fulfillment of the requirements for
the degree of **Doctor of Philosophy in Polimer Science and Technology, Middle
East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Necati Özkan
Head of the Department, **Polymer Science and Technology**

Prof. Dr. Akın Akdağ
Supervisor, **Chemistry Department, METU**

Assoc. Prof. Dr. P. Zeynep Çulfaz Emecen
Co-Supervisor, **Chemical Engineering, METU**

Examining Committee Members:

Prof. Dr. Necati Özkan
Polymer Science and Technology, METU

Prof. Dr. Akın Akdağ
Chemistry Department, METU

Assoc. Prof. Dr. Erhan Bat
Chemical Engineering, METU

Prof. Dr. Atilla Cihaner
Chemical Engineering, Atılım University

Prof. Dr. Adnan Bulut
Chemistry Department, Kırıkkale University

Date: 10.09.2021



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Name Last name : Sadia Tul Munna

Signature :

ABSTRACT

SYNTHESIS OF NOVEL MATERIALS FOR HIGH PERFORMANCE POLYMERIC MEMBRANE APPLICATIONS

Tul Munna, Sadia
Doctor of Philosophy, Polimer Science and Technology
Supervisor: Prof. Dr. Akın Akdağ
Co-Supervisor: Assoc. Prof. Dr. P. Zeynep Çulfaz Emecen.

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The polymeric membranes for antimicrobial applications are in high demand nowadays, since the bacteria had developed the resistance against the antibiotics. The membrane technology could become an efficient tool to cope with the antibiotic resistant bacteria which are becoming an inevitable problem on the health and pocket of a common man. To address this issue, the current research is carried out to synthesize polymer with antibacterial properties. Norbornene and oxonorborne monomers with special modification to achieve antimicrobial properties through *N*-halamine functional group of hydantoin was synthesized in this thesis. The monomers were polymerized using Ring Opening Metathesis Polymerization (ROMP). The polymer norbornene dicarboximide hydantoin P(NDH) and polymer oxonorbornene dicarboximide hydantoin P(ONDH) were saturated to s-P(NDH) and s-P(ONDH) with *p*-toluenesulfonyl hydrazide. Polymer s-P(NDH) successfully fabricated into membrane. While s-P(ONDH) could not be fabricated into membranes by itself, the s-P(ONDH) was blended with the polyether sulfone (PES). The s-P(NDH) was also blended with PES, along with PES modified cyanuric acid

for comparisons. Hence four membranes were studied, one was homopolymer and three were the blend polymer membranes. The synthesized polymers and the blend membranes were characterized using NMR, IR, DSC, and TG/DTA respectively. The properties of membranes were measured: with SEM, pore sizes were determined: with contact angle measurements, hydrophobicity of surface was determined. Besides, tests for the pure water permeance PWP and BSA protein rejection were conducted. The membranes were studied for their antimicrobial properties against *E. coli* and *S. aureus*. The respective membranes were efficient enough to give 100% kill of both bacteria, providing the controlled release of active chlorine and restricted the biofilm formation at the surface of membrane.

Keywords: Ring Opening Metathesis Polymerization (ROMP), *N*-halamine, norbornene polymers, oxonorbornene polymers, modified PES, phase inversion, UF membranes, antibiotic resistance bacteria.

ÖZ

YÜKSEK PERFORMANSLI POLİMERİK MEMBRAN UYGULAMALARI İÇİN YENİ MALZEMELERİN SENTEZİ

Tul Munna, Sadia

Doktora, Polimer Bilim ve Teknolojisi

Tez Yöneticisi: Prof. Dr. Akın Akdağ

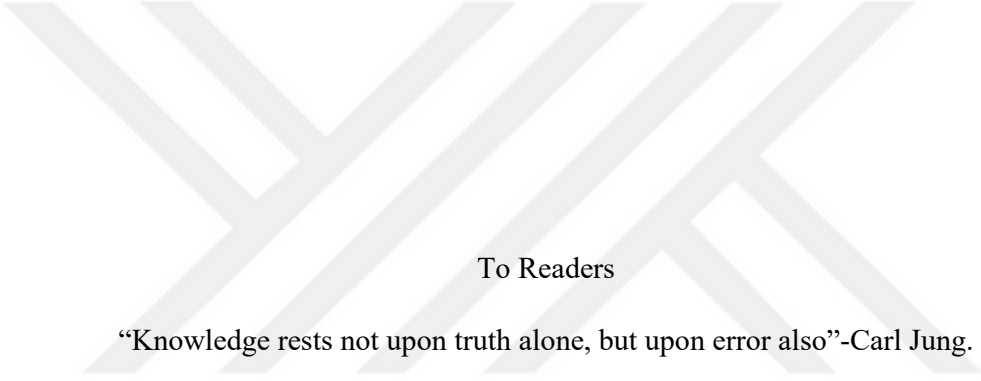
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Antibiyotiklere dirençli bakteriler nedeniyle antimikrobiyal uygulamalarda kullanılan polimerik membranlar günümüzde yoğun talep görmektedir. Membran teknolojileri, sağlık ve ekonomi açısından insanlar için önemli bir sorun haline gelen antibiyotiklere dirençli bakterilerle mücadelede önemli bir araç haline gelmektedir. Sorunun çözümüne yönelik olarak hidantoin yapısındaki *N*-halamin fonksiyonel grubu aracılığıyla antimikrobiyal özellikler elde etmek için bu çalışmada; esnek siklik olefin maddesinin Halka Açılım Metatez Polimerizasyonu (ROMP) reaksiyonu ile polimer sentezi gerçekleştirilmiştir. Çalışma kapsamında, norbornen dikarboksimid hidantoin P(NDH) ve oksonorbornen dikarboksimid hidantoin P(ONDH) polimerleri sentezlenmiştir. Ardından, bu polimerler s-P(NDH) ve s-P (ONDH) olarak doymuş hale getirilmiştir. s-P (ONDH) polimerinin koagülasyon banyosunda membran oluşturmaması nedeniyle, s-P (ONDH) ile polietersülfon (PES) polimeri karışımı hazırlanmıştır. Ek olarak s-P(NDH) polimeri, karşılaştırma amacıyla siyanürik asit ile modifiye edilmiş PES polimeriyle karıştırılmıştır. Sonuç olarak çalışmadaki dört membranın, biri saf olup diğer üç membran polimer karışımlarından oluşmaktadır. Sentezlenen polimerlerin ve karışımlardan hazırlanan membranların karakterizasyonu sırasıyla DSC, TG-DTA, NMR ve IR kullanılarak gerçekleştirilmiştir. Ek olarak, taramalı elektron mikroskobu (SEM) ile temas açısı karakterizasyonu da yapılmış, membranın saf su geçirgenliği ve bovin serum albumin (BSA) protein tutulumu ölçülmüştür. Üretilen membranların *E.*

coli ve *S. Aureus* bakterilerine karşı antimikrobiyal özellikleri test edilmiştir. İlgili membranların, kontrollü aktif klor salınımı sağlayarak ve membran yüzeyinde biyofilm oluşumunu engelleyerek iki bakteri türünün de tamamını öldürebilecek yeterlilikte olduğu gözlemlenmiştir.

Anahtar Kelimeler: Halka Açma Metatez Polimerizasyonu (ROMP), *N*-halamin, norbornene polimerler, oksonorbornene polimerler, modifiye PES, faz inversiyonu, UF membranları, antibiyotik direnci bakterileri.



To Readers

“Knowledge rests not upon truth alone, but upon error also”-Carl Jung.

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LIST OF ABBREVIATIONS

ABBREVIATIONS

NO _x	Nitrogen Oxides
SO _x	Sulfur Oxides
CO _x	Carbon oxides
MF	Micro Filtration
UF	Ultra Filtration
NF	Nano Filtration
RO	Reverse Osmosis
IE	Ion Exchange
ED	Electro Dialysis
GS	Gas Separation
PV	Pervaporation
L-S	Loeb-Sourirajan
EPA	Environmental Protection Agency
<i>E. coli</i>	Escherichia coli
<i>S. aureus</i>	Staphylococcus aureus
ROMP	Ring Opening Metathesis Polymerization
DA/ H-DA	Diels Alder/ Hetero Diels Alder
m-NDH	Monomer Norbornene Dicarboximide Hydantoin
m-ONDH	Monomer Oxonorbornene Dicarboximide Hydantoin
P(NDH)	Polymer Norbornene Dicarboximide Hydantoin

s-P(NDH)	Saturate Polymer Norbornene Dicarboximide Hydantoin
P(ONDH)	Polymer Oxonorbornene Dicarboximide Hydantoin
s-P(ONDH)	Saturated Polymer Oxonorbornene Dicarboximide Hydantoin
PES	Polyether sulfone
PESmCA	Polyether sulfone modified Cyanuric Acid
ATR-IR	Attenuated Total Reflectance Infrared Resonance
¹ H NMR	Proton Nuclear Magnetic Resonance
¹³ C NMR	Carbon 13 Nuclear Magnetic Resonance
SLS	Static Light Scattering
M _w	Weighted average Molecular Weight
DSC	Differential Scanning Calorimetry
TG	Thermogravimetry
DTA	Differential Thermal Analysis
DDTA	Differential Differential Thermal Analysis
SEM	Scanning Electron Microscopy
CA	Contact Angle
PWP	Pure Water Permeance
BSA	Bovine Serum Albumin
PBS	Phosphate Buffer Saline
LB	Lysogeny Broth
CFU	Colony Forming Unit
TMP	Trans-membrane Pressure

LIST OF SYMBOLS

ΔT (delta upper case) Change in Temperature

ΔH Change in Enthalpy

μ micron

δ (delta lower case) chemical shift scale in NMR presented in parts per million (ppm).

CHAPTER 1

INTRODUCTION

The high-performance polymers are polymer materials exhibiting the ultimate class of efficiency in terms of temperature, pressure, solvent resistance, and mechanical strength [1]. With the extraordinary properties of such polymers as bulk materials; even when fabricated into thin films, coatings, or membranes, they retain their properties of high-performance temperature, pressure, and solvent resistance even after many operational cycles thus being sustainable and durable.

Membrane technology that we are venturing these days is inspiration that came from keen observation of nature. The biosphere and living beings are marvelous examples of numerous membranes that are surrounding them from inside and outside from the environment. Hence, membrane is a semipermeable barrier between two phases. If one component of a mixture moves through the membrane faster than another mixture component, a separation can be accomplished [2]. Example of biological solid membranes are all seen in living beings such as amniotic membrane which is the outer most thin and selectively permeable membrane of all the living being's embryo. Every cell within the living beings is also covered by a cell membrane. All the eukaryotes and prokaryotes; including the microorganisms, consist of this semipermeable solid membrane. The synthetic solid membranes are the basis of modern membrane technology, which has transformed from laboratory development (1960) into proven industrial applications [2-4].

The idea of separations/filtrations from solid material (membranes) had emerged from the separation examples seen in our surroundings. The barriers of separation in common observation are earth strata which is a selective barrier of gasses, and non mixing of Pacific ocean and Atlantic ocean due to density function [5]. The

advancement in separations science has taken its basis from keen observation of these natural phenomena. The inspirations obtained from above examples had established the science of liquid/liquid separation in extractions and gas separations in cryogenic separations, where the barrier of polarity and weights help in completing the separations [3]. The advanced shape of such technology is Soxhlet separation and size exclusion chromatography. The structural simplicity of separation is seen in membrane bound separations, where the degree of separation is dependant upon the membrane retention ability only.

Broadly; *a membrane is a barrier between two identical or different phases, which allows the selective transport of the species depending upon its nature and activity bearing tunable characteristics for improvement and usability in other operations as well.* Hence it is obvious that one type of membrane is not applicable for all or other kinds of systems. Each system requirement and specifications change and this adaptation in the properties of membranes had led to the development and advancements in membrane technology owing to the increased demand of separation in various processes of common routine life such as water and atmosphere cleaning techniques.

Due to human activity, we had faced a huge impact of environmental damage which is coined with industrial revolution, and we came to know it later that we had ended up damaging our environment to an extent that now we are facing an enormous impact of Global Warming and health issues due to the contamination of our basic utilities: water and air.

The chemical species of industrial waste that had contaminated water sources and air simultaneously, had resulted in wide range of diseases being mild like allergies to enormous like gene mutation (radioactive contamination) and cancer. So, humans had not only put themselves but the lives of other living beings at stake. A serious need emerged to check back and cope with this contamination in our environment, conclusively the field of membrane technology and green chemistry emerged with a greater impact, and it caught immediate attention of all the chemists and physicists throughout the world. In order to protect our environment from adverse effects of

harmful wastes; approach of Green Chemistry and to undo the damage already done, Membrane Technology are the two routes/strategies adopted by the researchers.

The contaminations that are widely seen in water are due to discharge of chemical wastes in streams and rivers containing chemical species, household waste and sewerage wastes having viruses and bacteria that are their growth places. If radioactive waste; the resultant would be gene mutation and various kinds of cancers, with discharge of NO_x, SO_x and CO_x by industries [6,7] or by vehicles; the poor quality of air, resulting in smog [8] causing problems and issues in respiratory tract, while discharge of other fumes (organic/ inorganic) from industries polluting the environment resulting in aerosol-based infections and cancers [9]. Other than these contaminations induced to our freshwaters; the huge quantity of sea water is not usable due to high concentration of salt. The water desalination plants are also inevitable to provide the need of clean water to every living being [3], where sea water is the only source of water available.

The membrane technology had uplifted those undermined parts of our life that needed the restoration and recovery from the man-made damages. Precisely, membrane technology is thus focusing to cope with all the unwanted species that are present in our water and air in order to improve and reduce the environmental pollution impact. Shaping up of materials into finished membrane of desired properties is the unique contribution of synthetic material's researchers worldwide. According to the need and area of operation the membranes could be fabricated as porous, dense, or composite and used in solid/liquid, liquid/liquid, gas/gas separations (**Table 1.1**).

After every fabrication, the membrane is characterized and allocated into the unique group according to its pore sizes, whether the pore sizes are well defined through channels or being dense [10, 11]. The material selection and fabrication method or technique are also dependent upon the area of action where the membranes would be used.

Table 1.1: *Salient features of Porous and Dense Membranes.*

Membrane	Processes	Average pore Size	Area of Application
Porous	MF	100-1000 nm	bacteria and suspended particles.
	UF	2–100 nm	proteins, polysaccharides, viruses, and bacteria.
Porous and Dense	NF	1–2 nm	small organic molecules (drugs, dyes, sugars) and multivalent salts.
Dense	RO	Non porous	Ionic compounds
Charged	IE	Nonporous	ED, desalinization, production of NaCl, production of acids and bases using bipolar ED.

The separation through membranes involves the unit operations which are dependent upon respective principle and theories of separation [11]. The separation property of each membrane is governed through one of the principle/models of separation, respectively. There are two governing principles / models upon which the industry of membrane technology depends and helps in understanding the separation mechanism through the membranes. For any membrane one of the two basic models are adopted, which follow the different principles of flow. The pore flow is simply the flow through capillary and the pore size is separating the undesired species. To understand the pore flow model, it is most often assumed to be capillary, however, in usual practice all the pores in porous membranes are tortuous and the specific calculation for tortuosity is applied when determining the porous characteristics. In solution diffusion the solute permeates the membrane by dissolving in the membrane material and diffusing down the concentration gradient and separation occur due to difference in solubilities and diffusivities. Ion exchange is also a sub mechanism of solution diffusion with a difference that the driving force across the membrane is electric potential and this separation of ions take place through the ion exchange membranes [11, 12]. Each transport type has its mathematical representation and the type of external force such as pressure, diffusion, or potential difference that a

membrane/s experience to conduct the separation (**Figure 1.1**). The strength of the membrane against pressure in pore flow, solvent resistance in solution diffusion and ionic characteristic in ion exchange membranes are ensured to conduct the respective process of separation successfully.

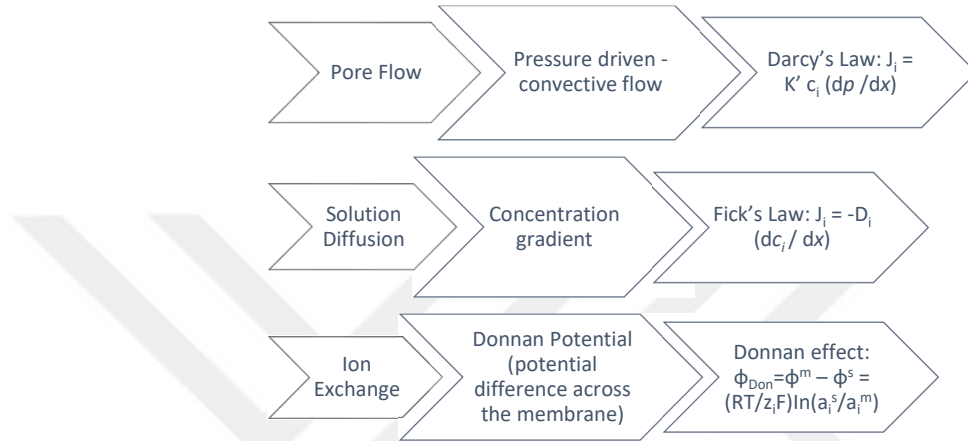


Figure 1.1: Transport in Membranes

As discussed, the desired strength in each operation type is inevitable for the membrane process, hence the selection of material of interest for membrane processing is circling around the chemistry of materials. Materials for membrane processing are selected from organic, inorganic, or hybrid sources; due to this reason classification of membranes is also diverse.

Organic membranes are usually polymers of organic nature providing cost effectiveness and easy processability. Almost 90% of industrially used MF, UF, NF and RO membranes are polymeric. Moreover polymer membranes are seen sensitive to high temperature and oxidize easily. Fouling is also observed in hydrophobic types, which with specific modifications could be used in pervaporation or membrane distillation, where the hydrophobic characteristic is specially a requirement when separating organic solvents or for avoiding water permeation, respectively. The hydrophilic ones mark their utility in RO where water purification is required from salts thus providing easy permeation of water and rejecting the solutes (salt). In this process good strength is especially required [13]. Thus, the utility polymers have their service temperature upto 300 °C, while the commodity polymers having characteristic high temperature stability greater than 300 °C. Such

materials are called high performance polymers [1], where high temperature resistance, pressure and solvent resistance are their defining features.

Inorganic membranes are made from silica, glass, or alumina. These are chemically inert and show thermal stability up to 500°C. Porous Ceramic membranes are used in food and beverage industries widely. However, they have marked their importance in NF, UF, MF and RO also. The membranes made from inorganic materials show very good stability over number of operation cycles, but these types of membranes are very expensive to process and exhibit low mechanical strength due to their brittle behavior. However, their cost could be regulated when low-cost inorganic materials are used in processing, hence this field requires more research and review [13].

Hybrid membranes are the combination of both organic and inorganic material. These could be ion exchange membranes, composites, or metal-organic framework membranes. The hybrid membranes are mostly used for gas separation applications. Once the membrane is fabricated from either material, could further be classified according to the morphology as well (**Figure 1.2**). Being symmetric and non-symmetric in morphology also further helps in the identification of suitable area of application for membrane. Isotropic symmetric membranes could be totally porous or totally dense throughout the width and length of membrane. While anisotropic are asymmetric membranes having top thin layer of porous or dense material and underneath thick support layer of big pores (composite) or could be small and big pores of same material which are called Loeb–Sourirajan (L–S) membrane [3, 13].

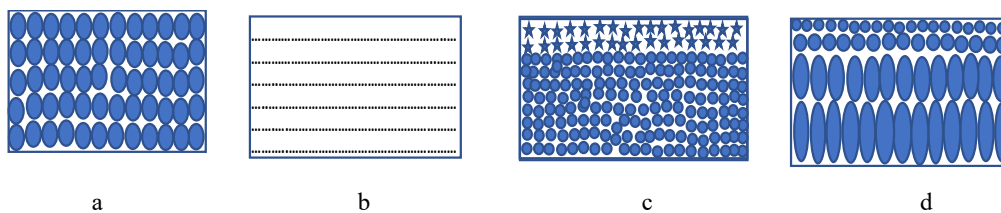


Figure 1.2: Membrane Morphologies: Isotropic: (a) porous and (b) dense. Anisotropic: (c) composite and (d) Loeb–Sourirajan (L–S) membrane.

The symmetric (isotropic) and asymmetric (anisotropic) membranes could be fabricated by several techniques which are briefly discussed below.

Symmetric membranes can be synthesized by sintering, casting, and track-etching. Sintering is adopted for ceramics where high temperature binding operation is required. This process is used for production of microporous membranes. In casting the polymer melt is casted over the substrate and after casting the solvent evaporation is done to get the membrane. It is used in manufacture of IE membranes and membranes for PV. In track-etching the polymer film is etched to create desired surface roughness and porosity. The manufactured materials function as porous membranes and are used in MF [3,14].

Asymmetric composite membranes are fabricated by interfacial polymerization, dip coating and plasma polymerization while with phase inversion and grafting; L-S membranes are fabricated. For interfacial polymerization two reactive monomers in organic and aqueous solvent react at interface and form the polymer then it is immersed in second reactive polymer and reaction is completed by crosslinking via irradiation. In dip coating UF membrane is dipped in dilute coating solution of polymer, prepolymer or monomer which is further set to evaporation to generate pores either at ambient conditions or by heat treatment. In phase inversion the required material is dissolved in suitable solvent and casted over a substrate with required thickness. Further the substrate is immersed in non-solvent to get the membrane [10].

Membranes are materials which are enhanced in their scope of application by using the discussed methods and desired improvements are done in their properties. It is the only technology that is equally useful in industrial and household applications. Every new membrane material is designed while keeping the industrial application in mind first since the later do not require high temperature, pressure, and solvent resistance sophistications. If a membrane is not suitable for industrial application, it is usually viable for lab-scale or household applications.

In industrial applications there are numerous kinds of membranes that are in common practice and are covering a wide range of water purification, solvent purification, gas separation and desalination processes. The membranes with good pressure resistance and temperature stability are used in water filtration processes of UF, MF NF, while

those with additional chemical resistance are used for PV. The membranes that could resist the solvents, and high pressures are used in RO process in desalination plants since the back washing of such membranes require such kind of stability. The membranes with oleophobic properties are used in the oil/water separation in the sea water oil spills. Membrane scaffolds in bio-medical implants are those materials of selection which have mechanical stability, pH, microbial and solvent resistance [15]. So far, the membranes have been derived from both synthetic and biological sources as well and are used in the specific area of their defining properties (**Table 1.2**).

Table 1.2: *Renowned Polymer materials, their defining resistances [1,3, 15] and process use.*

Membrane Material	Defining resistance attributes								Process	[Ref]
	Temperature Resistance or service temp.	Pressure	Chemical	Radiation	Mechanical	Flame	Micro-organisms	pH		
PVDF	150 °C	☑	☑	☑	☑	☑	☑	☑	MF UF MBR	[15, 16 ^{a,b}]
PP	☑	☑	☑				☑		MD	[15, 17 ^{a,b}]
PTFE	☑	☑							MF MD	[15, 18 ^{a,b}]
PEEK	260 °C	☑	☑	☑	☑	☑	☑	☑	MF UF Biomed	[15, 19 ^{a,b}]
SPEEK	☑	☑			☑		☑	☑	UF NF IE	[15, 20]
PSF	☑	☑			☑			☑	UF IE	[15, 21 ^{a,b}]
PES	☑	☑	☑		☑	☑	☑		MF UF Biomed	[15, 22 ^{a,b}]
PA	100 °C	☑	☑			☑			GS RO	[3, 15, 23]
PVA	☑		☑					☑	GS Biomed	[15, 24]

Table 1.2 Contd..

Membrane Material	Defining resistance attributes								Process	[Ref]
	Temperature Resistance or service temp.	Pressure	Chemical	Radiation	Mechanical	Flame	Micro-organisms	pH		
PAN	☒	☒	☒		☒	☒			UF NF	[3, 15, 25]
PDMS	☒	☒	☒						GS	[15, 26]
TEFLON composite		☒							PV GS	[1, 3 27]
PLA	☒	☒			☒		☒		MF UF Biomed	[1, 28]
PHA			☒				☒		Biomed	[15, 29]
PBS/ Cellulose triacetate	☒	☒							PV	[15, 30]
CA/ ceramic composite		☒					☒	☒	UF	[15, 31]
Alginate/ ferro ferric oxide	☒	☒							PV	[15, 32]
P.isoprene	☒	☒			☒				G.S	[15, 33]
Starch/ chitosan		☒			☒				UF	[15, 34]
Chitosan	☒	☒					☒		Bio.med Dialysis	[15, 35]

All the membrane undergoing the operational cycles, after some time face the performance decline due to the reason of fouling [36]. This fouling is “substance fouling” in which the pile of rejected species is formed at the surface of the membrane. This occurs due to inorganic salts and sugars and other organic molecules by initially clogging the pores, further to this the cake/gel layer of rejected species starts to build up at the surface of membrane. With continuous operation cycles this layer becomes very thick, and the quality and time of separation reduces. This is cleaned after certain cycles of operations by a usual procedure of back washing. Hence “Substance Fouling” is defined as: *The substance fouling is the non-biological physical barrier that builds up at the membrane and clogs the pores resulting in the reduction of the efficiency of membrane to an extent of malfunctioning.*

The UF, NF membrane [37] specifically and other membranes generally; face another type of fouling during the operational process, called biofouling, this is also dealt with ultrasonic waves [38] and back washing [36]. *Any fouling at the membrane that is of biological source; either as living biological source such as biofilm, and non-living biological sources such as mosaic formation when effect the efficiency of the membrane by shielding the pores to an extent of malfunctioning is called biological/biofouling.* The biological fouling is further discriminated into living biological fouling and non-living biological fouling. In the text further the unique terminology shall be used where necessary.

The non-living biofouling is the mosaic formation at the membrane due to the concentrated pilling of humic materials, proteins and lipids suspended in the system by any means. This is often dealt with backwashing or by using a suitable solvent to remove adsorbed unwanted substances. While the living biological fouling is the biofilm formation which is purely because of the adhesion of bacteria to the retentate side of the membrane. This biofilm easily forms at the surface of UF membranes if they are hydrophilic, that even the back washing of the membrane does not completely break down this layer, which has capability of growing back again and eventually replenishes.

Much of the problem is being faced in the industry due to the *living biological fouling*

of membrane, where providence of clean water for the masses is the ultimate priority. The biofilm is the unique environment of survival for bacteria; where they grow in colonies since they are unicellular in organization.

The unicellular organisms have their existence history since unknown, and they had remarkably shown their presence by creating an environment which is beneficial for their growth and existence. Most of these unicellular are benign to humans and other living organisms, but some of them are harmful to humans, animals, and plants as well. Sometimes they exist as a host parasite relation with the living beings, sometimes like symbionts, while sometimes as decomposers of dead organic matter into the soil. The microbes of class viruses and bacteria are those unicellular that effect the human body while causing diseases if they somehow reach inside. These viruses and bacteria which are already colonizing the damp places or living in water thus can easily target any living being if that water is consumed. In some cases, the ingestion is not necessary, superficial contact with contaminated sites or water, having microbes can also lead to numerous types of bacterial and viral infections. Since bacteria have a quality to create the biofilm at the surface where the required environment is of its easy adhesion and multiplication, a lot of bacterial infections are prone to humans from its surrounding since the biofilm growth is equally favorable in water and soil/land environment [39].

The most vulnerable area to get any kind of bacterial/viral infection are the hospitals and surgical facilities itself, which is a hub of visitors of all ages. The equipment that is used in hospitals during various treatment methods such as scissors, knives, tweezers, wound stitching (sutures), needles and for implantation purpose (dental and bone implants); they are usually difficult to sanitize. Now polymer materials are best option for hospital equipment and implant materials [40, 41], except needles that need to be heat treated or melted and refabricated to be sanitized. The polymer-based materials do not need sterilization due to their characteristic chemical composition providing non adhesive surface for bacteria especially. Some natural polymer materials such as cellulose with antimicrobial coating or grafting [42, 43], can effectively prevent the bacterial adhesion. Such materials should be used in

hospitals where the cloth is in direct contact with the patients as bedsheets, covers and curtains [44, 45].

The antibacterial surfaces and fabrics that have been developed so far are by grafting quaternary ammonium salts, molecularly engineered peptides, *N*-halamines [46-48] or compounds releasing bactericidal moieties such as metal ions and nanoparticles [49] and nanofibers [50] based membranes are widely in use. Fabricating and designing of photoactive surfaces by incorporating photosensitizers as coating in polymers, which upon exposure to light become active and the coating behaves like a sanitized surface [51], while the sanitized surface cleaners and hand washes are also the variant examples of commercially available products.

If the surface has the property to behave like a non-adhesion site for a bacterium, the result is obvious that there will not be any chance of biofilm growth, hence the colonization would not be possible. Research about surface coatings and surface modifications; to behave like antimicrobial, are also an interesting area of sanitized surfaces [52, 53]. Some coated polymeric surfaces with biocidal agents such as silver, gold, copper nanoparticles, antimicrobial ammonium compounds and antibiotics which enhance antibacterial properties in leaching or non-leaching systems [54].

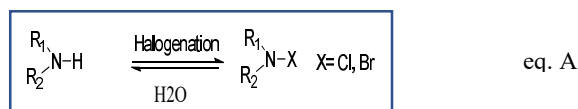
All the cited literature is the effort done by the researchers to reduce the biofilm formation at the surfaces that we use commonly. The presence of bacteria in wet areas such as water systems is also a growing challenge. Since bacteria can multiply simultaneously while being sessile or planktonic [39], with the availability of nutrients for growth. In some regions of the world, scarcity of water, uneven distribution of water accompanied by the contamination due to fecal wastes has increased the chances of bacterial growth in the fresh waters along with resulting economic burden. The need of clean water in household consumption has become a subject of paramount importance. To understand the average health of a community, the local water source samples are analyzed for the presence of coliform bacteria, which is a standard method [55] chartered by Environmental Protection Agency (EPA). When this bacteria is present in the test sample the chances of getting affected by

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S.aureus*) is foreseen as a potential risk.

In common observation, none of the waters are devoid of presence of microorganisms unless a water channel is exposed with the chlorine, a common biocidal practice so far. Until now a desirable amount of chlorine is used in water tanks and swimming pools to kill the bacteria, however the huge amount of added chlorine to water is the reason of chlorine signature in water which is also a matter of question as it makes water unpleasant to drink. With the technique of water purification, either the filtration plant adopts the strategy of bacterial rejection by using required porous membrane (UF, NF), or by having a filtration membrane with a grafted chemical entity, that would release the required amount of disinfectant in water and induce necrosis in bacteria. In the first technique the chances of biofilm formation increase due to the living biological fouling at the membrane. More often, the later strategy is very well adopted to release metal ions such as silver ions, to make water drinkable at household level, while metal ions or halogens such as chlorine to kill the microbes at larger scale. An idea of release of controlled amount of chlorine opened a new dimension in the research for designing a N-halogenated specie incorporated into a membrane.

The concept of *N*-halamine took its shape from the *Worley S.D., et al.* research work reported in 1996. He introduced the concept of *N*-halamine substituting the previously used *N*-chlorinated sulphone amides moieties for antimicrobial purpose. He introduced styrenetriazinedione polymer as an effective antimicrobial agent [56]. His other prominent work comprising the *N*-halamine are the disinfecting of rotavirus[57], antimicrobial emulsion coatings as surface sanitizers [58], detoxification coatings, antimicrobial cotton with different types of *N*-halamine, *N*-halamide and *N*-halimide derivatives [45,59,60], cellulose based *N*-halamine grafting for antimicrobial purposes [62], chitosan with grafted *N*-halamine [63], cotton/polyester blends with *N*-halamine coatings [64], where he successfully established release of controlled amount of chlorine in the system of study. The chemistry of *N*-halamine/amide/imide; where usually but not necessarily, chlorine is

the required halogen (**equation. A**), is verily researched as the most suitable process for antimicrobial [65] action and sufficiently effective against potential living biofouling at membrane surface in UF membranes (**Table 1.3**).



Initially the precursor of *N*-Halamine is grafted into the filtration membrane or synthesized as part of polymer back bone or side chain. The precursor of *N*-halamine is the hydrogen associated N-group. This precursor group after attachment with the polymer is fabricated into membrane. The N-H precursor is usually the part of amine, amide, or imide groups (**Figure 1.3**) and is halogenated using the desired halogen group into *N*-halamine, *N*-halamide or *N*-halimide respectively. After consumption of the halogen during antimicrobial operation, it is replenished back using the same method of halogenation.

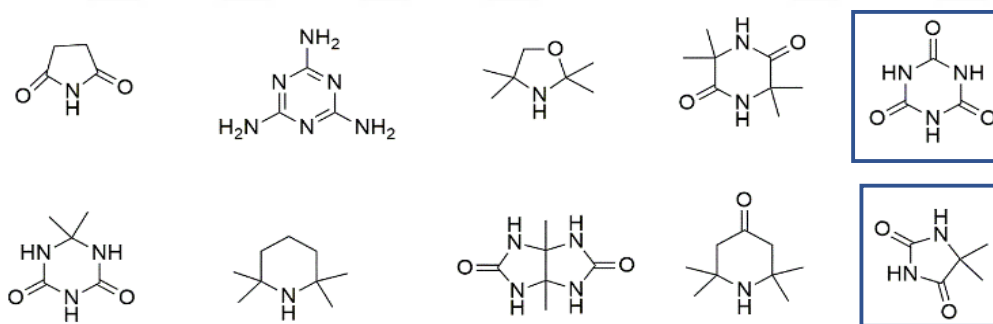


Figure 1.3: Representative cyclic precursors of *N*-halamine/amide/imide functionalities.

Table 1.3: Brief overview of precursor *N*-halamine in various polymer materials

Membrane	Target	Result	[Ref]
Chitosan-hydantoin	<i>S. aureus</i> <i>E. coli</i>	99% 99%	40
Chitin nano fiber- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	100% 100%	41
Cotton- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	100% 99%	42
Cotton- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	100% 100%	43
PET/cotton- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	99% 99%	44
Cotton- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	100% 100%	45
PES-SiO ₂ - <i>N</i> -Halamine nanoparticles	<i>E. coli</i>	60.2%	46
Iron oxide core with (poly(ADMH-co-MMA))	<i>S. aureus</i> <i>P. aeruginosa</i>	96% 96%	47
TiO ₂ / <i>N</i> -Halamine nano particles	<i>S. aureus</i> <i>E. coli</i>	100% 100%	49
polyethylenimine- <i>N</i> -Halamine-polyacrylic acid LBL	<i>L. monocytogenes</i>	99.9%	52
PVC- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	55% 68%	53
PP- <i>N</i> -Halamine	<i>S. aureus</i>	96%	61
Polyethyleneimine-co-styrenemaelicanhydride- <i>N</i> -Halamine	<i>E. coli</i>	99%	66
PS-co-Tertiarybutylamine- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	98% 96%	67
PS- <i>N</i> -Halamine-5-allylbarbituric acid	<i>S. aureus</i> <i>P. aeruginosa</i>	85% 94.5%	68
PES/PES- <i>N</i> -Halamine PES/PES- <i>N</i> -Halamide	<i>E. coli</i> <i>B. subtilis</i>	100% 100%	69
PMMA-Hydantoin-Silica nano particle	<i>S. aureus</i> <i>E. coli</i>	99% 98%	70
Polyuerthane- <i>N</i> -Halamine	<i>S. aureus</i> <i>E. coli</i>	100% 100%	71

The living biological fouling in other than *N*-halamine/amide/imide membranes is due to the fact that, although the membranes are designed to reject the microbes, but at the rejection side of membrane those rejected bacteria start cell adhesion at the membrane surface, causing biofilm formation and colonizing. The *N*-halamine/amide/imide (in-situ and ex-situ) polymeric membranes prevent the living biological fouling since the contact of chlorine with bacterial cell result in the cell lysis or necrosis (**Figure 1.4**). This results in the death of the bacteria and totally prevents the cause of living biological fouling at the membrane, while water also become sterilized for use.

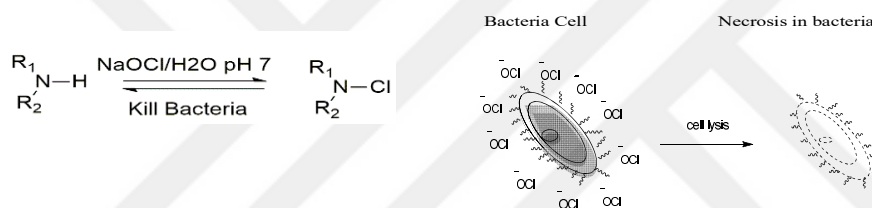


Figure 1.4: *N*-halamine and bacterial cell necrosis

The research on development of novel heterocyclic biocidal *N*-halamide/imide derivatives [66] is still a relatively new area of research. The covalently bonded N-X bonds have long-term stabilities in contact with aqueous solution, as well as in dry storage as compared to the acyclic (straight chain-based) *N*-halamine grafting at membranes [67-69]. The initial problem of such systems was water-soluble *N*-halamine, which was controlled by functionalizing insoluble polymers with *N*-halamine moieties [72]. In further reported work the improvement in attributes of temperature, pressure, and solvent resistance was achieved by using high performance membranes [46, 71] modified by grafting *N*-halamine. The repeated halogenation of such membranes over repeated cycles is also an advantage in making cost effective sustainable usage [65].

The controlled release of chlorine is essential, hence the polymeric membranes with the nitrogen group associated with halogen (*N*-halamine/amide/imide) became a

topic of interest with which the question of controlled release of disinfectant is actually solved by determining the number of workable cycles of a certain membrane and the log of bacteria killed during the bacterial challenge, thus had given the estimation about the quantity of water and bacteria versus the membrane work able cycle specifications.

During the filtration process the membrane durability, stability in solvents, higher temperatures and pressure is especially considered. Usually, the acyclic polymers are less stable than the cyclic ones. The presence of heterocyclic systems in polymers further enhance the stability, temperature resistance and mechanical properties [73]. The widely studied systems of norbornene or oxonorbornene derivatives are heterocyclic systems subjected to ring opening metathesis polymerization (ROMP). With these systems several desired modifications are possible and are reported in literature. Inherent chemistry in the monomer of such specialty polymers does not solely depends upon the reaction pathways but also on the choice of reactants. Diels Alder reaction is the very first reaction step in the monomer synthesis of norbornene and oxonorbornene systems. Diel's Alder reaction was discovered in 1928 by Otto Diels and his student Kurt Alder which in 1950 become the reason for their Nobel prize in Chemistry. The DA reaction has its uniqueness that it does not require any catalytic system to undergo the product formation which is a result of [4+2] cycloaddition reaction between a diene and a dienophile. Those reaction in which a heteroatom is part of diene, dienophile or both result in Hetero-Diels Alder reaction [73, 74] (**Figure 1.5**).

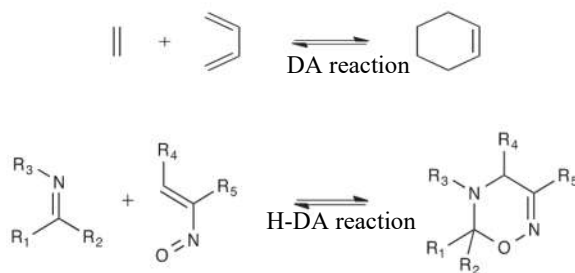


Figure 1.5: DA and H-DA reaction

Norborne and oxonorbornene carbic anhydrides are the example systems with first reaction being DA reaction. The product of DA reaction also governs an interesting stereochemistry as the product might be endo, exo or mixture of both. Such type of product is observed when cyclic dienes or dienophiles are involved. Another aspect of the presence of cyclic ring is a bridged product which governs the attribute of endo- or exo-orientation in the structure. In most cases, the endo-product is favored due to secondary orbital interactions (“endo-rule”). However, the ratio of endo to exo isomers can vary depending on whether the reaction is kinetically or thermodynamically controlled [74].

The active functionality which is present in ROMP monomer is its ring strain with unsaturation, while in the catalyst (transition-carbene complex) the transition metal and unsaturation of associated carbene thus easily do the ROMP with the energy which is enough to achieve the energy of activation for completion of reaction without any external stimuli like heating and pressure (**Figure 1.6**).

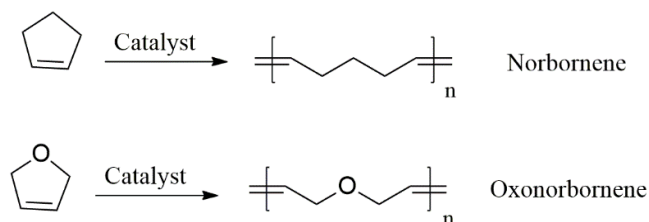


Figure 1.6: Systems of Norbornene and Oxonorbornene

The microstructure of polymers of norbornene and oxonorbornene derivatives depends on the type of connection between adjacent monomer units. There are different ways that the repeating units can be connected in the polymer chain. The double bonds in the structure may have *cis(c)-trans(t)* isomerism and the allylic methines on either side of the double bond may have the same chirality as racemic as syndiotactic (alternate arrangement) or with opposite chirality, giving meso (m) being isotactic (random) [74]. These polymers exhibit several unique chemical and physical properties, such as low water absorption, high thermal and chemical

corrosion resistance, high impact strength and resistance to crack propagation [75]. These polymers due to the unsaturation in their polymeric backbone are intrinsically hydrophobic and are least favorable for the microbial adhesions [76].

Cross linking is another competitive feature that is carried out with the availability of an extra functionality after polymerization, such as in dicyclopentadiene (DCPD). After ROMP the polymerizing chain having the functionality in each repeat unit is also repeating with each monomer unit. This system effectively produces the cross linking in the two growing polymeric chains with the facilitation of the same catalytic system [77] (**Figure 1.7**).

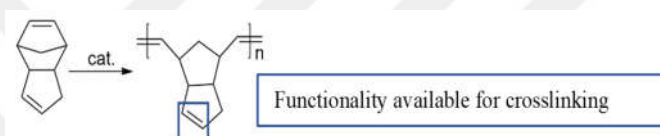


Figure 1.7: Crosslinking in ROMP

Polymerization of strained cyclic olefins with the help of a suitable catalyst system that carry out metathesis and polymerization at same time, while being stable from environmental effects, producing tacticity and stereo regularity is a likely definition of modern ring opening metathesis polymerization, as explained through it's mechanism (**Figure 1.8**). All the characteristics of ROMP that are achieved now are through a process of immense research which is done over the span of five decades [78].



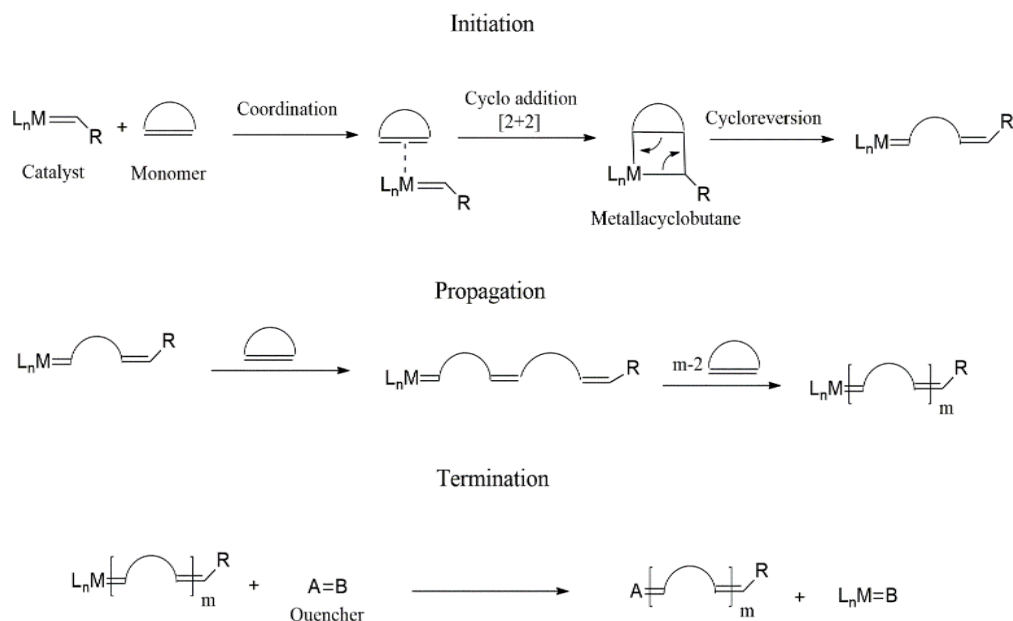


Figure 1.8: Mechanism of ROMP [79].

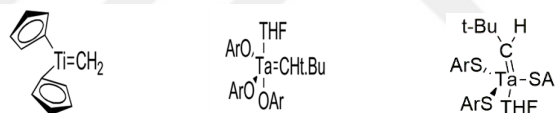
The ROMP technology allows the preparation of specialty polymers that are not accessible by other polymerization methods. The ROMP systems are living processes, due to their catalyst being the active center of monomer attachment. This provided efficacy in structure and molecular weight control, providing very low polydispersity index [80]. In addition, the development of new stable but very active catalysts, which are compatible with numerous functional groups, allows the polymerization of highly functionalized monomers. The importance of this polymerization technique is shown by the fact that it is used industrially to produce useful polymers such as polydicyclopentadiene, polycyclooctene and polynorbornene, polyoxonorbornene systems [78].

The first patent reported by Anderson and Merckling in 1955 for polymerization of norbornene using a catalyst, prepared in situ by mixing TiCl_4 with reducing agents such as Grignard reagents, metal alkyl or aryl, metal hydrides, alkali metals. However, the structure was elucidated in 1960 using IR revealing the ring opening metathesis polymerization. Until that time Lewis acid catalysts were used and were easily affected by air; thus, they were replaced by transition metal catalyst in

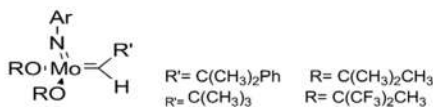
proceeding years of research. Typical systems being WCl_6 /EtOH/EtAlCl₂, WOCl_4 /Me₄Sn and MoCl_2 (NO)₂ L₂ /M₃ Al [78].

The polymerization carried out by these catalysts were done under inert atmosphere and the quenching of polymerization was done by exposing the system to air. Due to the presence of Lewis acids the side reactions along with ROMP were the major drawback of these classical catalysts [78,79].

The titanium-cyclo butane complexes and quasi-titanium carbene complexes were also studied for ROMP, lead to concept of “living ROMP”, depicting that the catalyst was the active site for attachment of coming up monomers and thus the termination of catalyst at the end of polymerization was required. The resultant polymers had good yield that also showed the narrow polydispersity for homopolymers only, while block copolymers did not had polydispersity with this system. The titanium-based catalyst was also less Lewis acidic as compared to the classical catalysts [78].



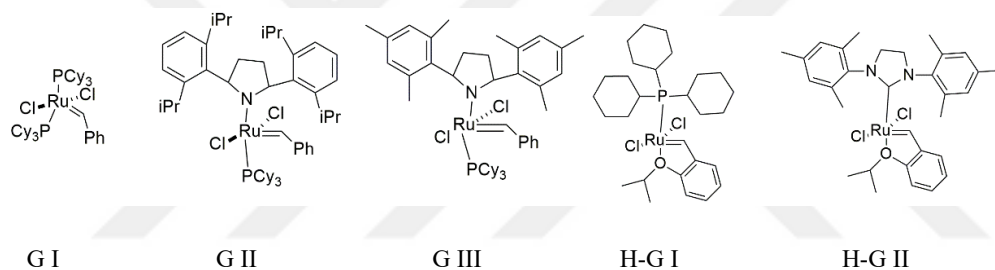
Taking inspiration from the titanium-based catalyst, the tantalum carbene complexes / tantalum alkylidene complexes were synthesized by Schrock and his coworkers. With this system monodisperse block copolymers were suitably synthesized [78].



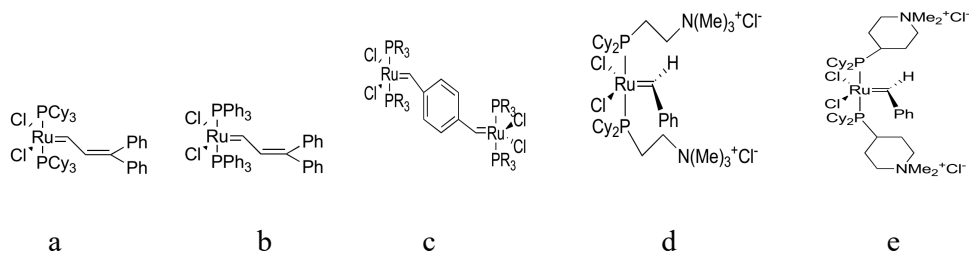
The research group of Schrock [79, 81] and coworkers also worked on molybdenum complexes known as new generation catalysts for ROMP. They successfully showed the ROMP in norbornene and norbornadiene derivatives bearing the example functional groups of ethers, esters, acetates, nitriles, halides, and acetals. With their work the insight in the steric effects and electronic properties were also revealed. The molybdenum catalyst was only a landmark in ROMP journey towards efficiency

as air and moisture resistance was still a question, although which had improved from the previous catalyst systems [78, 82].

In 1992 Grubbs and his coworkers synthesized ruthenium based vinylalkylidene complex for ROMP. This new catalyst was even able to polymerize the low strain cyclic olefins with good efficiency. It was also very stable in air, moisture, protic and acidic media, thus closing a chapter of usage of previous catalysts as researchers were more interested in modern carbene transition metal catalysts which had solved all the questions of stability. So far three generations of Grubbs catalyst had been introduced by Grubbs and his co-workers [83, 84], while Hoveyda-Grubbs are also popular class of catalysts in use [85].



While other ruthenium-carbene catalysts for ROMP are also synthesized and commercialized (**a-e**). Bimetallic Ruthenium catalyst (**c**) easily synthesized the tri block copolymer ABA. Further the water-soluble catalysts (**d,e**) were also developed for the synthesis of water-soluble monomers. These reactions accelerate with addition of HCl thus providing a reaction media absent in organic solvent and surfactant while providing low polydispersity product [78].



In classical catalyst systems the polymerization was inhibited till quenching, by exposing the reaction system to air. This exposure to air was effective to disable the Lewis acid catalyst while the modern systems, being stable in air, need a quencher to stop the “living polymerization”. Schrock used benzaldehyde as quencher [77], while other researchers have also used vinyl benzaldehyde [86] ethyl vinyl ether, etc. The polymer of ring opening metathesis is usually precipitated in toluene, methanol, chloroform, ether and ethanol depending upon each product’s solubility vs precipitation chemistry [77, 85].

After the stability and ease of reaction conditions achieved by researchers with minimal or no side product, ample of work had been reported by researchers showing versatile derivatives of norbornene / oxonorbornene with their potential properties and area of application (**Figure 1.9**).

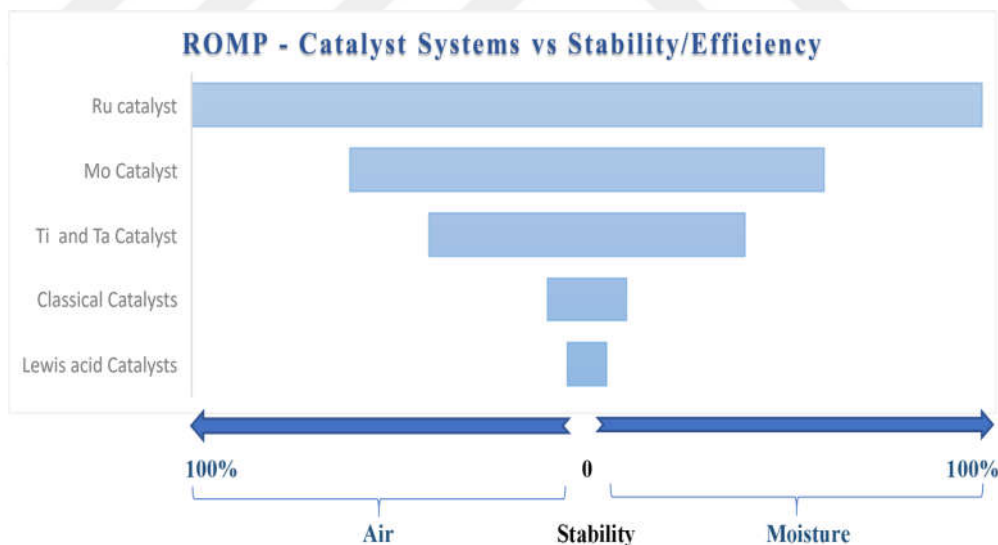
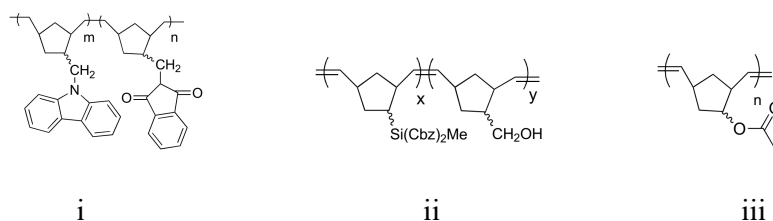
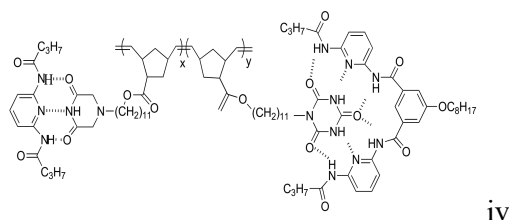


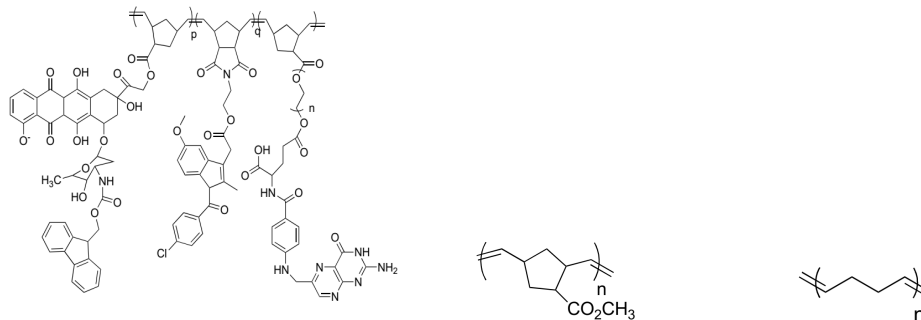
Figure 1.9: Graphical representation of stability/efficiency of transition metal catalyst for ROMP.

Using the G-I catalyst for norbornene polymers compound **i** was synthesized successfully as reported [82]. This di-block copolymer showed good stability in temperature range greater than 400 °C, while it also had good fluorescence properties and was soluble in wide range of solvents as well. The compound **ii** reported [74] as block copolymer of carbazole pendent norbornene polymer with narrow polydispersity and low molecular weight polymer. The reported copolymer showed the solubility in wide range of solvents where it had special solubility for chlorobenzene and chloroform. This solubility attribute had made it possible to use this polymer as glass coating via spin coating technique. While the crosslinking of the copolymer made it completely non soluble in the respective solvents, while on the other hand high temperature stability up till 300 °C was observed. The reported Compound **iii** [86] with series of other alkyne substituted norbornenes were polymerized using GI and II to see the polymer weight comparisons. It is reported that the range of 75 to 680 kDa of oligomers/ polymers are achieved by various used combinations and ratio of the catalyst and monomers.



The reported compound **iv** [87] is a copolymer exhibiting the self-assembling and self-sorting behavior. It was closely studied as an analogue to DNA strain in terms of stereoregularity, where the cyanuric acid and thiamine acid moieties self-assembling was studied. This compound has its potential application area in biomedical materials for drug delivery and as biomimetic material.



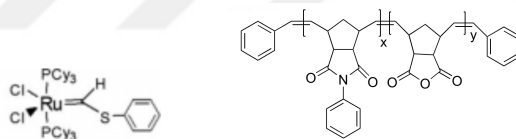


viii

ix-a

ix-b

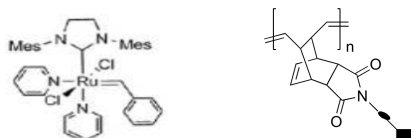
The reported [84] compound **x** was synthesized using the **catalyst-a**. The copolymer exhibited good optical transparency and was established to be used in optical devices while exhibiting good conducting behavior when deposited with Indium Tin Oxide (ITO), a potential electron carrier layer in organic light emitting devices.



catalyst-a

x

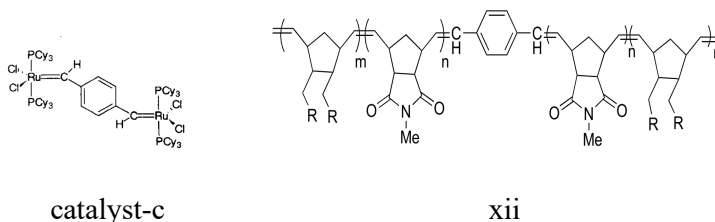
The compound **xi** was synthesized using the **catalyst-b** [93]. The structure **xi** is an example of certain gradient copolymer. With the sequential addition of the monomer the di-block polymer is produced, while the simultaneous addition of two monomers produced the dendron type homo polymer. The dendron homo polymer exhibited the rigidity and stiffness in the structure. This rigidity is maximum at the attachment point and reduces towards the end group towards the end of the chain length.



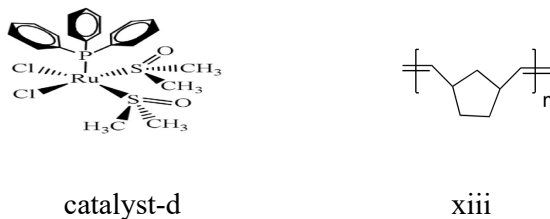
catalyst-b

xi

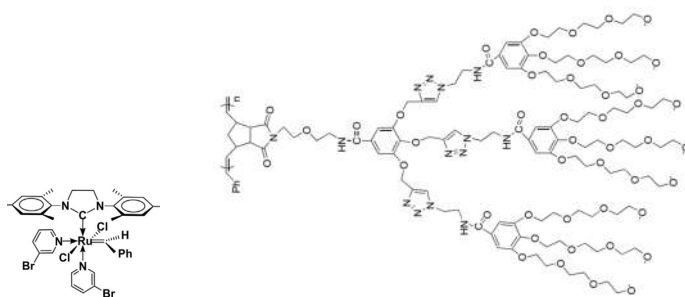
The compound **xii** was synthesized using the **catalyst-c** [94], as a triblock polymer. The research emphasized that the presence of bimetallic catalyst had facilitated the triblock copolymerization.



The compound **xiii** was synthesized from norbornene using the **catalyst-d** [95] establishing the successful polymerization in ethyl diazoacetate (EDA) solvent. It was reported that ROMP is dependent upon transition metal catalyst. If initiator is changed to ethyl 2-bromoisobutyrate, resultant product will be methyl methacrylate polymer P(MMA) and the mechanism of reaction will be Atomic chain Transfer Radical Polymerization (ATRP) while facilitating the acyclic acrylate product respectively.



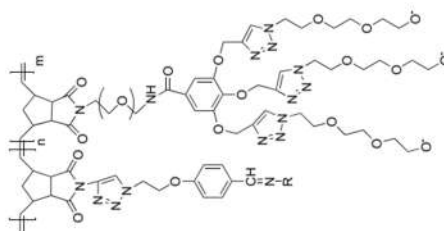
The reported compound **xiv** was synthesized using the **G-III-a** [96]. The dendronized norbornene system was reported to be very slow in conversion during the polymerization. The presence of 1,2,3-triazole-containing nanoreactors and the chains of PEG had provided the polymer to behave like self-assembly in neutral and ionic waters (one representative structure from four of such reported are presented here).



G-III-a

xiv

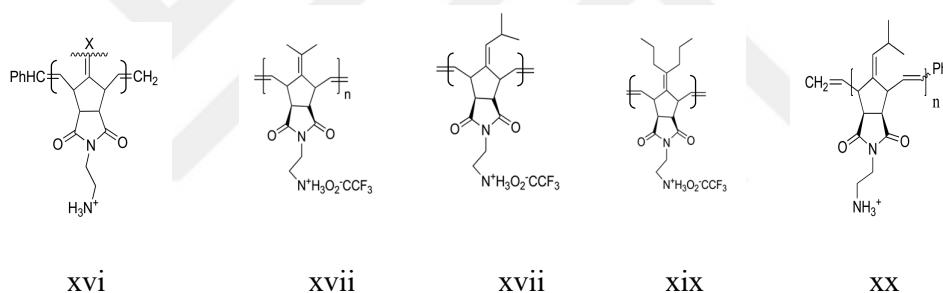
The compound **xv** reported in literature [97] is synthesized while using the **GIII-a** catalyst. This catalyst system exhibited successful ROMP while having dendrimers and conjugated copolymer structures. These were reported to be stable in aqueous and pH environment for drug delivery applications. Further at pH 2.5 to 4 the copolymer exhibits a micellar conformation and releases the O-benzylhydroxylamine (BHA), a drug conjugated with the dendrimer while being at neutral pH. Some other drugs release efficiency was also studied beside BHA, such as 1-hexadecanamine, tryptophan and benzocaine, using this copolymer system respectively.



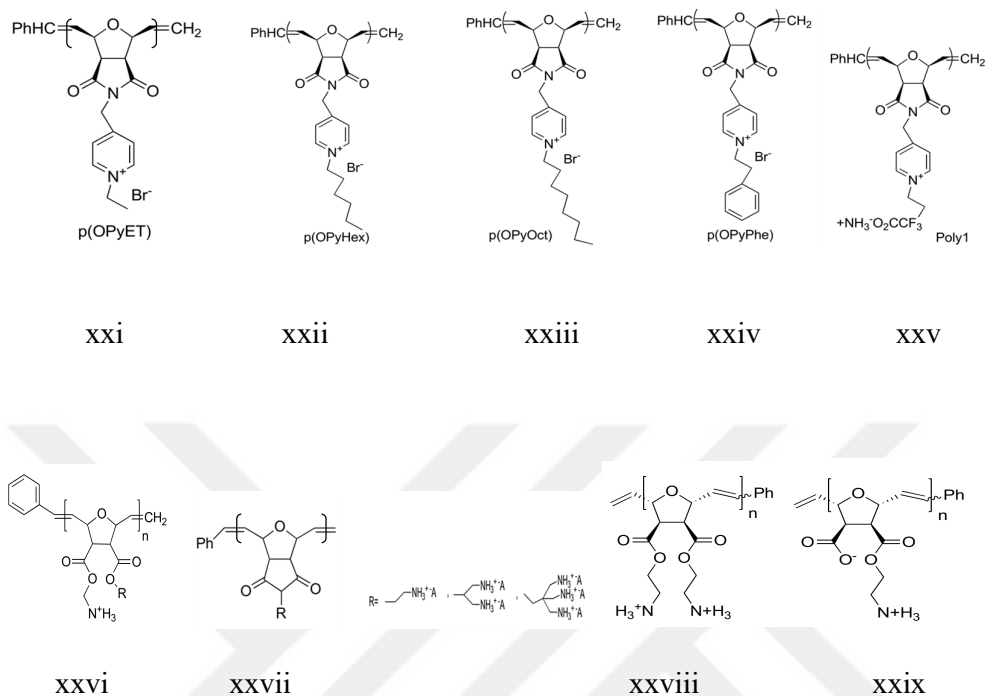
xv

ROMP is a very useful technique in polymerization reactions since it has established its benchmark in biomedical application and in conjugated polymer systems depending upon the type of grafting's being done to the norbornene or oxonorbornene systems as studied above. It is also established that one metal system being used now is ruthenium, either be of any type of catalyst system, the presence of Ru as a metal center is the potential new shape of ROMP based catalyst -polymers

being studied widely. Through ROMP one can add many functionalities as a side chain on the polymer. One such area of exploration in these systems are nitrogen bearing species as amine/amides/imides. Such systems are tunable to behave like antimicrobial when converting them into N-halamine/amide/imide. The literature study of ROMP based norbornene and oxonorbornene suggest that there are few examples as coating in biomedical applications but not as membranes. Antimicrobial norbornene and oxonorbornene polymers are widely used in biomedical applications where the biofilm prevention is the special focus. The mimicking peptides compound **xvi** [98], compound **xvii-xix** [99] and compound **xx** [100] are reported with antimicrobial property as material coatings of poly norbornene zwitterions.



The oxonorbornene based reported compounds **xxi-xxv** [54], **xxvi** [101] and **xxvii** [102], **xxviii** and **xxix** [103] zwitterions are among such examples. The antimicrobial efficacy of such polymers is vastly related to the repulsion of the bacteria and compatibility with human immune system in case of implants; are the major success of the norbornene and oxonorbornene antimicrobial polymers; ready to be used for biomedical applications so far [76].



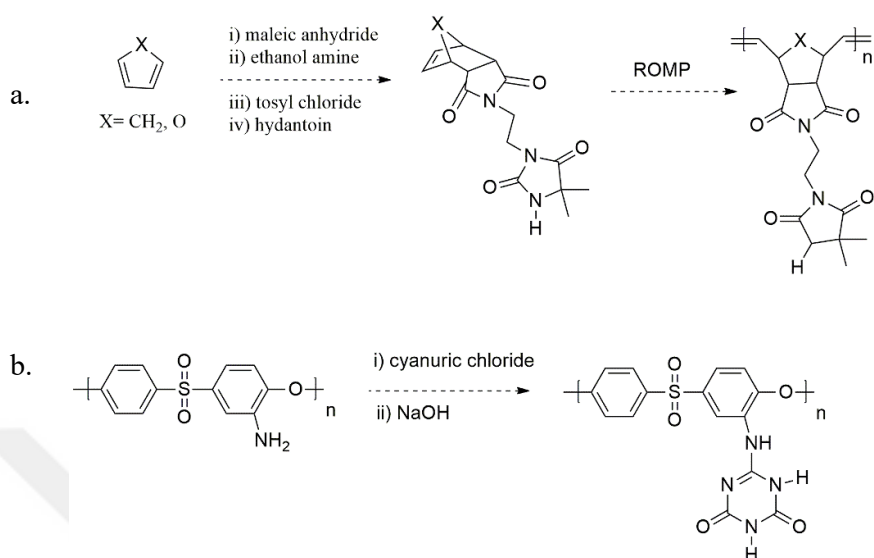
Upon compilation of literature study of these antimicrobial membranes polymerized through ROMP and the literature study on the modifications in industrially vogue membrane materials; presented in the **Table 1.2**; all these example polymers membranes and surfaces were used in UF and NF applications. Such reported work highlights the importance and need of antimicrobial membranes, nano particles, and coatings for ensuring a healthy living environment for human beings.

CHAPTER 2

AIM OF STUDY

All membrane materials, over the time for water-based applications, face the problem of biofouling. To overcome this problem, previously, our group modified PES [69], and studied its antimicrobial properties. In that study, hydrophobic PES became sufficiently hydrophilic after grafting of amine onto the polymer. Even though modified PES gave significant results in inhibiting the bacterial growth, it did not retain the oxidative chlorine long enough due to acyclic amine precursor. The derived target from that work is to fabricate membrane with cyclic *N*-halamine precursor, which could retain oxidative chlorine longer as it releases the chlorine in a controlled manner by preventing biofouling.

A cyclic amine/amide/imide precursor was needed for long term antimicrobial performance. Such high-performance polymers with antimicrobial properties could be synthesized by ring opening metathesis polymerization (ROMP) reaction. In this study, hydantoin moiety and cyanuric acid were installed onto novel monomers and polymer of PES. The hydantoin containing polymers were synthesized through ROMP while cyanuric acid grafted PES was obtained through modifications in amino-PES as summarized in **Scheme 1**.



Scheme 1: a) Synthesis of novel monomer and polymer, b) modification of PES.

With the polymers in hand, membranes were fabricated. After chlorination of the amide/imide moieties, the membranes were challenged against Gram positive and Gram-negative bacteria to access their antifouling effects. Moreover, classical membrane properties were also tested. The work done is summarized in **Figure 2.1**.

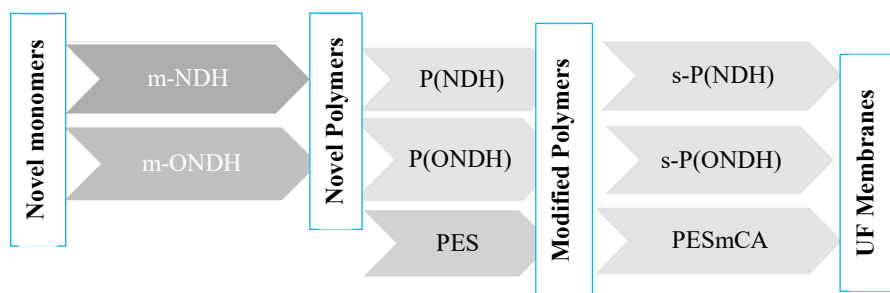


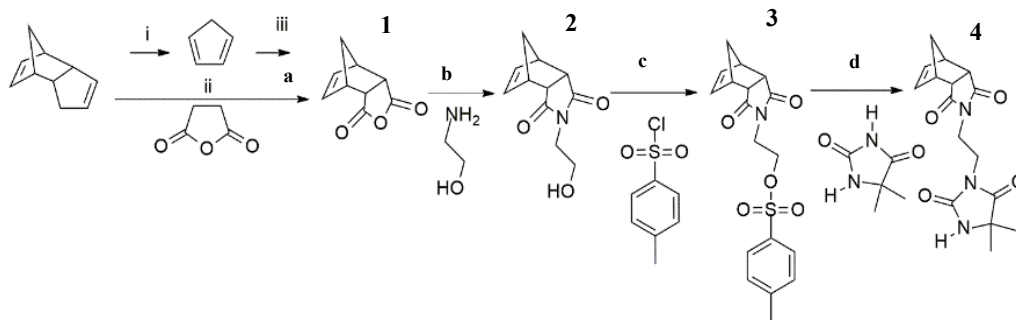
Figure 2.1: Flow chart of present work: Synthesis, Modifications, and Application.

CHAPTER 3

RESULTS AND DISCUSSION

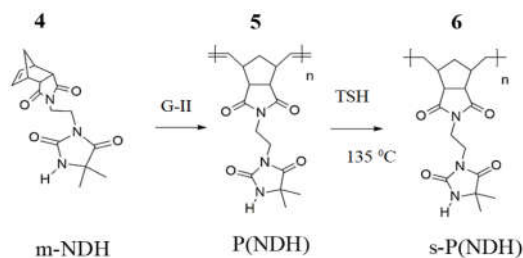
3.1 Synthesis of Novel Polymers

Synthesis of monomer **4** was accomplished through Diels Alder (DA) reaction. Dicyclopentadiene dimer was cracked at 138 °C, and cyclopentadiene was collected at -78 °C using acetone and dry ice to prevent a self DA reaction. Collected cyclopentadiene was stirred with dissolved maleic anhydride in 1:1 hexane and ethyl acetate solution. This stirring was performed in ice bath to get norbornene anhydride **1**. Compound **1** was treated with ethanolamine in toluene using Dean-Stark apparatus. In the reaction, when the system is made inert before refluxing, better yield of product **2** is observed. Compound **2** was tosylated using *p*-toluene sulphonyl chloride (TsCl) in presence of pyridine in DCM used as solvent. The amount of TsCl used was equimolar, to give better results in terms of yields and simplicity of workup procedure. It was noted that workup of product **3** using CuSO₄ solution had yield of 50%, while workup using 3 % HCl instead of CuSO₄ to remove pyridine had increased the product yield to *ca.* 78%. The tosylated compound **3** was treated with 5,5-dimethylhydantoin for an S_N2 reaction in the presence of a base, DBU. This reaction provided pure product **4** when reaction was conducted in DMF; which was suitably removed using high vacuum. Alternate solvent of this reaction was acetone (due to the high boiling point of DMF), which also provided easy recovery of final product at nominal vacuum but extensive work up for removal of *p*-toluene sulfonic acid by product was required. The reactions for synthesis of compound **4** are summarized in **Scheme 2**.



Scheme 2: (a) Diels Alder reaction, (i) DCPD cracking at 138 °C, (ii) receiving CPD at -78 °C (using acetone dry ice), (iii) Maleic anhydride, Hexane, ethyl acetate) stirring until product precipitated as white solid. (b). Ethanolamine, Toluene, Dean Stark reflux. 7h. (c) *p*-toluene sulphonyl chloride, DCM, Pyridine. Stirring >24h at room temp. (d) 5,5-dimethyl Hydantoin, DBU, DMF/Acetone, stirring for two hours then reflux 5h.

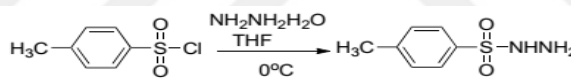
With compound **4** in hand, it was subjected to polymerization via ROMP. ROMP was carried out with Grubb's second-generation (G-II) catalyst in DCM. The previous attempts with Grubbs' first generation catalyst for similar system did not furnish polymers [104]. Therefore, G-II was employed in this study. The catalyst to monomer ratio was found to be crucial for the reaction (*vide infra*). The reaction was ceased using ethyl vinyl ether, there the polymers were capped with ethoxy group. The polymer was recovered by precipitating in diethyl ether. **Scheme 3** shows the sequence of these reactions.



Scheme 3: a) m-NDH/G-II [M/C 250/1] in DCM, quenching by ethyl vinyl ether. b) saturation of P(NDH) in DMF using *p*-toluene sulfonyl hydrazide, refluxing at 135 °C 5-7h.

In order to have one site available for halogenation in polymer P(NDH), the saturation of olefinic bonds was done since the double bonds can also react with hypochlorous acid to form chlorohydrins. Classical reactions of double bonds are done in alcohols. However, the polymer P(NDH) was not soluble in ethanol. It was further seen that it is also not soluble in methanol, 1-propanol, and 1-butanol. Nevertheless, The hydrogenation experiment with H₂ in the presence of Pd/C was performed, and was unsuccessful (**Table 3.1**). Therefore, *p*-toluenesulfonyl hydrazide (TSH) was employed to reduce **P(NDH)** successfully in DMF to **s-P(NDH)**.

Toluenesulfonyl Hydrazide (TSH): TSH reagent was synthesized from TsCl while stirring it at 0°C with hydrazine monohydrate in THF following a reported procedure [105] (**Scheme 4**). It was characterized using IR (Appendix C).

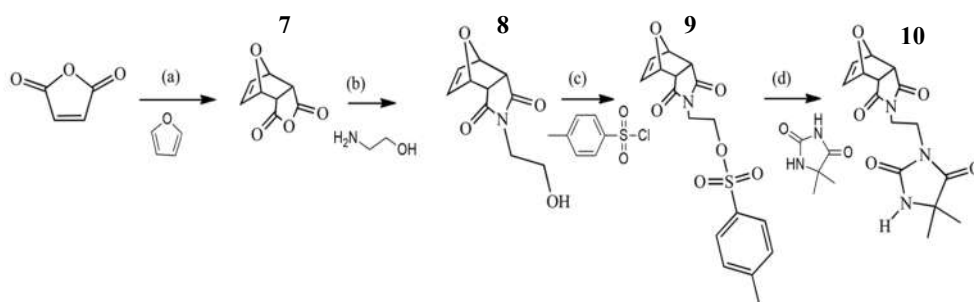


Scheme 4: Synthesis of *p*-toluenesulfonyl hydrazide

Table 3.1: *Experiments for saturation of polymer*

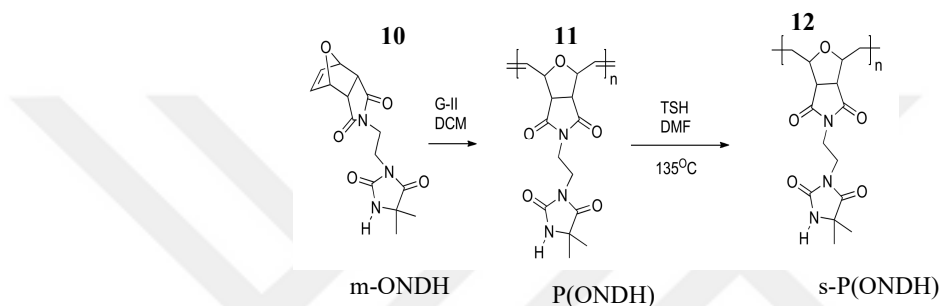
Solvent	Condition	Inference
Methanol / ethanol / 1-propanol / 1-butanol	Pd-Charcoal, Stirring at room temp, H ₂ inert atm.	No saturation
O-Xylene	TSH, Reflux	Partial saturation
DMF	TSH, Reflux	Complete saturation

The second novel monomer **10** was oxonorbornene dicarboximide hydantoin m-ONDH, which was synthesized and further polymerized using ring opening metathesis polymerization reaction. The synthesis started with DA reaction between furan and maleic anhydride. Product oxonorbornene **7** was obtained by stirring the reactants at ambient condition in the presence of toluene, the product precipitated as white solid. In the next step, **7** was treated with ethanol amine in presence of ethanol to produce oxonorbornene dicarboximide -2-ethanol (**8**). Product **8** was obtained with yield of 92% when the system was made inert before the addition of ethanolamine. The Dean Stark reflux was not possible since compound **7** was not soluble in toluene. The obtained product was re-dissolved in DCM and washed with water through extraction. Product was vacuum dried and was further treated with equimolar amount of *p*-toluene sulfonyl chloride in presence of pyridine as base, DCM as solvent, and stirred for 2-3 days at ambient conditions. Its reaction completion was monitored via TLC. The 5% HCl workup procedure was followed to get the **9**. The compound **9** was dissolved in acetone and DBU was added as base to conduct S_N2 reaction. The nucleophile 5,5-dimethyl hydantoin was introduced to reaction mixture. It was stirred at ambient conditions for 36 hours or refluxed for 7 hours. The two different reaction conditions provided similar results (**Scheme 5**).



Scheme 5: (a) Diels Alder reaction: MA and Furan, stirring 2h. (b). Ethanolamine, ethanol, reflux 24h. (c) *p*-toluene sulphonyl chloride, DCM, pyridine, stirring at room temp for >24 h. (d) 5,5-dimethyl Hydantoin, DBU, Acetone, stirring / refluxing for 7h.

Monomer **10** was polymerized using the Grubbs-II catalyst, following the same procedure of polymerization and saturation as done for P(NDH), in the presence of DCM and inert media to get polymer P(ONDH) (**Scheme 6**). Further the polymer was saturated using *p*-toluene sulfonyl hydrazide (TSH) to get saturated polymer s-P(ONDH).



Scheme 6: a) m-ONDH/G-II [M/C 250/1] in DCM, quenching by ethyl vinyl ether. b) saturation of P(ONDH) in DMF using *p*-toluene sulfonyl hydrazide, refluxing at 135 °C 5-7h.

3.1.1 Catalyst to Monomer Ratio

Monomers **4** and **10** were polymerized using variable amounts of G-II catalyst. The ratio of 1/500 catalyst/monomer was used. This ratio provided the successful polymerization, but the polymer did not form into membranes. Then, catalyst/monomer ratio changed to 1/250, this provided a polymer with the essential strength for the polymer to fabricate into a successful membrane. It was observed that the polymer obtained from 1/500 ratio was like a viscous material at room temperature. This is probably due to their low molecular weight on the contrary to our expectations. This might be due to the fact that all catalyst molecules not being active. While the polymer from 1:250 ratio was a solid at room temperature. All the characterizations and membrane fabrications were done using this concentration. It

was observed that the polymers were not soluble in THF. For molecular weight determination SLS technique was used, being soluble in DMSO (*vide infra*).

3.1.2 Recovery of Quenched Catalyst

In ROMP systems the ruthenium carbene catalyst (G-II in this research) during reaction go through double decomposition because of effective ring strain that is available in the norbornene and oxonorbornene systems.

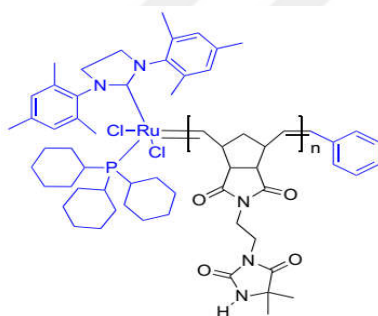


Figure 3.1: Assumed structure during polymerization

The catalyst forms a connectivity with the monomer in such a way that one end of polymer is sealed by one of its decomposed parts: for G-II being phenyl group (**Figure 3.1**). While the other end of monomer is the active center (living polymerization) of connectivity for coming up monomer due to the presence of Ru carbene complex. For this reason, a quencher is required which could perform the dual decomposition just like catalyst, to provide the end capping to both Ru carbene complex and to the monomer chain undergoing polymerization. In this study during one of the polymerization reactions quencher was used in excess. It was observed that the polymer precipitated without any other solvent as white polymer free from Ru complex. Further after the filtration of polymer, Ru complex is recoverable from the filtrate through vacuum evaporation that has DCM and little amount of remaining

quencher only. Hence easy recovery of Ru complex is possible which is a deactivated initiator only not a catalyst (**Figure 3.2**).

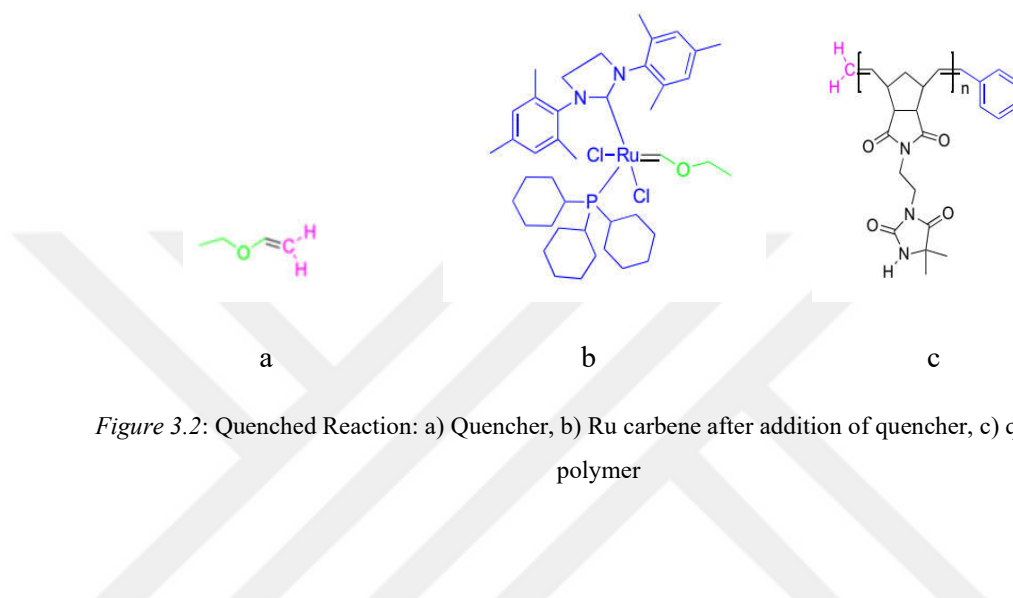
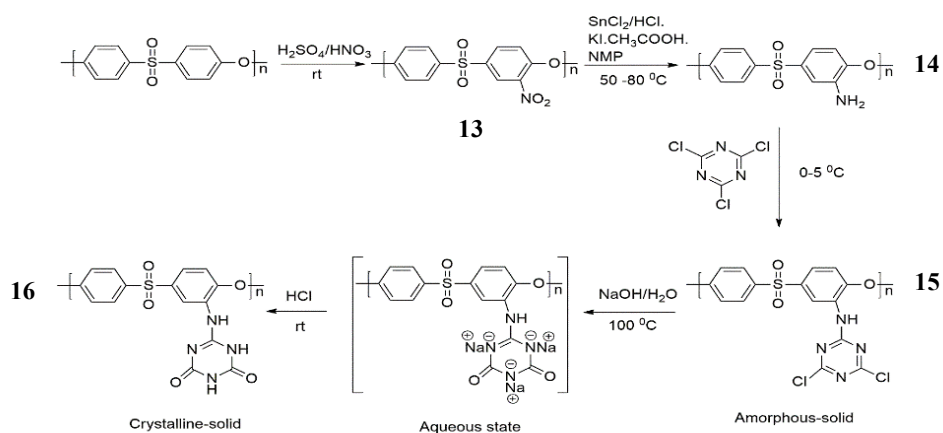


Figure 3.2: Quenched Reaction: a) Quencher, b) Ru carbene after addition of quencher, c) quenched polymer

3.2 Modification of polyether sulfone (PES)

The high-performance polymer PES was grafted with cyclic amide for antimicrobial studies. PES was nitrated in the presence of sulphuric acid and nitric acid. This stirring media turned PES into semi solid gel, and nitrated product **13** was obtained after dilution with water. The nitroso group was further reduced to amine using the methanol or NMP as solvent. Stannous chloride was added as catalyst to facilitate reduction by protons from acetic acid, forming acetate ion in presence of KI and HCl. Complete reduction was seen with NMP, as **13** was fully soluble in it. Product **14** was precipitated after alkalinity, to reduce the acidified effects of reaction medium. The cyanuric chloride which is a six membered cyclic chlor-amide was then grafted to the amine of PES while stirring it in NMP at temperature of 0-5 °C. The product **15** was obtained by precipitation in water, providing fine powder when dried. This PES amine cyanuric chloride **15** was further modified into PES amine cyanuric acid **16** with acidification, making it N-amide available for the halogenation (**Scheme 7**).



Scheme 7: Modification of PES: i) stirring for 7 days at room temperature, ii) reduction catalyst SnCl_2 facilitated by addition of KI and proton donating group of acetic acid, full conversion due to NMP solvent. iii) cyanuric chloride grafting to primary amine of PES, by product being HCl, polymer precipitated in water iv) refluxing in sodium hydroxide solution at 100 °C, intermediate aqueous state, upon acidification precipitated product PES-NH-CA or PES-modified-Cyanuric acid (PESmCA) obtained as crystalline solid.

3.3 Characterizations of monomers and polymers

Compounds **4** and **10** were fully characterized with ^1H NMR, ^{13}C NMR, IR and HRMS. The polymers were characterized with ^1H NMR, ^{13}C NMR, and IR. ^1H NMR peaks were significantly different than the monomer as expected. The signals broadened in the ^1H NMR of polymers. The ^{13}C NMR of the polymer is different than that of the monomer. After reduction of olefinic bonds of polymer P(NDH), the disappearance of olefinic protons was noted (**Appendix C**). For P(ONDH) similar pattern was observed. The modified PES (PESmCA) polymer was mainly characterized with IR. The nitrated PES showed a peak at 1530 cm^{-1} which corresponds to nitro group. The PES- NO_2 showed the disappearance of NO_2 peak and appearance of NH_2 around 3300 cm^{-1} . Addition of cyanuric chloride and its conversion to cyanuric acid was proved by appearance of a peak around 1742 cm^{-1} .

The molecular weights of P(NDH) and P(ONDH) were measured with static light scattering (SLS) in DMSO (**Table 3.2**). It was seen that the molecular weight of P(NDH) was around 1800kDa. When it was reduced to s-P(NDH) its molecular weight also reduced to 540kDa. Initially, it was thought that *p*-toluenesulfonyl hydrazide reacted with the polymer chain to disintegrate the polymer. However, this is probably due to rigid polymer P(NDH) forming aggregates in DMSO. When double bonds are reduced the conformational space gets bigger and solvation increases. In turn, aggregates are broken. Therefore, the saturated P(NDH) molecular weight is more reliable. For the polymer P(ONDH) the molecular weight obtained was 148 kDa. For s-P(ONDH) molecular weights were not determined due to its hazy solution in DMSO.

Table 3.2: SLS characterizations of homo polymers

	P(NDH)	s-P(NDH)	P(ONDH)	s-P(ONDH)
M_w (g/mol)	1869000	541500	148200	Solubility problem

The TG analysis of polymer P(NDH) showed beginning of degradation at ~ 419 °C and all of it degraded at the temperature around 500 °C as one component. The residual weight was noted to be 14% at the temperature of ~ 530 °C. The polymer s-P(NDH) showed the beginning of degradation at ~ 450 °C and all the polymer degraded at ~ 510 °C as a single component. The polymer P(ONDH) showed a thermal event at ca 60 °C with 1.1 % weight loss which is of residual solvent. The decomposition of 3.1 % started at temperature of 427 °C, further to this it was quite stable at the temperature of 550 °C with the total weight loss of only 3.1 %. In s-P(ONDH) the weight loss started from 50 to 60 °C till 400 °C with weight loss of 2 % as a solvent evaporation. Then after 400 °C fast decomposition was observed. The polymer degraded as one component. The final temperature observed was 480 °C at 15% of residual weight.

DTA analysis shows that the polymer P(NDH) start to decompose at ca 400 °C. When it is reduced to s-P(NDH) the decomposition temperature increased to 420 °C. This shows that the double bonds are more reactive than the single bonds as expected. For P(ONDH) the decomposition temperature at 400 °C did not meet the total decomposition event even after 500 °C. While like the previous saturated polymer, the s-P(ONDH) decomposition temperature increased. These results show that the decomposition of unsaturated polymers begin from the double bonds. The results are compiled in table with the DSC of monomer presented in **Appendix C**.

Table 3.3: Thermal events in monomer and polymers of norbornene/oxonorbornene dicarboximide hydantoin.

Component	T _s (°C) ^a	T _d (°C) ^b
m-NDH	250	i. 250-309 ii. 309-500
P(NDH)	400	400-500
s-P(NDH)	450	450-510
m-ONDH	175	i. 175-350 ii. 350-525
P(ONDH)	550	427-550 3% only
s-P(ONDH)	425	425-480

a. TG/DTA: Temperature of stability.

b. TG/DTA: Decomposition temperatures of components.

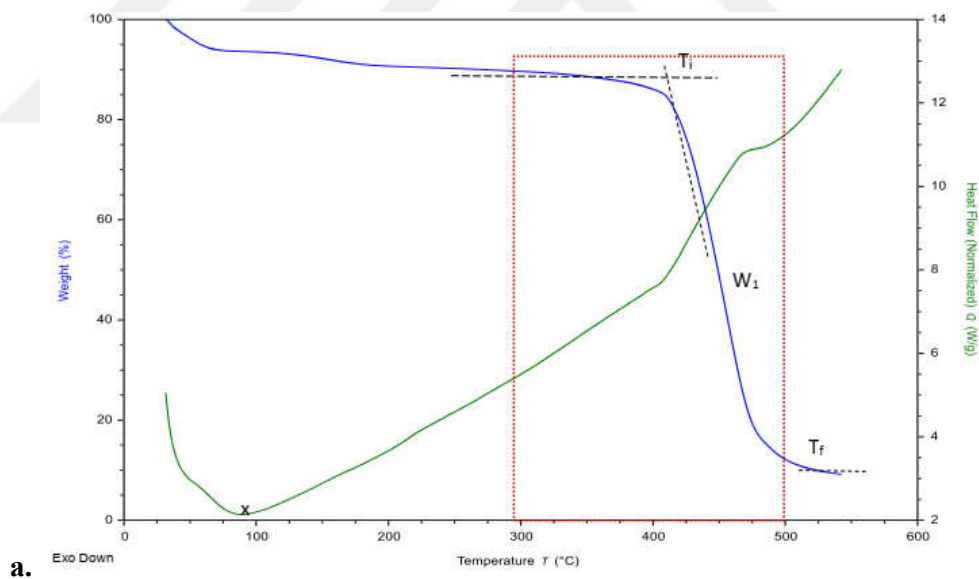
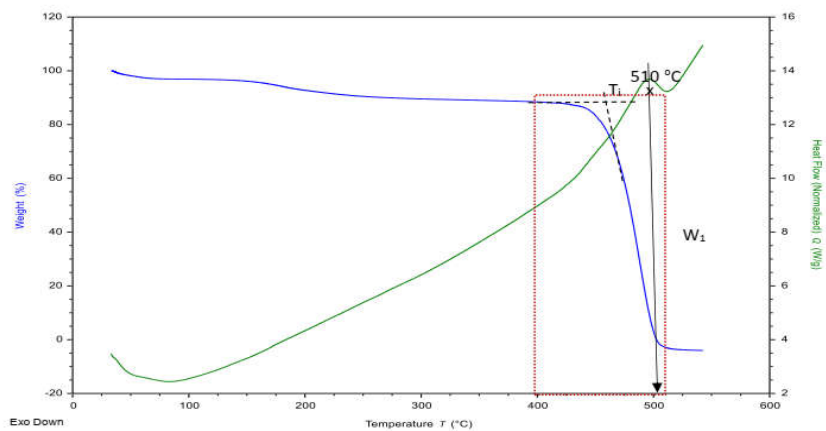
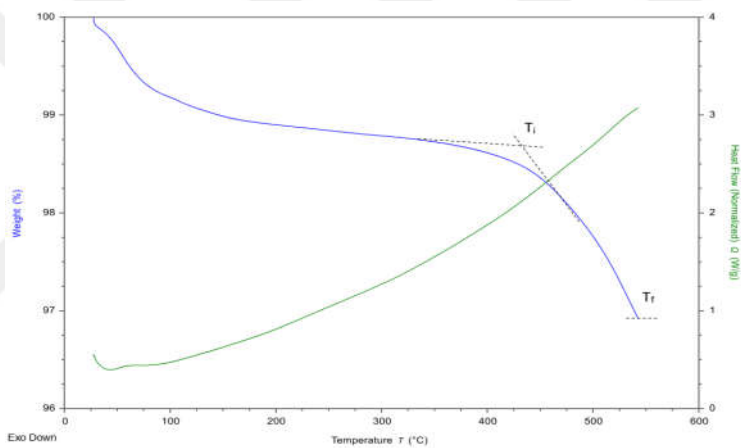


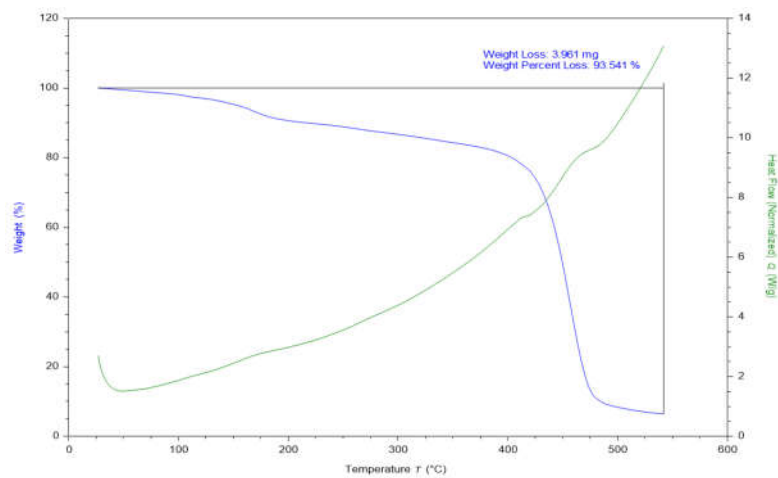
Figure 3.3 TG/DTA of homopolymer a) P(NDH)



a.



b.



c.

Figure 3.4. TG/DTA of homopolymers **a)** s-P(NDH), **b)** P(ONDH) and **c)** s-P(ONDH)

The modification of PES to PESmCA was performed through sequence of reactions. The modifications were characterized using IR. Initially PES was nitrated using general nitration procedure using sulphuric acid/nitric acid mixture. The nitration of PES was confirmed with the peak of 1531 cm^{-1} , which is the characteristic IR peak for nitration. The nitro group was further reduced using stannous chloride in presence of acid. The reduction was fully achieved as respective nitro peak disappeared and a broad peak of amine was observed in the IR around 3100 cm^{-1} . The amino PES was treated with cyanuric chloride in NMP at temperature interval of $0\text{--}5\text{ }^{\circ}\text{C}$. The IR spectrum showed the respective IR peak. The conversion of cyanuric chloride cyanuric acid (PESmCA) was carried out by refluxing it in NaOH. The IR peak of 1742 cm^{-1} showed the presence of carbonyl bond in the spectrum. The IR spectrum are stacked in **Figure 3.5**.

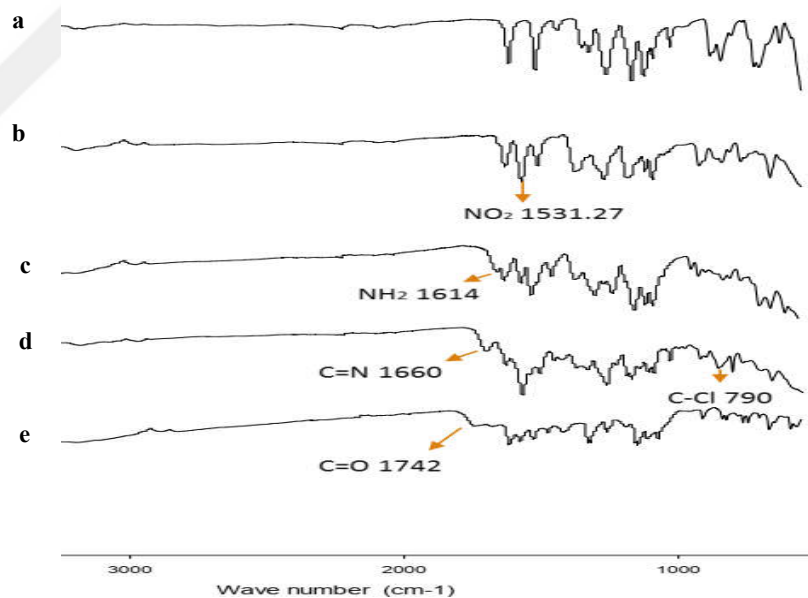


Figure 3.5: ATR-IR of modified PES: **a)** PES, **b)** PES-NO₂, **c)** PES-NH₂, **d)** PES-NH-Cyanuric chloride, **e)** PES-NH-Cyanuric Acid (PESmCA).

The morphology of bulk polymers **15** and **16** were further confirmed through the SEM characterization. It was seen in the micrograph of **15** that it comprises of nanosized pores. It was seen that **15** was amorphous while **16** is ordered due to the hydrogen bonding (**Figure 3.6**). This could be explained by the fact that cyanuric acid forms interchain hydrogen bonds and become well-arranged structure (**Figure 3.7**). The comparative XRD of compound **15** and **16** is presented in (**Appendix C**).

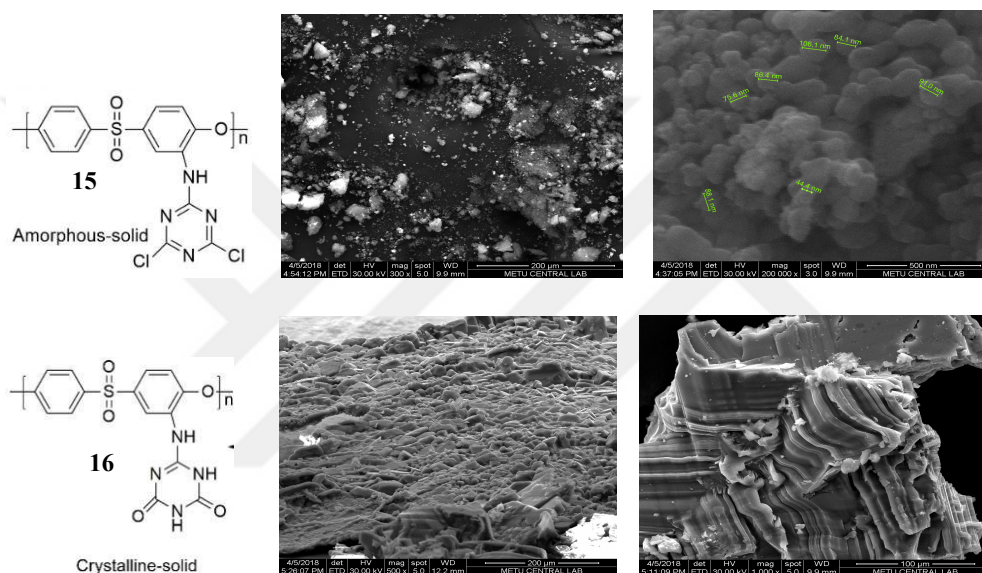


Figure 3.6: SEM characterization of compound **15** and **16**

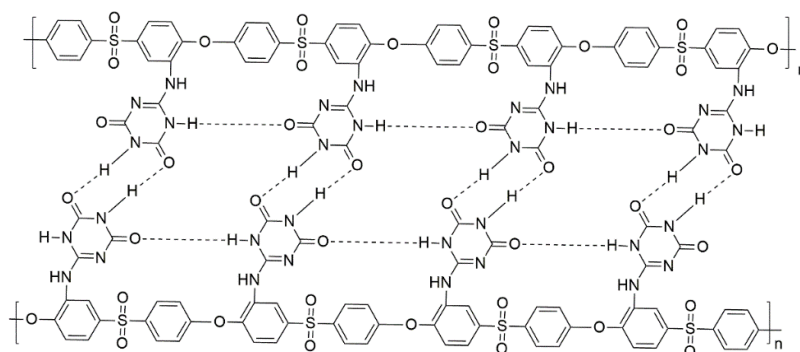


Figure 3.7: Hypothetical ordered structure of compound **16**.

3.4 Membranes from unsaturated/saturated homopolymers and blend polymers

The unsaturated polymers membranes were fabricated initially with 15%, 25% and 35% compositions of P(NDH) in DMSO. The membranes of 15% and 25% composition provided successful membrane coagulation, while the 35% composition did not coagulate with numerous trials. Following the same procedure, the casting solutions of P(ONDH) did not coagulate into membrane except 5% solution providing a membrane of low flexural strength as determined manually. Further 10% and 20% compositions were also tried choosing the variable substrate of cellulose fiber and plastic other than glass tab, the material behaved like coating rather than a membrane on each type of surface. The saturated polymer s-P(NDH) with 20% composition was successfully fabricated into membrane. While the s-P(ONDH) 20% did not coagulated into membrane, rather it stayed at the substrate like a coating even after more than 7 days of immersion in water. The structures of polymers tested for membrane fabrication are presented in **Figure 3.8**.

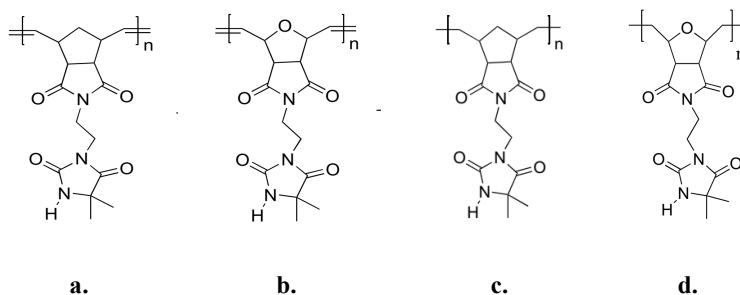


Figure 3.8: Novel Polymers **a)** P(NDH), **b)** P(ONDH), **c)** s-P(NDH), **d)** s-P(ONDH).

The s-P(ONDH) was blended with the PES to prepare the blend membrane and for comparison the s-P(NDH) and modified PES was also blended with the PES. The PES and the pure polymer were blended with 3:1 composition and 20% weighted casting solution was prepared using DMSO. The blend solutions were PES:s-P(NDH), PES:s-P(ONDH) and PES:PESmCA, successfully fabricated into respective membranes (**Figure 3.9**). Blend Composition of 1:1 PES:s-P(ONDH) was also prepared but contrary to above it was not a well-mixed system provided the membrane with textured pattern. The membranes from blend ratio 3:1 were used in further characterizations and bacterial challenge experiments.

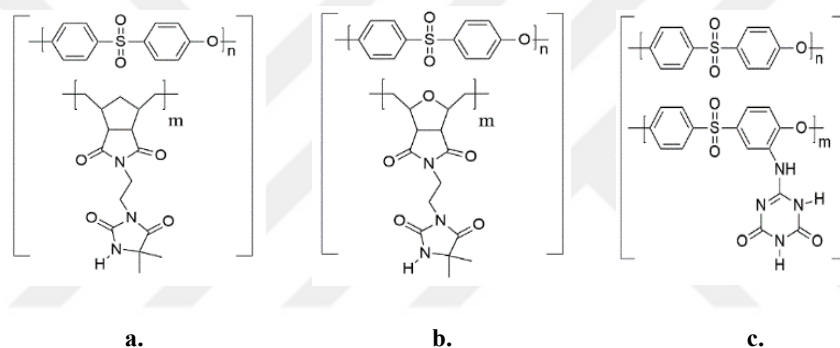


Figure 3.9: Blend Polymers: **a)** PES:s-P(NDH), **b)** PES:s-P(ONDH), **c)** PES:PESmCA.

3.4.1 Characterization of blend membranes

The blend membranes were characterized using IR, while TGA was conducted to see if the polymers were well mixed composition through their decomposition pattern.

The ATR-IR showed the representative peaks of PES between 1500-1600 cm^{-1} while the carbonyl groups of the s-P(ONDH), s-P(NDH) and PESmCA were seen ca. 1600 to 1700 cm^{-1} in all the three blend compositions. Imide bond was also present between 2100 to 1900 cm^{-1} in each IR spectrum. Presented in **Figure 3.10** are the ATR-IR of the blend polymer membranes.

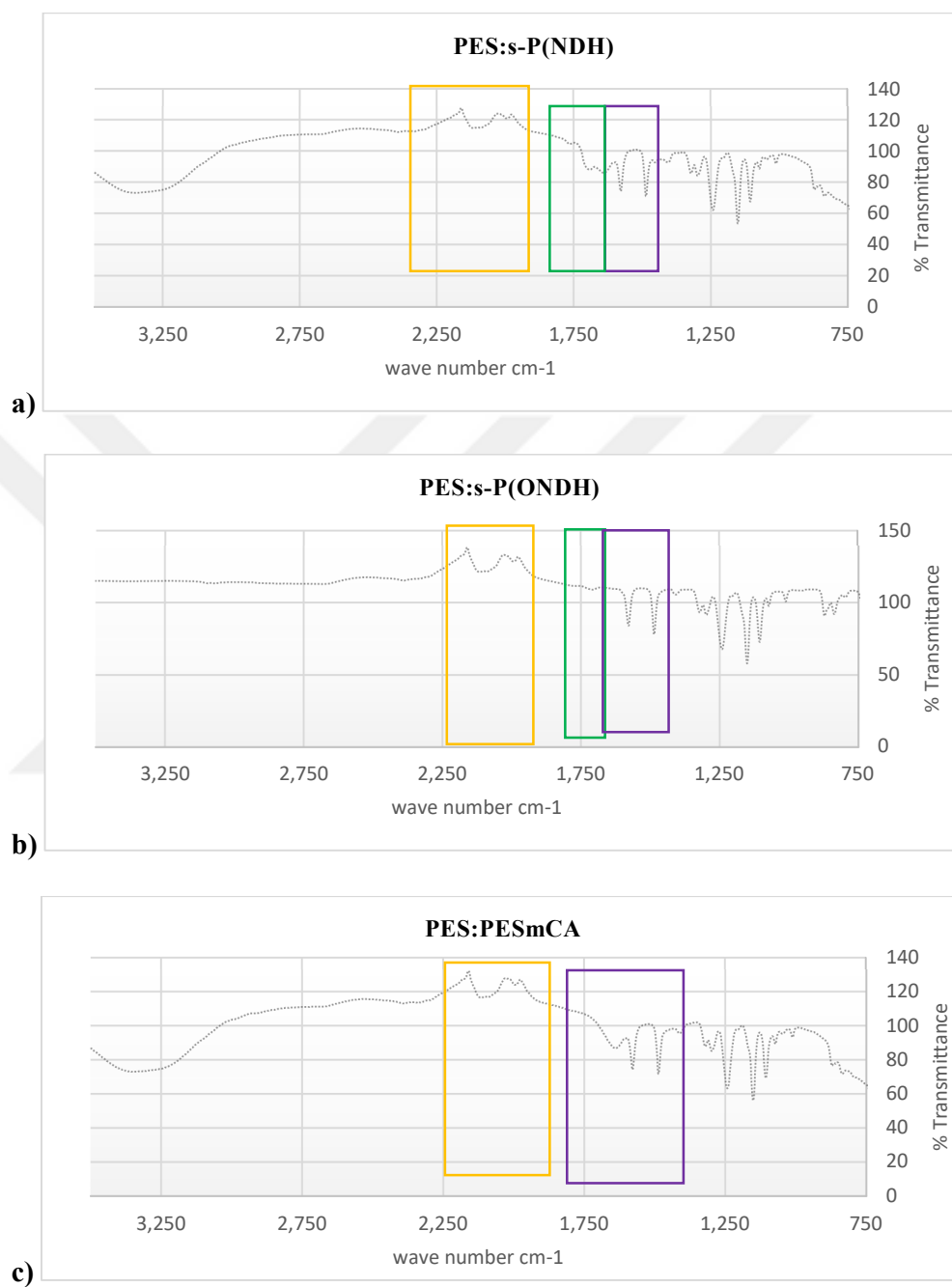


Figure 3.10: ATR-IR of Blend Membranes : **a)** PES:s-P(NDH), **b)** PES:s-P(ONDH) and **c)** PES:PESmCA

The thermal characterization of blend polymer membranes was conducted through the TGA analysis and their derivative TGA were calculated. The decomposition temperatures of blend membranes PES:s-P(NDH), PES:s-P(ONDH) and PES:PESmCA were ca. 475, 510 and 540 °C respectively. All the three blends decomposed in one step degradation. In all the three blend polymer membranes the residual solvent (DMSO) entrapped in pores of membranes was observed between the temperature range of 100-200 °C. The decomposition temperature of PES polymer was seen at 510 °C. **Figure 3.11a** and **3.11b** represents the TGA and derivative TGA of blend polymer membranes.

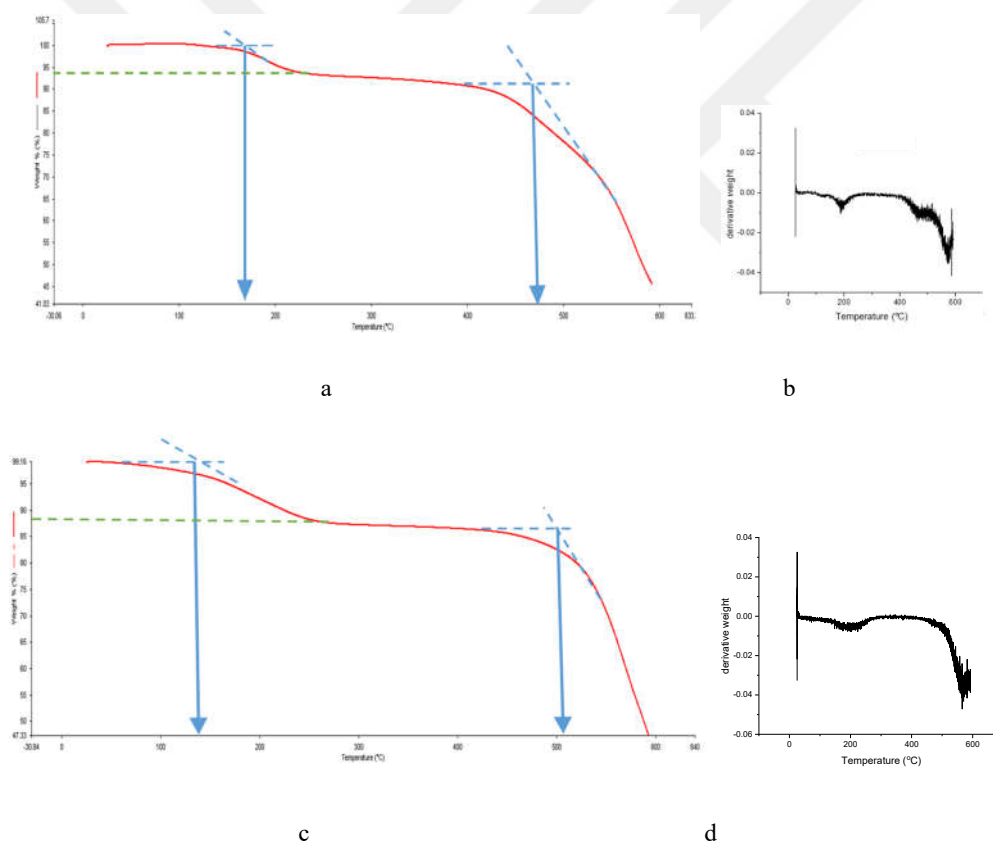
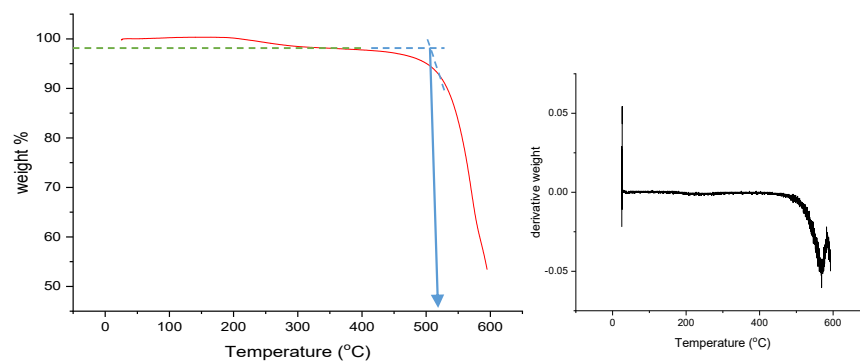
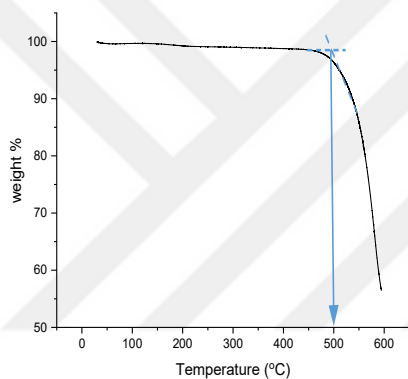


Figure 3.11-a: TGA and derivative TG of blend PES:s-P(NDH) (a,b); PES:s-P(ONDH) (c,d).

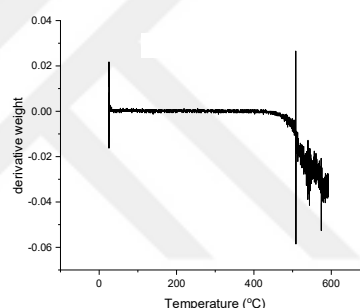


a

b



c



d

Figure 3.11-b: TGA and derivate TG of PES:PESmCA (a,b); and PES (c,d).

The thermal events observed during TGA of blend polymers are compiled in **Table 3.4** respectively.

Table 3.4: *Thermal events of Blend Polymers*

Blend Polymer	T _s °C	T _d °C
PES:s-P(NDH)	465	475
PES:s-P(ONDH)	500	510
PES:PESmCA	530	540
PES	490	500

TGA (10°C/min) in air : T_s stability temperature, and T_d is decomposition temperature.

3.5 SEM morphological study of membranes

The membranes P(NDH) 15%, P(NDH) 25%, s-P(NDH) 20% membranes of homopolymers and the 20% PES:s-P(ONDH), PES:s-P(NDH) and PES:PESmCA; three blend compositions, morphology was studied. Membrane visualization showed the presence of smaller pores at the active side and relatively bigger pore voids at the non-active side of the membrane being asymmetric Loeb-Sourirajan membranes.

The P(NDH) 15% and P(NDH) 25% membranes morphology revealed the presence of asymmetric structure with smaller pores at the active side and relatively large pores at the nonactive side of the membrane. For P(NDH) 25% membrane the average pore size of active side was half a micron (**Figure 3.12-a**).

The membrane fabricated from the saturated polymer s-P(NDH) 20% composition; SEM morphology study revealed the asymmetric structure for the membrane with all nano sized pores with smaller pores at active side. The average pore size at active side was 89 nm with the cross section revealing the tortuosity in the structure. The blend composition of PES:s-P(NDH) had the average pore size of 27 nm at the active side. The membrane structure has macro voids as revealed from its cross section with smaller sized pores at the active side of the membrane (**Figure 3.12-b**). The blend composition of PES:s-P(ONDH) had pore size of 16 nm at its active side which was the smallest of the pores obtained in the blend compositions of membranes. The cross section was tortuous as seen from the cross section. The blended composition of PES:PESmCA had the pore size of 25 nm at active side. The cross section showed the presence of relatively straight channels with smaller pores at the active side of membrane (**Figure 3.12-c**). The pore sizes are compiled in **Table 3.5** respectively.

The non-active side of all the membranes showed very large pore which were showing the asymmetric structure of the membrane.

Table 3.5 *Average pore sizes of pure and blend membranes*

Polymer	Weight content %	Average pore size at Active side (nm)
P(NDH)	15	>10 μ m
	25	0.5 μ m
P(ONDH)	-	-
s-P(NDH)	20	89
PES:s-P(NDH)	20	27
PES:s-P(ONDH)	20	16
PES:PESmCA	20	26

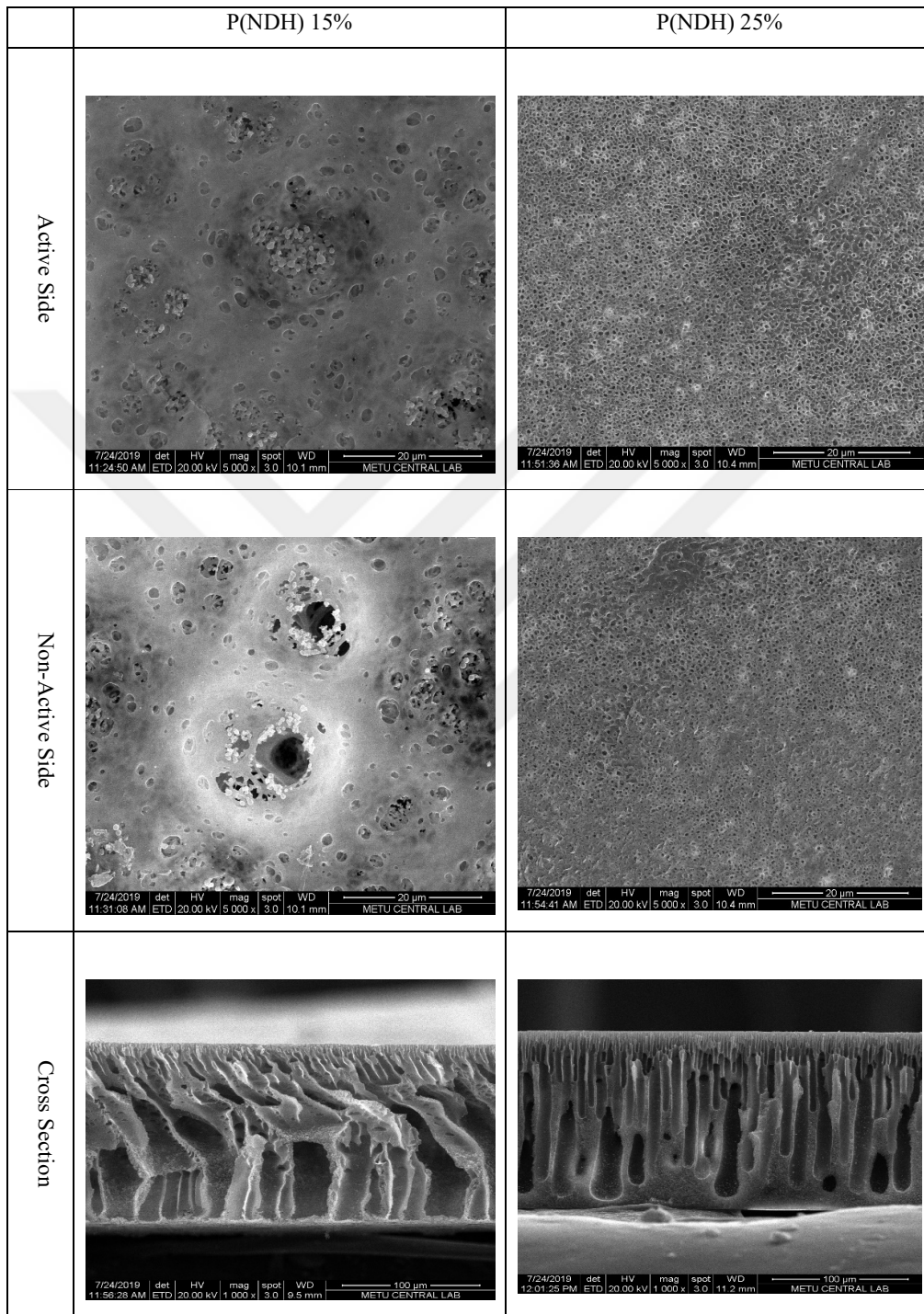


Figure 3.12-a : SEM micrographs of unsaturated membranes

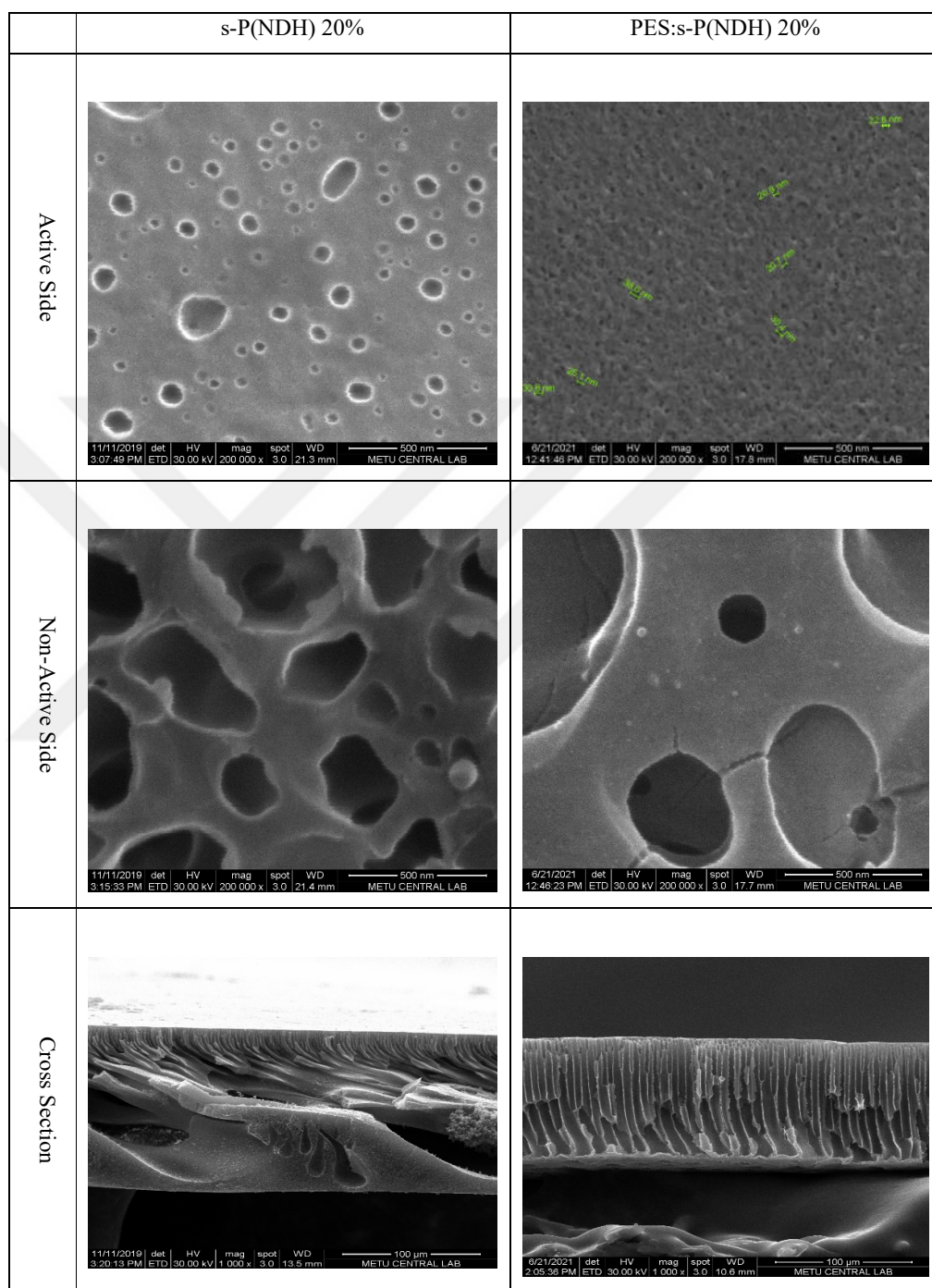


Figure 3.12-b SEM of pure and blend membrane of s-P(NDH)

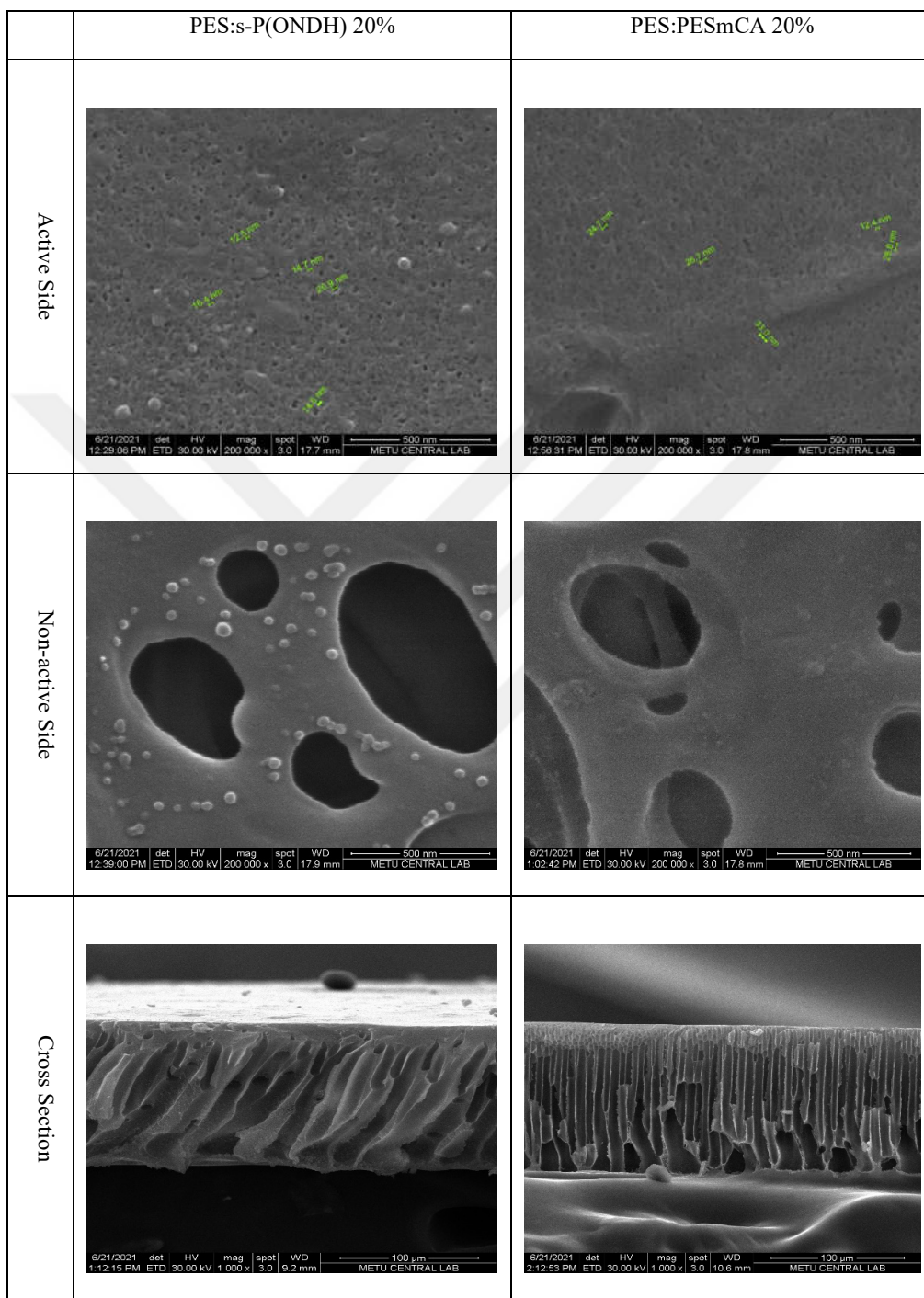


Figure 3.12-c: SEM of blend membrane PES:s-P(ONDH) and PES:PESmCA.

3.6 Membrane Performance Tests: Pure Water Permeance (PWP) and Bovine Serum Albumin (BSA)

The water permeance is related to the porosity, pore size and skin layer thickness of the membrane, while the membrane selectivity is related to the pore size. The rejection of solutes is due to the pore size. The protein rejection test was conducted using Bovine Serum Albumin (BSA; 66.5 kDa), for the membranes at 0.2 bar pressure using the same cell and membrane area as used during PWP test.

The unsaturated membrane P(NDH) 25% had the PWP of 278 l/h.m².bar with null rejection of BSA protein. The saturated membrane s-P(NDH) 20%, showed PWP of 19 l/h.m².bar and BSA rejection of 93%. When this homopolymer was blended with PES making 3:1 composition of PES:s-P(NDH) 20% weighted membrane had the PWP of 320 l/h.m².bar which was greater than the s-P(NDH) 20% membrane's. BSA rejection of PES:s-P(NDH) was 93% , providing the similar result as the s-P(NDH) 20% membrane had. The other blend membranes of 3:1 compositions PES:s-P(ONDH) 20% and 3:1 composition of PES:PESmCA 20% provided the PWP of 177 l/h.m².bar and 46 l/h.m².bar. Their BSA tests provided the rejection of 83% and 88% respectively (**Table 3.6**).

Table 3.6: PWP and BSA Test of pure and blend membranes

Polymer	Polymer Weight % in DMSO	PWP l/(h.m ² .bar)	BSA Rejection %
P(NDH)	15	-	-
	25	278	0
P(ONDH)	5	186	-
s-P(NDH)	20	19	93.8
PES:s-P(NDH)	20	320	93.5
PES:s-P(ONDH)	20	177	83.6
PES:PESmCA	20	46	88.1

3.7 Chlorination of Membranes

The membrane s-P(NDH) was tested for different chlorination times and ten mins chlorination was adopted for all the membranes. It was observed during chlorine release rate test for all the membranes that the membranes had covalently bonded chlorine N-Cl bonds, which do not break down during stirring of membranes in water. Only the adsorbed chlorine was identified in iodometric tests (APENDIX A).

The saturated membranes pure and blend were chlorinated for ten minutes in 15% NaOCl solution adjusted to pH 7 by using few drops of acetic acid (Figure 3.13). After chlorination, the membranes were washed in distilled water for 20-30 mins to remove the adsorbed chlorine and IR was checked.

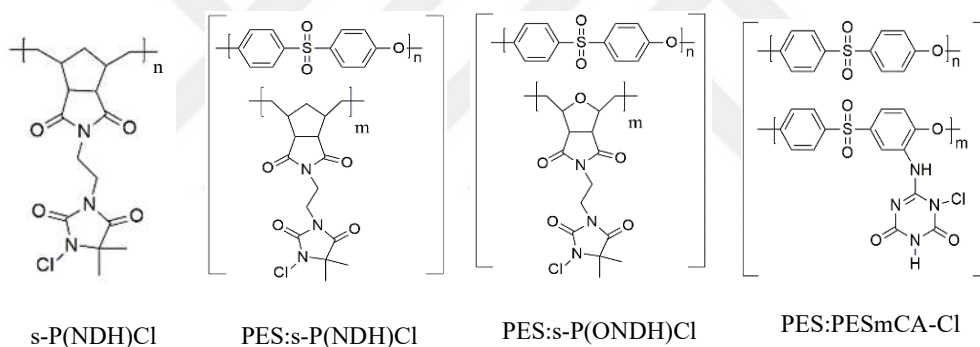


Figure 3.13: Chlorinated Pure and Blend Membranes

3.7.1 ATR-IR of Chlorinated Membranes

The membranes were dried at ambient conditions and ATR-IR was checked. It was seen that the chlorinated N-Cl peak was observed at 756 cm^{-1} in s-P(NDH) while in blend membrane this N-Cl peak was seen at 717 cm^{-1} in PES:s-P(NDH) and PES:PESmCA, while in PES:s-P(ONDH) at 721 cm^{-1} respectively. Their IR are presented in Figure 3.14.

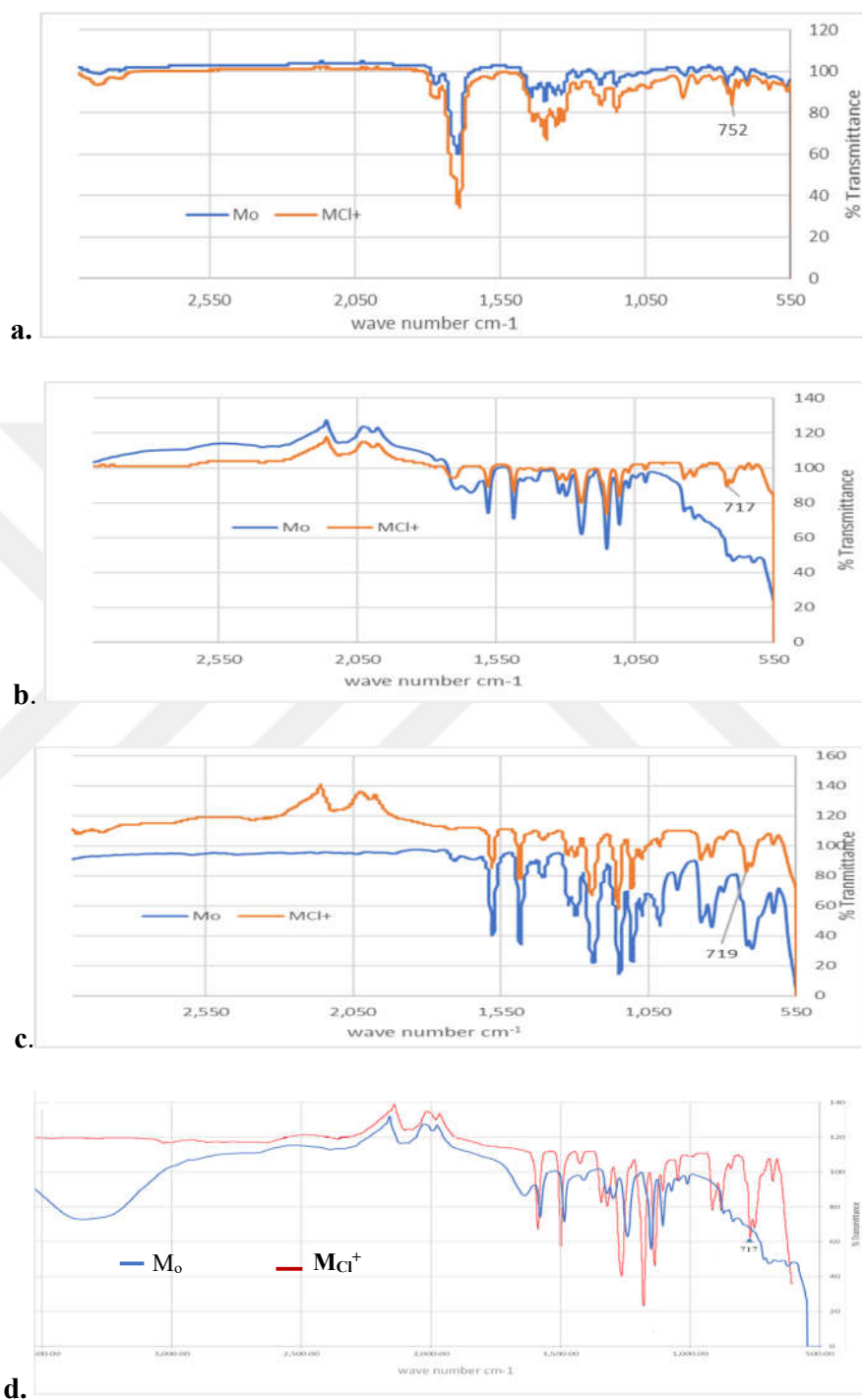


Figure 3.14: ATR-IR of blend membranes of M_o and M_{Cl}^+ **a)** s-P(NDH), **b)** PES:s-P(NDH), **c)** PES:s-P(ONDH), **d)** PES:PESmCA.

It was seen that the amount of chlorine that was identified in iodometric testing (see **Appendix A**) after stirring of membrane was the adsorbed chlorine that was trapped in the porous structure of membrane. The actual membrane bonded available sites of N-H bonds is rapidly filled in first ten mins of chlorination. Rest of chlorine uptake is only adsorbed in the membrane. The maximum absorption limit for active chlorine in membrane s-P(NDH) is 3 hours stirring as seen in the Table A-1 (**Appendix A**), but this stirring is unnecessary since the adsorbed chlorine in membrane will give error in membrane performance test during antimicrobial study.

It was seen that the amount of adsorbed chlorine in s-P(NDH) after 60 minutes of stirring is 2.02 mg and in ten minutes it is 0.48mg, providing the insight that even with reduced time of chlorination the membrane still adsorbed the active chlorine. While the estimation of bonded chlorine in s-P(NDH) and blend membrane was a variable depending upon the number of bonded sites available. However in membrane sacrificial test the bonded chlorine in membrane (4.1 cm²) of s-P(NDH) was 1.12mg (**Appendix A: Table A-6**).

It was observed that the membrane N-Cl bond is covalently stable. Thus, the chlorine from membrane's bounded sites is only depleted when any charged specie like KI (in iodometric test) or bacteria itself, reaches to the close proximity to the membrane. From these tests the stable N-halamine bonds presence is seen in all the ATR-IR analysis after removal of adsorbed chlorine Figure 3.14.

Ten mins chlorination is enough time of chlorination for s-P(NDH) N-amide precursor to have covalently bonded chlorine in membranes; while 15 to 20 mins of stirring of the chlorinated membrane in distilled water (washing) is enough to remove any adsorbed chlorine trapped in the membrane. For blend membranes same stirring time of chlorination and washing time was adopted (**Appendix A: Tabel A-7**).

3.7.2 Membrane Performance test PWP, BSA rejection.

The chlorinated membranes were also subjected to the PWP and BSA rejection test. Contrary to the non-chlorinated membrane performance result, it was observed that the chlorination had increased the pore sizes in the membrane structure which is not related to the membrane hydrophilic/hydrophobic behavior. Hence the water passed and flow without time count in slightest pressure through the membranes. The BSA rejection test was also close to zero for all the membranes with exception of PES:s-P(ONDH) whose rejection average was 8.1% respectively.

3.8 Antimicrobial Study of Membranes

The antimicrobial studies of the chlorinated membranes were performed in three different experiments. Initially the control experiments for the Gram positive *S. aureus* and Gram negative *E.coli* were performed and suitable uncountable colony of growing bacteria was selected. The detail of reagent preparations and sterile protocols during all these experiments is presented in **Appendix B**.

3.8.1 Control Experiment for *E.coli* Top 10 and *S.aureus* 25923

The control experiment provided the contamination free growth of respective bacteria due to the followed protocols of sterile procedure presented in Appendix B. It was seen that in 10^6 dilution the bacteria colonies were uncountable for both *E. coli* and *S. aureus* (**Table 3.7**). For antimicrobial test 10^6 dilution factor was selected for bacterial challenge experiments.

Table 3.7: Result of Control Experiment of *E. coli* and *S. aureus*

Agar Plate + Constituent	Result	Inference
Empty	CF	CF
HEPES BUFFER	CF	CF
<i>E. coli</i> 10 ³ CFU	<25>	CF growth
<i>E. coli</i> 10 ⁴ CFU	<224>	CF growth
<i>E. coli</i> 10 ⁶ CFU	UC	CF growth
Empty	CF	CF
HEPES BUFFER	CF	CF
<i>S. aureus</i> 10 ³ CFU	3 colonies	CF growth
<i>S. aureus</i> 10 ⁴ CFU	29 colonies	CF growth
<i>S. aureus</i> 10 ⁵ CFU	UC colonies	CF growth
Key: CF= contamination free UC= uncountable CFU=colony forming unit		

3.8.2 Bacterial challenge

For the four membranes; pure polymer s-P(NDH) and blend polymers PES:s-P(NDH), PES:s-P(ONDH) and PES:PESmCA, three types of bacterial challenge test were conducted. TEST I was the contact of chlorinated membranes with the solid agar surface with sessile bacteria growth. TEST II was the contact of chlorinated membranes with the liquid media in planktonic growth where the leaching and non-leaching membrane systems were identified. The TEST III was dead end filtration of bacterial suspensions respectively.

3.8.2.1 TEST I: Static Agar

This test was contemporary agar plate test [69]. This static test was conducted with *E. coli* and *S. aureus* one by one. In this test the inhibition zone was only under the chlorinated membrane or slightly out of the circumference.

3.8.2.1.1 Chlorinated Membranes Challenge Against *E. coli* Top 10

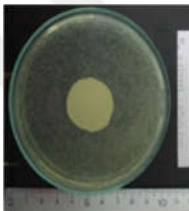
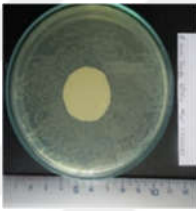
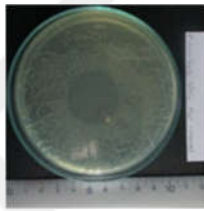
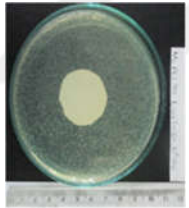
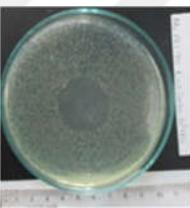
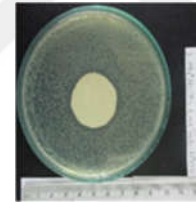
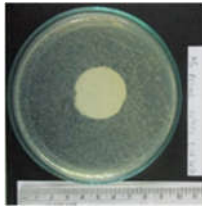
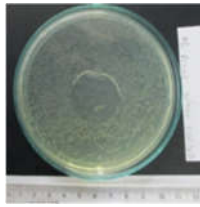
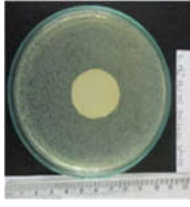
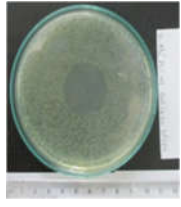
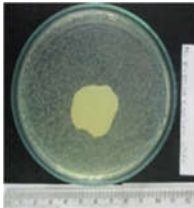
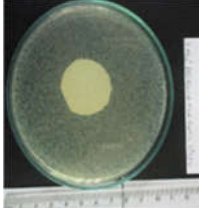
After the incubation time the agar plates were noted for bacterial growth. It was seen that they were contamination free. Agar plates with M_0 and M_{Cl}^+ membranes were examined after removal of membranes from the surface. It was seen that for all the M_0 had the bacterial growth under the membrane, while the M_{Cl}^+ had the zone of inhibition under the area of membrane. Some membranes also had the zone of inhibition greater than the area of membrane for which the TEST II was conducted to find out which of the membranes are leaching chlorine systems. The test result is presented in **Table 3.8-a** and representative photographic result is presented in **Table 3.8-b** respectively.

Table 3.8-a: TEST I: Chlorinated pure and blend membranes vs. *E. coli*

Agar Plate + Constituent	Control Experiment	Chlorinated Membranes vs <i>E.coli</i> Top 10			
		s-P(NDH)	PES:s-P(NDH)	PES:s-P(ONDH)	PES:PESmCA
Empty	C	C	C	C	C
HEPES	C	C	C	C	C
10^6 CFU	UC, UC, UC	-	-	-	-
10^4 CFU	254, 203,216	-	-	-	-
10^3 CFU	24, 21, 30	-	-	-	-
M_0 + 10^6 CFU	-	UC,UC,UC	UC,UC,UC	UC,UC,UC	UC,UC,UC
M_{Cl}^+ + 10^6 CFU	-	IZ,IZ,IZ	IZ,IZ,IZ	IZ,IZ,IZ	IZ,IZ,IZ
membrane system	-	NL	L	NL	NL

KEY: C=Contamination free UC = uncountable, IZ = Inhibition zone NL=non leaching
L=leaching

Table 3.8-b: Photographic Illustrations of TEST I: Membranes vs *E.coli*

	EMPTY	HEPES BUFFER	<i>E. coli</i> 10 ⁶ CFU
Control			
	M ₀		M _{Cl} ⁺
s-P(NDH)	 		 
PES:s-P(NDH)	 		 
PES:s-P(ONDH)	 		 
PES:PESmCA	 		 

3.8.2.1.2 Chlorinated Membranes Challenge Against *S. aureus* 25923

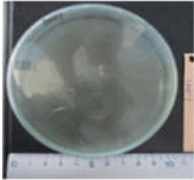
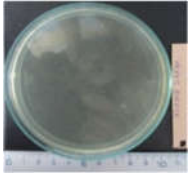
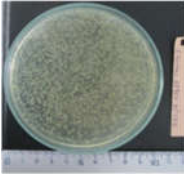
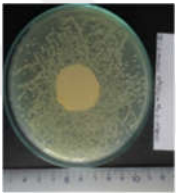
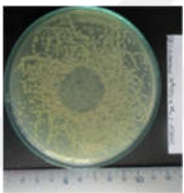
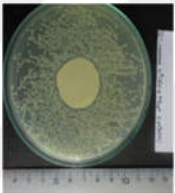
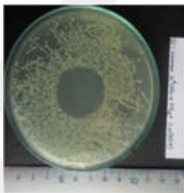
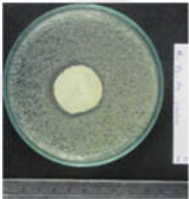
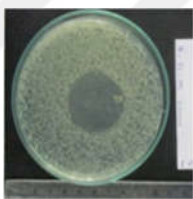
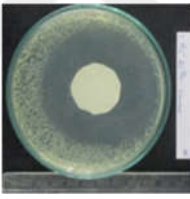
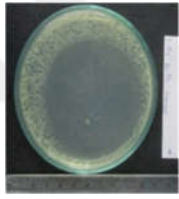
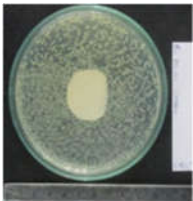
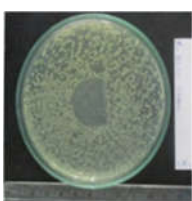
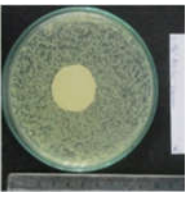
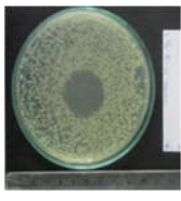
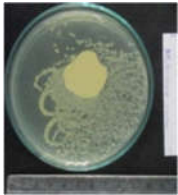
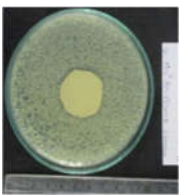
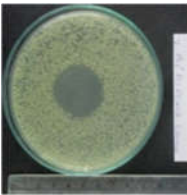
The chlorinated and non-chlorinated membranes were examined after the incubation time. The control plates of solo agar, HEPES buffer were contamination free. The plate with 10^6 CFU were also contamination free. The agar plates with membranes were examined after removal of membranes from the surface. It was seen that there was bacterial growth under the M_0 membranes while M_{Cl}^+ membranes had the zone of inhibition precisely under the area of contact. Follow up repeat experiments were also conducted for each type of membrane. The accumulated data is tabulated in **Table 3.9-a** and representative photographs are presented in **Table 3.9-b** respectively.

Table 3.9-a: TEST I: *Static agar test result: Membranes vs S.aureus*

Agar Plate + Constituent	Control Experiment	Chlorinated Membranes vs <i>S.aureus</i> 25923			
		s-P(NDH)	PES:s-P(NDH)	PES:s-P(ONDH)	PES:PESmCA
Empty	C	C	C	C	C
HEPES	C	C	C	C	C
10^6 CFU	UC, UC, UC	-	-	-	-
10^4 CFU	29				
10^3 CFU	3				
M_0 10^6 CFU	-	UC,UC,UC	UC,UC,UC	UC,UC,UC	UC,UC,UC
M_{Cl}^+ 10^6 CFU	-	IZ,IZ,IZ	IZ,IZ,IZ	IZ,IZ,IZ	IZ,IZ,IZ
CI- membrane system type	-	NL	L	NL	NL

KEY: C=contamination free, UC = uncountable, IZ = Inhibition zone NL=non leaching
L=leaching

Table 3.9-b: Photographic Illustration of TEST I: Membrane vs *S.aureus*

	EMPTY	HEPES BUFFER	<i>S. aureus</i> 10 ⁶ CFU
Control			
	M₀		M_{Cl}⁺
s-P(NDH)	 		 
PES:s-P(NDH)	 		 
PES:s-P(ONDH)	 		 
PES:PESmCA	 		 

3.8.2.2 TEST II: Dynamic (to and fro) to identify leaching/non leaching system.

The membranes often behave like leaching systems at their own during the agar tests. This phenomenon needs to be characterized before any agar test. Since this test will conclude that which membrane is a true leaching system. Sometime the chlorinated membranes kept in sterile water; might take water drop if not properly dried, could cause the greater inhibition zone. Hence a membrane needs to be specified as leaching / non -leaching **Figure 3.15**. This test was designed after one of the repeat tests of membrane s-P(NDH) provided bigger zone of inhibition, being contrary to its other repeat test. Another result of bigger zone of inhibition was observed in membrane of blend composition PES:s-P(NDH) during all of its repeat tests. To find out which of the chlorinated membranes is a leaching system of chlorine and which is non leaching, the dynamic to and fro test was designed and conducted.

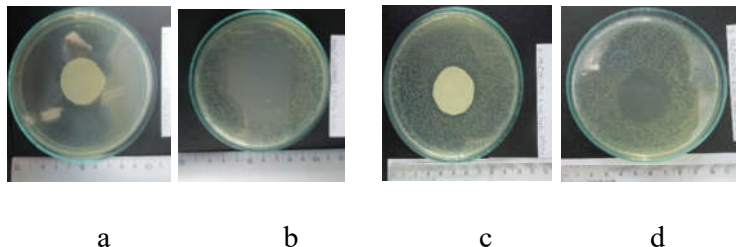


Figure 3.15: False positive result (a. b) of s-P(NDH) as it is **NL** system, (c, d) True positive result of PES:s-P(NDH) which is true **L** systems

The dynamic to and fro test was conducted. It was observed that since only PES:s-P(NDH) membrane provided 100% kill after 60 mins being the leaching (L) system of chlorine **Figure 3.16**. While the other three membranes did not favor any killing of bacteria at any selected instance until final, being the non-leaching (NL) system **Figure 3.17**.

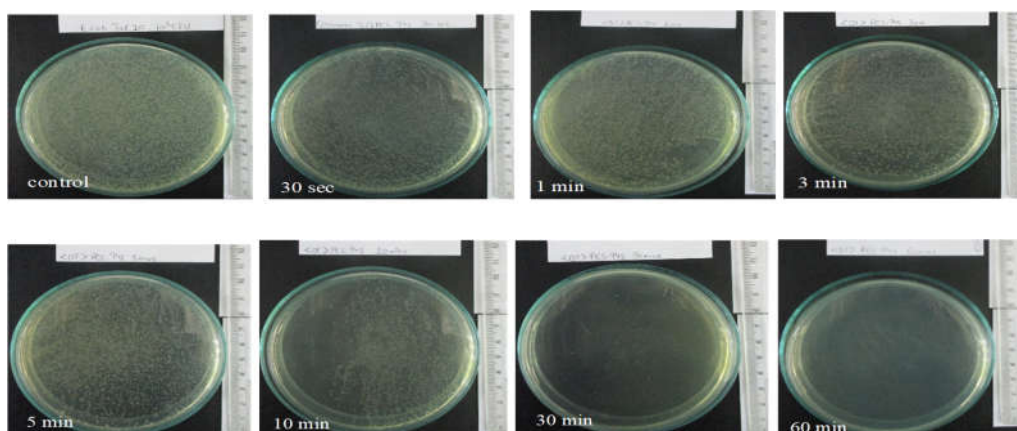


Figure 3.16: Dynamic test of membrane PES:s-P(NDH)

The bigger inhibition zone in static test (TEST I) and in dynamic test (TEST II) further show that PES:s-P(NDH) release “Cl⁺” more readily than the others. It was observed this with initial number of colony reduction seen after five minutes of contact time which lead to complete kill in 60 minutes. The results of test are tabulated in **Table 3.10**. It was seen that during static agar test the NL membranes had zone of inhibition precisely under the membrane which did not provide any reduction in this test.

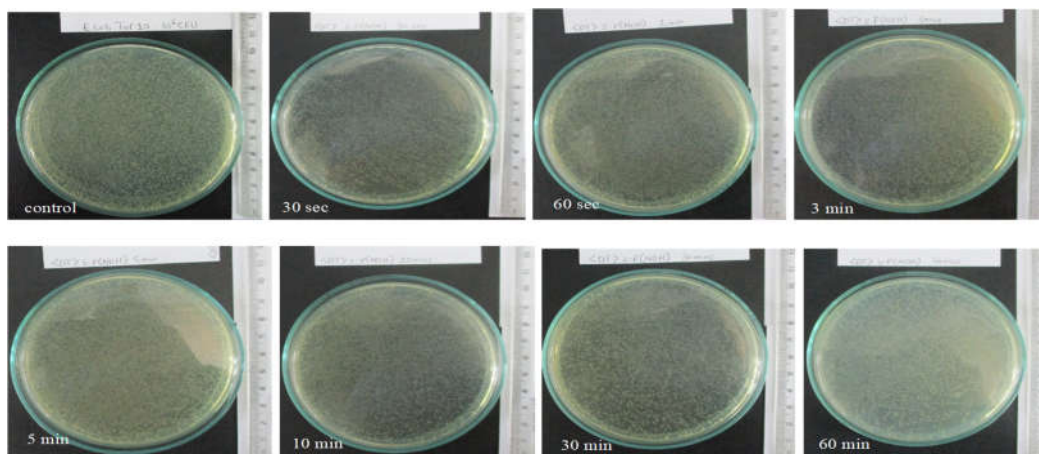


Figure 3.17: Dynamic test of s-P(NDH): Representative of NL system, same result was obtained from PES:s-P(ONDH) and PES:PESmCA.

Table 3.10 *TEST II: Chlorine leaching/non-leaching system: Dynamic to and fro result*

Membrane contact time	Systems of Chlorinated Membranes either Leaching L or Non-leaching NL)			
	s-P(NDH)	PES: s-P(NDH)	PES: s-P(ONDH)	PES: PESmCA
	NL	L	NL	NL
30 sec				
60 sec		No kill		
3 min	No Kill		No Kill	No Kill
5 min		Colony reduction		
10 mins		30% kill		
30 mins		99% kill		
60 mins		100% kill		

3.8.2.3 TEST III: Antimicrobial dead-end filtration

After chlorination the PWP tests of membranes were conducted. During that test the flow of water was unusual as compared to non-chlorinated membranes. Further the BSA rejection test showed null rejection. After this the suspension of bacteria were passed through the chlorinated membranes via dead end filtration to see the efficacy of these membranes. It was seen that the membrane s-P(NDH) provided the 100% kill to both *E.coli* and *S.aureus*. The membrane PES:s-P(NDH) provided 100 %kill and 99.9% kill in case of *E.coli* and *S.aureus*. Further the PES:s-P(ONDH) provided the 99.9 % kill and 100 % kill of *E.coli* and *S.aureus*. The membrane PES:PESmCA provided 100% and 99.9 % kill of *E.coli* and *S.aureus* respectively. The obtained result is tabulated in the **Table 3.11** below and their photographs are presented in **Figure 3.18** respectively.

Table 3.11 Antimicrobial dead end Filtration TEST III result

Chlorinated Membranes	Dead-end Filtration Antibacterial Test	
	<i>E.coli</i>	<i>S.aureus</i>
s-P(NDH)	100% kill	100% kill
PES:s-P(NDH)	100% kill	99.9% kill
PES:s-P(ONDH)	99.9 %kill	100% kill
PES:PESmCA	100% kill	99.9% kill

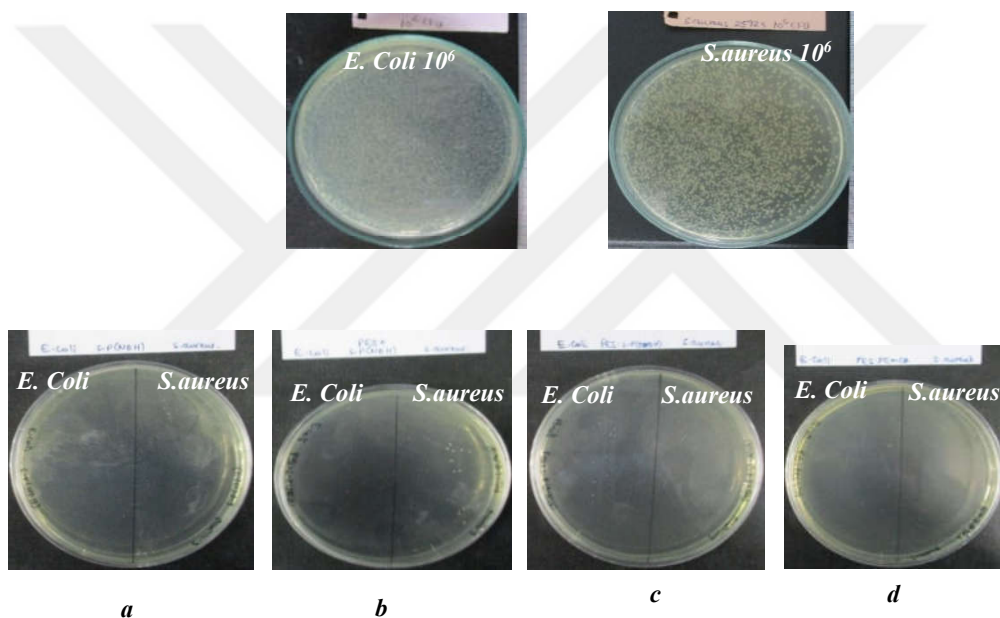


Figure 3.18: Result of *E.coli* / *S.aureus* filtrate seeded agar plates after incubation **a)** s-P(NDH), **b)** PES:s-P(NDH), **c)** PES:s-P(ONDH) and **d)** PES:PESmCA.

3.8.2.3.1 Examination of Biofilm Formation at membranes

The membranes after dead end filtration were placed at the agar plates such that the surface of interaction with bacteria was placed for agar surface contact to facilitate the growth of possible bacteria and biofilm. The plates were incubated at same conditions and the agar plates were examined after removal of the membranes from them. It was seen that none of the membranes had any alive bacteria at its surface

and the agar plates were contamination free. The membranes resisted the biofilm formation at their surface and inside due to stable N-Cl bonds (**Figure 3.19**).

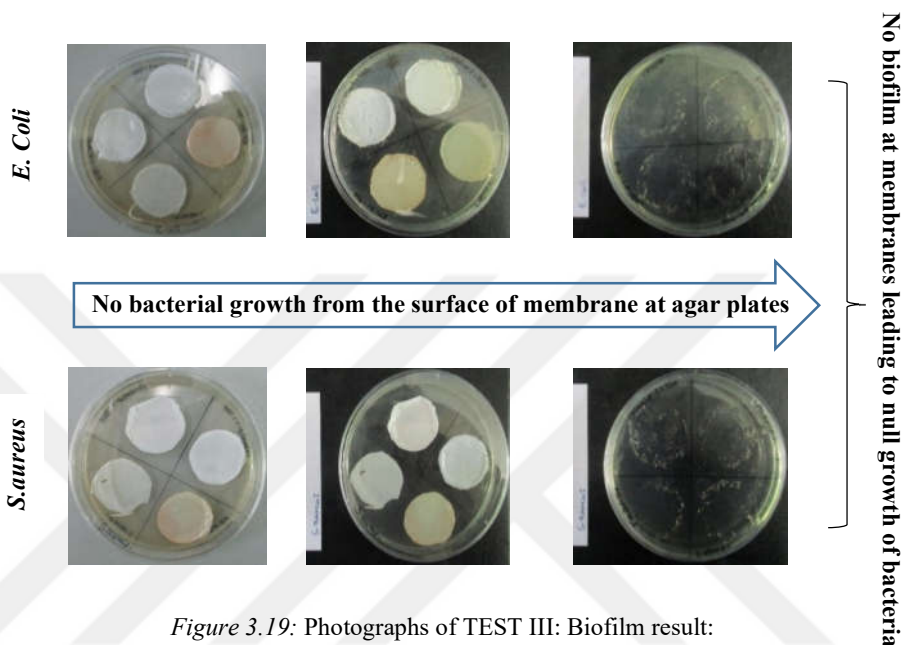


Figure 3.19: Photographs of TEST III: Biofilm result:

Agar incubation of membranes after *E. coli* and *S. aureus* filtration

3.9 Reuse of membranes

All the membranes of M_0 and M_{Cl}^+ after antimicrobial test were washed in 5% NaOCl solution for 2-3 hours. Further the membranes were neutralized while stirring in $Na_2S_2O_3$ solution for one hour. After drying the membranes in vacuum oven, the membranes were kept in sterile water. Half of the membranes were used for another cycle of chlorination as M_{Cl}^+ and other half as M_0 for repeat antimicrobial experiments. The membrane performance of pure and blend membranes when reused, were without any change during all the three types of antimicrobial test.

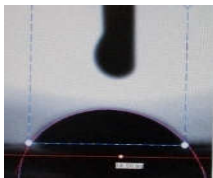
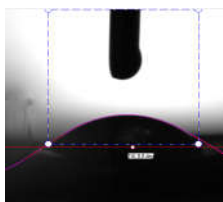
3.10 Contact angle (CA) of Pure and Blend membranes.

The contact angle measurement was done to determine the hydrophobic/hydrophilic property of membrane during various interaction to understand the surface. The pure polymer s-P(NDH) was hydrophobic with CA of 97, this character gradually reduced with the lowest CA was observed when the membranes were chlorinated hence becoming super hydrophilic, while actual angle was not readable from the device. After the antimicrobial test the membrane character changed from super hydrophilic to hydrophilic with CA of 35 and 45 respectively.

It was seen that the blend membranes were hydrophilic with average contact angles of fresh membranes as 63, 66 and 33 of PES:s-P(NDH), PES:s-P(ONDH) and PES:PESmCA respectively. In PES:s-P(NDH) the chlorinated membrane was also super hydrophilic, while the hydrophilic character reduced at surface after antimicrobial test and BSA rejection test. It was seen that the chlorinated membrane when tested for PWP and BSA the contact angle reached to 78, which was highest of observed surfaces of the respective polymer. Further the contact angle in PES:s-P(ONDH) reduced more after chlorinated and antimicrobial tested surfaces showed the hydrophilicity with CA <5.

The PES:PESmCA was most hydrophilic among the two other blend compositions after fabrication with CA of 33. The hydrophilicity of the membrane was reduced when chlorinated to superhydrophilicity, however it was reduced when checked after antimicrobial tests. The chlorinated membrane after PWP and BSA rejection, reached to the borderline of hydrophilic/hydrophobic at average value of 90.15 respectively. The CA of membranes are presented in **Table 3.12**.

Table 3.12: *Contact Angle of Membranes after interaction with different species*

Membrane	Surface Specification	Contact angle
s-P(NDH)  CA= 97	Chlorinated	<5
	Chlorinated + <i>E.coli</i> test	35.21
	Chlorinated + <i>S.aureus</i> test	45.10
	Chlorinated +PWP and BSA	81
PES: s-P(NDH)  CA= 63	Chlorinated	9.76
	Chlorinated + <i>E.coli</i> test	11.41
	Chlorinated + <i>S.aureus</i> test	36.77
	Chlorinated +PWP and BSA	78.15
PES:s-P(ONDH)  CA= 66	Chlorinated	Hydrophilic could not measure
	Chlorinated + <i>E.coli</i> test	<5
	Chlorinated + <i>S.aureus</i> test	<5
	Chlorinated +PWP and BSA	26.41
PES:PESmCA  CA=33	Chlorinated	Hydrophilic could not measure
	Chlorinated + <i>E.coli</i> test	70.12
	Chlorinated + <i>S.aureus</i> test	58.27
	Chlorinated +PWP and BSA	90.15

It was seen that the pore sizes were independent of the hydrophilic and hydrophobic behavior of the membrane surface. In case of these polymers the hydrophilic/hydrophobic character is only dependent upon the chemical makeup of the membrane and its interaction with the other species, unlike in metals where the generated porosity effects the hydrophilic/hydrophobic character of the material. Further it was noted the membrane changes after chlorination; with zero rejection of BSA protein. The literature study [106] has revealed that the presence of halogen group in the polymer has caused the wetting (hydrophilic) behavior in the polymers. When halogen is neutralized by sodium thiosulphate the polymer had leaned towards contra wetting (less hydrophilicity).

CHAPTER 4

CONCLUSIONS

Summary of Synthesis/ modification of polymers and Fabrication of membranes

The two novel polymers s-P(NDH) and s-P(ONDH) were successfully synthesized and characterized. Introduction of cyanuric acid onto PES was also accomplished.

Polymer s-P(NDH) was capable of membrane formation while the second polymer s-P(ONDH) did not coagulate as respective membrane but stayed adhesive like coating material. When the former polymer was blended with PES (1:3) as a support material, membrane was fabricated successfully. Moreover, two more blend membranes of PES with s-P(NDH) and PESmCA were also prepared. The membranes were characterized, and their performances were determined followed by study of their antimicrobial properties.

Summary of antimicrobial membrane performance

The inhibiting of antibiotic resistant bacteria (*E.coli* and *S.aureus*) achieved successfully with 99 to 100 % kill. Controlled release of oxidative chlorine (Cl^+) from membranes was testified from static agar test (TEST I) and dynamic to and fro test (TEST II). Further the total absence of biofilm at membrane surface was observed after antimicrobial dead end filtration test (TEST III) agar plating results.

The membrane of pure s-P(NDH) in the static agar tests (TEST I) showed that this membrane has zone of inhibition precisely under the membrane area. On the contrary to this, PES:s-P(NDH) provided the zone of inhibition greater than the membrane area. It was observed that this polymer s-P(NDH) has good antimicrobial performance when used as pure or as blend membrane, however the system become leaching (L) from non-leaching (NL) after mixing with PES.

It was observed that all the three blend membranes had given 100% kill in static test (TEST I). The inhibition zone in case of PES: s-P(ONDH) and PES:PESmCA were strictly under the membrane just like the membrane of s-P(NDH), contrary to PES:s-P(NDH) results.

The dynamic test (TEST II) resulted in the 100% killing of *E.coli* after 60 minutes only in case of PES: s-P(NDH) which was a controlled leaching system of chlorine. While the other three membranes s-P(NDH), PES-s-P(ONDH) and PES:PESmCA however chlorinated but failed to give killing even after 60 minutes of agitation. It was seen that s-P(NDH), PES:s-P(ONDH) and PES:PESmCA had very stable N-Cl bond. While the PES:s-P(NDH) had chlorine leaching property of releasing the chlorine slowly into the system with in time of one hour precisely. It was the reason of bigger inhibition zone during TEST I (static agar test) of this membrane.

Since it was observed that the membrane after chlorination has some contraction in material increasing the pore size at some areas. This caused the water to flow during dead end PWP test, without any applied pressure. The BSA rejection also nullified in all the membranes. Hence the bacterial rejection through the membrane became a question owing to the pore size. When dead end antimicrobial test (TEST III) was conducted, it was seen that although the pore sizes have increased after chlorination of membranes; the passed through bacterial suspension provided 99 to 100% kill in all the membrane permeates. All the chlorinated membranes are efficient enough for the bacterial challenge, but they cannot reject the BSA protein as compared to the non-chlorinated counter parts which showed the rejection average of 88 to 93% in all the membrane.

All of the polymers were found to be efficient as a good vehicle to keep the active chlorine intact, without uncontrolled release of chlorine in the system; found effective against *E.coli* and *S.aureus*, the antibiotic resistant pathogens. The membranes are recyclable since most of the times the material was washed and re-chlorinated effectively during various tests performed.

CHAPTER 5

EXPERIMENTAL

5.1 Materials

All the specialty reagents used in various stages of post polymerization modifications of m-NDH and m-ONDH are as follows; dicyclopentadiene (cyclopentadiene dimer, DCPD), Furan ($\geq 99\%$), maleic anhydride (2,5-Furandione 99%), ethanolamine (2-Aminoethanol 98%), *p*-toluenesulfonyl chloride (Tosyl chloride 98%), 5,5-dimethyl hydantoin (4,4-Dimethyl-2,5-dioxoimidazolidine, DMH 97%) were purchased from Sigma Aldrich. Reagents used during polymerization of m-NDH and m-ONDH are Grubbs second generation catalyst (M204), for quenching of polymerization ethyl vinyl ether (Ethoxyethylene 99%) and reagent hydrazine monohydrate (Hydrazinium hydroxide) were purchased from Sigma Aldrich.

Reagents used during modification of PES such as sulphuric acid (H_2SO_4 , 95%) nitric acid (HNO_3 , 65%) cyanuric chloride were purchased from Sigma Aldrich. Stannous chloride (SnCl_2) and N-methyl pyrrolidone (NMP) were purchased from MERCK. Polyethersulfone (PES, Ultrason E6020P) was purchased from BASF chemicals.

The materials used during membrane fabrication and performance tests are Dimethyl sulfoxide (DMSO) purchased from MERCK. Bovine Serum Albumin (BSA) and Phosphate Buffer Saline (PBS) were purchased from Sigma Aldrich. For chlorination of membranes common house-hold bleach (NaOCl , $\text{Cl}^+ < 5\%$) was used.

5.2 Characterizations

The novel monomers during post polymerization modifications and polymers (novel and modified PES) were characterized by following instrumentation techniques; ATR-IR characterization was done using Thermo Scientific Nicolt: S10 instrument. The ^1H NMR and ^{13}C NMR data was recorded from Bruker's AVANCE 3 ultra-shield 400 MHz instruments, using the solvents CDCl_3 and DMSO-D_6 respectively. The High-Resolution Mass spectrometry (HRMS) for monomers m-NDH and m-ONDH was done using Waters SYNAPT G1 MS instrument using ESI-TOF-MS method in positive mode (ES+) with 50-1000 Da range analysis. The molecular weight of novel polymers was analyzed by Dynamic Light Scattering spectrometry instrument using the method of static light scattering SLS. The analysis provided the information about the weight average molecular weight of the polymer (M_w).

Thermal properties of monomers, polymers and blend membranes were analyzed by TG/DTA, while DSC technique was used for blend polymer membranes. Thermogravimetric Analysis and Differential Thermal Analysis for monomers and polymers were done using the SDT650 TGA-DSC instrument. The analysis was done in nitrogen inert environment with temperature range of 25 °C to 600 °C at 10 °C/min. Differential Scanning Calorimetric analysis was done using Perkin Elmer DSC8000 in presence of nitrogen inert environment. The analysis was between -35 °C to 600 °C.

The Scanning Electron Microscopy was done using instrument FEI Quanta-400F to study the morphology of membranes. The membranes morphology was determined by using SEM. For surface characterization of active and non-active sides; the membrane was dried by purging nitrogen, while the cross sections were prepared in liquid nitrogen. The prepared samples were dried in vacuum oven for 24 hours, sputtered coated with Pd/Au before analysis.

5.3 Synthesis and Preliminary Characterization of novel norbornene polymer P(NDH) and s-P(NDH)

5.3.1 Synthesis of Monomer

5.3.1.1 Compound 1: 5-norbornene-endo-2,3-dicarboxylic anhydride [107].

Cracking of cyclopentadiene was carried out at $\sim 138^{\circ}\text{C}$. The cracked cyclopentadiene was collected at -78°C in a round bottom flask. From the collected cyclopentadiene almost 3.9 g (59.5 mmol) was added into ethyl acetate and hexane (1:1, 10ml:10ml) having dissolved maleic anhydride (3.6 g, 36.7 mmol) kept at 0°C . The contents were stirred until solid precipitates were formed which were filtered, and dried. The product yield was qualitative. The product obtained was endo kinetic product. Product weight 5.4 g, 84.5%; mp $165\text{--}167^{\circ}\text{C}$; **IR (ATR, cm^{-1})** 1580, 1892, 1760; **^1H NMR** (CDCl_3 , 400 MHz): δ 6.31 (t, 2H, $J = 2.1\text{ Hz}$), 3.58 (dd, 2H, $J = 3.0, 1.64\text{ Hz}$), 3.51 (m, 2H), 1.78 (dt, 1H, $J = 9.0, 1.7\text{ Hz}$), 1.57(m, 1H).

5.3.1.2 Compound 2: 2-(2-hydroxyethyl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3 (2H)-dione [108].

Compound **1** (3.5g, 21.3 mmol) was dissolved in toluene (120 ml) and ethanolamine (3.03g, 49.6 mmol) was added dropwise. After half hour of stirring, it was refluxed using Dean-Stark apparatus. After 6 hours the reaction completion was confirmed by TLC. The solvent was evaporated, and crude product was redissolved in DCM and washed three times with water. The organic phase was dried using magnesium sulphate and solvent was evaporated to get (**2**) as a crystalline product. The weight of pure compound was 4.1g with 90.9% yield, mp = $80\text{--}90^{\circ}\text{C}$; **IR (ATR, cm^{-1})** 1580, 1760, 1690, 2980; **^1H NMR** (CDCl_3 , 400MHz): δ 6.12 (t, 2H, $J = 2\text{ Hz}$), 3.64 (dd,

2H, $J=5.2, 3.9$ Hz), 3.56 (dd, 2H, $J=5.3, 3.9$ Hz), 3.40 (m, 2H), 3.29 (m, 2H), 2.17 (d, 1H, $J=6.2$ Hz), 1.74 (dt, 1H, $J=8.8, 1.8$ Hz), 1.55 (d, $J=8.8$ Hz, 1H).

5.3.1.3 Compound 3: 2-(1,3-dioxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl) ethyl 4-methylbenzenesulfonate [109].

Compound **2** (3g, 14.5 mmol) was dissolved in DCM (70 ml) and *p*-toluene sulphonyl chloride (2.6g, 13.6 mmol) and pyridine (3 ml) was added. The contents were stirred at room temperature for 24 hours. The reaction was monitored by TLC. After reaction completion extraction of pure compound was done by washing with 5% HCl and brine solution. The organic phase was dried over magnesium sulphate, filtered and evaporated to get (**3**) as off-white solid (4g) with yield 78 %, mp= 100-110 °C ; **IR** (ATR, cm^{-1}) 1580, 1760, 1690, 1172, 1600; **$^1\text{H-NMR}$** (CDCl_3 , 400 MHz): δ 7.77 (d, 2 H, $J=8.4$ Hz), 7.35 (d, 2H, $J=7.92$ Hz), 6.07 (t, 2H, $J=1.88$ Hz), 4.07 (t, 2H, $J=5.46$ Hz), 3.59 (t, 2H, $J=5.46$ Hz), 3.37 (m, 2H), 3.25 (dd, 2H, $J=1.64, 1.52$ Hz), 2.45 (s, 6H), 1.72 (dt, 1H, $J=5.28, 1.75$ Hz), 1.52 (d, 1H, $J=8.8$ Hz).

5.3.1.4 Compound 4: 2-(2-(4,4-dimethyl-2,5-dioxoimidazolidin-1-yl) ethyl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione [110].

Compound **3** (3 g, 8.3 mmol) was dissolved in DMF (20 ml) and 5,5-dimethylhydantoin (3 g, 23.2 mmol) was added followed by addition of DBU (2.54 g, 16.7 mmol). After two hours of stirring the reaction contents were refluxed for 3 hours. After reaction completion the DMF was removed using high vacuum. The dried raw product was re-dissolved in DCM and washed with water. Organic phase was dried using magnesium sulphate and filtered. The vacuum dried light yellow to white crystalline product (**4**) 2.17 g was obtained (82.2 % yield), mp = 170 °C; **IR** (ATR, cm^{-1}) 1760, 1690, 1580; **$^1\text{H-NMR}$** (CDCl_3 , 400 MHz): δ 6.07 (t, 2H, $J=1.88\text{Hz}$), 5.51 (s, 1H), 3.60 (m, 4H), 3.34 (h, 2H, $J=1.73$ Hz), 3.25 (m, 2H), 1.71 (dt, 1H, $J=5.28, 1.7$ Hz), 1.52 (d, 1H, $J=8.64$ Hz), 1.42 (s, 6H). **$^{13}\text{C NMR}$** (CDCl_3 , 100

MHz): δ 177.70, 177.28, 156.02, 134.31, 58.65, 52.19, 45.90, 37.07, 36.48, 24.51. **HRMS** (ESI) m/z , $[M+H]^+$ calcd for $C_{16}H_{20}N_3O_4$ 318.1446; found 318.1454.

5.3.2 Polymerization of m-NDH and Saturation.

5.3.2.1 Compound 5: polymer (norbornene dicarboximide hydantoin) P(NDH) [111].

In a dried Schlenk line monomer m-NDH **4** (5g, 15.7mmol) was added in DCM (25ml). System was made inert by purging Argon for 30 mins. Grubbs 2 (C/M 1/250 molar ratio) was dissolved in DCM (5ml) and added in the system. It was stirred for 24h at room temperature. Polymerization was quenched with ethyl vinyl ether (2ml) and stirred further for 3h [24]. Solvent was removed from reaction medium by vacuum and raw product was re-dissolved in DMSO. Product was precipitated as white polymer using methanol and water. Better precipitations were observed using diethyl ether. White polymer P(NDH) was dried in vacuum oven at 60°C overnight; weighed as 4.6 g (92% yield). **IR** (ATR, cm^{-1}) 3314, 1760, 1690, 1580; **^1H NMR** (DMSO- d_6 , 400MHz): δ 5.55, 5.41, 4.13, 3.36, 3.16, 2.54, 2.09, and 1.25. **^{13}C NMR** (DMSO- d_6 , 100 MHz): δ 177.69, 177.10, 155.38, 128.42, 58.02, 40.69, 24.69 ppm.

5.3.2.2 Compound 6: saturated polymer (norbornene dicarboximide hydantoin) s-P(NDH) [105].

The polymer P(NDH) (**5**) 1 g was dissolved in 35 ml of DMF and (1g, 5.37 mmol) of TSH was added. The reaction contents were set to reflux for 5-7 hours. The saturated polymer s-P(NDH) (**6**) was obtained by simple precipitation using water and diethyl ether. The filtered product was washed with water repeatedly to remove the tosylic acid byproduct. Weight of polymer dried = 1.1 g. **IR** (ATR, cm^{-1}): 2920, 2850, 1770, 1680; **^1H -NMR** (DMSO- d_6 , 400MHz): δ 4.39, 2.89, 2.73, 2.59, 2.28,

2.09, 1.23, 1.05 ppm. ^{13}C NMR (DMSO- d_6 , 100MHz): δ = 177.91, 177.66, 155.32, 57.98, 47.85, 36.61, 36.32, 24.56 ppm.

5.4 Synthesis and Preliminary Characterization of novel oxonorbornene polymer P(ONDH) and s-P(ONDH)

5.4.1 Synthesis of monomer

5.4.1.1 Compound 7: oxonorbornene (3a,4,7,7a-tetrahydro-4,7-epoxyisobenzofuran-1,3 dione) [112].

Maleic anhydride (10g, 121.9 mmol) and Furan 8.5ml were added to Erlenmeyer flask and set to stir at room temperature for 2 hours. The flask was placed in ice bath and 10 ml of toluene was added to induce the crystallization of the product. Product was white solid, which was redissolved in DCM and washed with simple extraction method using distilled water. The organic layer was dehydrated using magnesium sulphate and vacuum dried to get white crystalline product. Weight= 17.1g; 85.5% yield. m.p = 112 °C; IR (ATR, cm^{-1}) 1860, 1780, 1020. ^1H NMR (CDCl_3 , 400 MHz): δ 6.58 (t, J = 1.0 Hz, 2H), 5.46 (t, J = 1.0 Hz, 2H), 3.18 (s, 2H).

5.4.1.2 Compound 8: oxonorbornene 2-(2-hydroxyethyl)-3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione) [113].

Compound 7 (4g, 24.09 mmol) was stirred in ethanol (30ml) and ethanolamine 3ml added drop wise. The reaction contents were stirred and set to reflux as a modified procedure reported in literature. It was noted that the 7 was completely soluble after the addition of ethanol amine. The contents were refluxed for 7 hours. The reaction completion was noted by the TLC. The ethanol was removed from the reaction

medium by vacuum evaporation. The crude product was dissolved in DCM and washing-extraction procedure was done to remove the remain of by product water. The organic phase was dehydrated using magnesium sulphate and vacuum dried to get crystalline product 4 g; 80%yield. m.p = 130 °C. **IR (ATR, cm⁻¹)** 3470, 1760, 1680, 1020. **¹H NMR** (CDCl₃, 400MHz): δ 6.46 (t, J= 0.94 Hz, 2H), 5.22 (t, J= 1.0 Hz, 2H), 3.69 (t, J= 5.26 Hz, 2H), 3.62 (t, J= 5.34 Hz, 2H), 2.88 (s, 2H), 2.29 (s, 1H).

5.4.1.3. Compound 9: oxonorbornene (2-(1,3-dioxo-3a,4,7,7 a-tetrahydro-1H-4,7 epoxyisoindol-2 (3H)-yl) ethyl 4-methylbenzenesulfonate)
[114].

The compound **8** (1g; 4.78mmol) was dissolved in DCM (20ml), and pyridine (1.5ml) was added followed by addition of *p*-toluenesulphonyl chloride (0.9g, 4.72mmol). The medium was set to stirring from zero degrees to room temperature while using the self-modified reported procedure. The completion of reaction was determined by using TLC. The product was extracted by sequenced washing with 5%HCl solution three times, three times washing with sodium bicarbonate solution, one time with brine and one time with water. The organic phase was dehydrated using magnesium sulphate. The filtered organic phase was vacuum dried and left to crystalize. The product **9** weighted as 1.61g; 92.5% yield. m.p = 230 °C. **IR (ATR, cm⁻¹)** 1780, 1700, 1590, 1170, 1010. **¹H NMR** (CDCl₃, 400 MHz): δ 7.76 (d, J=8.28 Hz, 2H), 7.33 (d, J= 8.28Hz, 2H), 6.46 (m, 2H), 5.24 (t, J=1.0 Hz, 2H), 4.19 (t, J= 5.52 Hz, 2H), 3.74 (t, J=5.22 Hz, 2H), 2.85 (s, 2H), 2.44 (m,3H).

5.4.1.4. Compound 10: oxonorbornene (2-(2-(4,4-dimethyl-2,5-dioxoimidazolidin-1-yl) ethyl) 3a,4,7,7a-tetrahydro-1H-4,7-epoxyisoindole-1,3(2H)-dione).

The compound **9** (1g, 2.75mmol) was dissolved in acetone (35ml) in round bottom flask. In a beaker 15 ml of acetone and DBU (1.5 ml) was added followed by addition

of 5,5-dimethyl Hydantoin (0.5 g; 3.91 mmol) added the contents of beaker were added to the reaction flask and were left to stir at room temperature until the reaction completion; monitored by TLC. The solvent was evaporated by vacuum drying and re-dissolved in DCM for extraction washing procedure using sodium bicarbonate solution three times and consecutive washing with water. The organic phase was dehydrated using magnesium sulphate, filtered and again vacuum dried to obtain the monomer as light yellow to white crystalline solid weighted as 0.71g; 80% yield. m.p = 173 °C. **IR (ATR, cm⁻¹)** 3330, 1760, 1690, 1480, 1020. **¹H NMR** (400 MHz, CDCl₃): δ = 6.48 (s, 2H), 5.97 (s, 1H) 5.22 (s, 2H), 3.71 (s, 4H), 2.84 (s, 2H), 1.40 (s, 6H). **¹³C NMR** (CDCl₃, 100MHz): δ 177.27, 176.27, 136.37, 80.56, 58.72, 47.53, 37.38, 36.82, 24.52 ppm. **HRMS (ESI)** m/z. [M+H]⁺ calcd for C₁₅H₁₈N₃O₅ 320.11246; found 320.1246.

5.5 Polymerization of m-ONDH and Saturation.

5.5.1 Compound 11: polymer (oxonorbornene dicarboximide hydantoin) P(ONDH) [111].

In a dried Schlenk line monomer m-ONDH (0.5g, 1.56 mmol) was dissolved in DCM 25 ml and the contents of reaction flask were purged with Argon. The G-II (C/M 1/250 molar ratio) was dissolved in DCM (3ml) and added to the medium dropwise [6]. The content was left to stir for two days. The polymerization reaction was quenched using ethyl vinyl ether while adding excess of quencher. The polymer precipitated and was filtered off. It was dried in vacuum, providing 0.4g polymer. **IR (ATR, cm⁻¹)** 1779, 1704, 1597, 1174. **¹H NMR** (DMSO-d₆, 400 MHz): δ 6.51, 5.93, 5.70, 5.12, 3.51, 3.38, 2.80, 2.51, 1.21. **¹³C NMR** (DMSO-d₆, 100 MHz): δ 177.28, 175.32, 155.14, 136.27, 80.07, 57.07, 54.74, 46.94, 24.04 ppm.

5.5.2 Compound 12: saturated polymer (oxonorbornene dicarboximide hydantoin) P(ONDH) [105].

In a round bottom flask compound **11** (0.9g) was dissolved in 35 ml of DMF and TSH (1.5g, 8.06mmol) was added. The contents of reaction flask were stirred and set to reflux for 7 hours. After the reaction completion the contents of the flask were precipitated using ether and water. The white polymer was obtained which was filtered and dried. weight of saturated polymer = 1.1g. **IR (ATR, cm⁻¹)** 1730, 1690, 1140. **¹H NMR** (DMSO-d₆, 400 MHz): δ 1.65, 1.88, 2.04, 2.49 ppm. **¹³C NMR** (DMSO-d₆, 100 MHz): δ 177.71, 176.67, 162.65, 155.18, 79.41, 57.81, 35.82, 30.82, 24.48 ppm.

5.6 Modification of polyethersulfone (PES)

5.6.1 Compound 13: nitrated polyethersulphone PES-NO₂ [69].

In round bottom flask PES (5g) was added followed by addition of sulphuric acid (20ml) and stirred for 90 minutes. Nitric acid (15ml) was added drop wise into the reaction medium using the dropping funnel and set to stir for seven days to ensure maximum nitration in PES which was a gelatinous colloid, and not completely soluble in the reaction medium. The nitrated PES was precipitated using distilled water, filtered, and washed repeatedly, dried at ambient conditions. **IR (ATR, cm⁻¹)** 1570, 1531, 1485.

5.6.2 Compound 14: polyethersulphone amine PES-NH₂ [115].

In a dropping funnel, dissolved solution of compound **13** (1g) and NMP 10ml was assembled. In a three-neck round bottom flask stannous chloride (2g, mmol) and HCl (20 ml) was added and stirred for few minutes, while setting the condenser and dropping funnel; to the third neck the system was sealed with the septum. Dissolved

solution of glacial acetic acid (10ml) and KI (0.5g, mmol) was added into the system drop wise using syringe and heated to 50 °C. Slowly the contents of the dropping funnel were added into the system, stirred and refluxed for three hours. After cooling down the system, initially 50ml of 1 M NaOH solution was added and stirred. Further the contents were transferred to beaker and more batches of 1M NaOH was added until the alkalinity was detected at pH strip. The contents were filtered, and modified polymer was washed with distilled water and dried. **IR (ATR, cm⁻¹)** 1614, 1570, 1480.

5.6.3 Compound 15: polyethersulphone amine cyanuric chloride PES-NH-CCl [116].

In a round bottom flask compound **14** (0.5g) was added and dissolved in NMP (10ml), followed by addition of cyanuric chloride (0.5g, mmol). The system was kept stirring for two hours at 0-5 °C. After this the polymer was precipitated in distilled water and filtered through sintered glass via vacuum and left to dry. Weighed product was 0.66g powder. **IR (ATR, cm⁻¹)** 1660, 1572, 1479, 790.

5.6.4 Compound 16: polyethersulphone amine cyanuric acid PES-NH-CA or PESmCA [117].

Compound **15** (0.2g) was added in a round bottom flask and dissolved solution of NaOH (1g) in distilled water (10ml) was added and refluxed for 3 hours. After cooling the reaction contents, the product was in a transition state of polyelectrolyte (aqueous state). The contents were treated with 3ml HCl to precipitate the product. Product was filtered and dried, 0.18g crystalline solid. **IR (ATR, cm⁻¹)** 1742, 1571, 1482.

5.7 Membrane Fabrication

Membranes were fabricated using immersion precipitation technique of phase inversion method. Casting solutions were prepared in DMSO. The casting surface was glass tab and casting roll of 250 μm thickness was used. The solution was casted immediately after complete solubility. The glass tab was immersed in the water trough following the immersion precipitation technique of phase inversion. The water (RO) of trough was refreshed after two hours interval and three to four times, until the membrane fully precipitated and left the substrate. Membranes were washed and kept in 20% ethanol solution for further use.

5.8 Pure Water Permeance Test

The PWP test was conducted in 10ml Amicon cell with permeance area of 4.1 cm^2 . Initially water was passed through the membranes at pressure of 1.5 bar unchecked until the pore sizes reduced to their original size. Since the membranes after fabrication are kept in 20% ethanol; the membranes lean towards hydrophilicity, until all the adsorbed ethanol is removed (**Figure 5.1**).

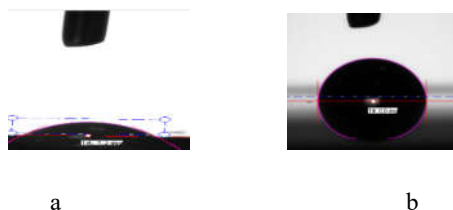


Figure 5.1: s-P(NDH) membrane condition in ethanol and dried : (a) CA 43 $^{\circ}$ when in ethanol solution. (b) CA 97 $^{\circ}$ washed and dried.

After membrane stability the water flux per unit area of membrane with variable pressures of 1.5, 1.0 and 0.5 bar were recorded and calculated (**equation B**). The flux calculated at each pressure was obtained from the ten times repeat of volumetric flow

with stabilized numerical value. The slope of Flux vs TMP graph provides the permeance of respective membrane.

$$Permeance = \frac{Flux \left(\frac{l}{m^2.h} \right)}{pressure (bar)} = \frac{\Delta V}{A \cdot \Delta P \cdot t} \quad \text{eq. B}$$

Where, ΔV = volume of water, A = area of membrane, ΔP = trans membrane pressure and

t = time during flow of water

5.9 Protein Rejection Test

BSA protein was used as rejection solute in this test. The stock solution of BSA was prepared with concentration of 0.2g/200ml in UP water (pH 7.4 with PBS Buffer). The UV spectrophotometer was used to check the absorbance of the stock solution, permeate and retentate. The absorbance of the instrument was adjusted at 280 nm for BSA, and ultra-pure (UP) water was used as reference. During analysis pressure was set to 0.2 bar rendering a deviation of ± 0.02 at pressure gauge. The solution was stirred at 100rpm. The collection of permeate was halted at 3ml, the retentate from cell was also completely poured into test tube and UV absorbance was checked for both. In this manner the dead-end cell was re-filled with stock solution and the analysis was repeated three times. The absorbance of permeate and retentate were noted and converted to concentration by using the extinction coefficient of 1.62 obtained from the standard calibration curve of BSA already available in literature [118]. The solute rejections were calculated by using the **equation C**.

$$Rejection \% = 1 - \left(\frac{C_P}{C_R} \right) \quad \text{eq. C}$$

5.10 Chlorination of Membranes

The saturated polymer membranes s-P(NDH), and blend membranes of PES:s-P(NDH), PES:s-P(ONDH) and PES:PESmCA were chlorinated for ten mins in NaOCl solution adjusted to pH 7 using few drops of acetic acid. Further the membranes were washed in distilled water with stirring for 20 to 30 minutes. The membranes were kept in sterile water before antimicrobial tests to ensure the contamination free conditions.

5.11 Bacterial Challenge

Three types of antimicrobial tests were conducted to see the performance of chlorinated membranes. Control experiments of *E.coli* and *S.aureus* were done before these tests to select the suitable colony forming concentration from these bacteria to be prepared throughout the antimicrobial tests.

5.11.1 Control Experiment

The *S. aureus* with strain ID 25923 and *E.coli* strain name Top 10, were inoculated separately in 5 ml of Lysogeny Broth (LB) medium in test tube with the help of inoculum loop. Inoculated tubes were kept in shaking incubator for 18-19 hours and 16-17 hours respectively. The standard optical density at 600 nm for *S.aureus* 25923 was $0.4 = 5 \times 10^8$ CFU/ml and for Top 10 *E.coli* was $0.125 = 5 \times 10^8$ CFU/ml. The bacteria were harvested after respective time of inoculation following the procedure of repetitive washings using 1 ml of HEPES buffer using 2 ml volume of eppendorf tube. Each time the suspension was well mixed by pipetting and then centrifuged to pelletize the healthy bacteria. After that the suspension was removed and fresh 1 ml of HEPES was added. The OD was checked after fourth wash, while resuspending the bacteria in HEPES buffer 1000 μ l, 100 μ l was added into UV sample cuvette with 1900 μ l of HEPES and pipetted to mix. The OD was noted and multiplied by factor

of 20 to get the optical density of the healthy bacteria. The dilutions of 5×10^8 , 10^8 , 10^7 , 10^6 , 10^5 , 10^4 and 10^3 were prepared in the seven eppendorf tubes (**Table 5.1**) respectively [119].

Table 5.1: Dilutions of Control Experiment of *E.coli* and *S.aureus*

Experiment	Control	OD ₆₀₀ nm	OD x20 = Z	$\frac{5 \times 10^8}{(Y)(5)/Z}$	$\frac{10^8}{10^8/5 \times 10^8}$	$\frac{10^7}{10^7/10^8}$	$\frac{10^6}{10^6/10^7}$	$\frac{10^5}{10^5/10^6}$	$\frac{10^4}{10^4/10^5}$	$\frac{10^3}{10^3/10^4}$
<i>E.coli</i>		0.103	2.06	0.303 ml = 303 μ l	0.2ml= 200 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l
<i>S.aureus</i>		0.27	5.4	0.370ml = 370 μ l	0.2ml= 200 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l	0.1 ml = 100 μ l

From the dilutions prepared the 10^6 , 10^4 and 10^3 were selected to be plated for the bacterial colonies control check. The agar plates were seeded with 50 μ l from each selected concentration and spread at the surface with the help of glass beads. After 2 minutes the glass beads were removed, and the bacteria plated agar plates were incubated in heating oven at 37 °C for 18-20 hours. After the required time of incubation, the plates were examined.

5.11.2 Antimicrobial TESTS I: Static Agar

Three membranes were chlorinated for 10 mins in 15% NaOCl solution adjusted at pH of 7.4. Non chlorinated M_o and chlorinated M_{Cl^+} membranes were washed in sterile water with stirring of 30 mins. The bacterial inoculum was prepared in LB and kept incubated at 37°C for 16-17 hours. The bacteria were washed in HEPES buffer, and the UV was checked. From the optical density the bacterial concentration was adjusted to 5×10^8 CFU. Further the concentration was adjusted to 10^6 CFU and 50 microliter was seeded on the respective agar plates. One control plate was empty

agar plate, second control plate was agar plate with HEPES buffer, and seven agar plates were seeded with the actual bacterial concentration selected for the test. Three plates having non-chlorinated membrane M_0 , and three having chlorinated membranes M_{Cl}^+ while one was kept as bacterial control. The Membranes were placed at the top of agar surface and incubated for 18 hours at 37°C. Hence from each of the four membrane compositions six membranes of 4.1cm² were used, respectively in individual test. The tests were conducted and repeated at variable days, with new inoculum and same dilution procedure for *E.coli* and *S.aureus* respectively.

5.11.3 Antimicrobial TEST II: Dynamic (to and fro)

The dynamic test was conducted in small petri dishes capable of having 10ml of HEPES buffer with 10⁶ CFU of *E. coli*. The chlorinated membrane from each polymer were subjected to the to and fro motion of these petri dishes. This agitation was manual, and the careful periodic plating of the bacteria sample was done on 7 individual agar plates (the procedure was conducted for each membrane).

E.coli bacteria was selected for this test, and same procedure of inoculation and bacterial washing was adopted. The OD was determined and the bacterial concentration of 10⁶ CFU was adjusted in 10 ml of HEPES buffer. This concentration was placed in four small petri dishes and chlorinated membranes from each composition was placed in it, in consecutive tests. The plates were subjected to slight agitation, manual with to and fro motion. The 50 microliter of bacteria was plated from test plate after 30 sec, 60 sec, 3 min, 5 min, 10 min, 30 min and 60 mins respectively in seven agar plates having mixing beads. The seeding was spread with the help of glass beads and were removed after two mins. Agar plates were incubated for 17 hours at 37°C (**Figure 5.2**).

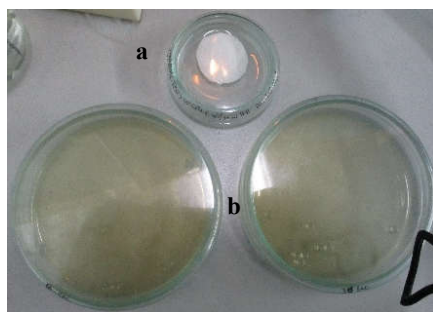


Figure 5.2: Dynamic test: a) Test plate, b) agar plates (two of seven during seeding)

5.11.4 Antimicrobial TEST III: Dead-end filtration

In this test 10^6 CFU from bacteria *E.coli* and *S.aureus* were adjusted separately in 10ml of HEPES buffer prepared in (4+4) x 10 ml large falcon tubes. The eight chlorinated membranes; two from each composition, were subjected to the dead-end flow of 10 ml suspension of 10^6 CFU of bacteria without any external pressure. The permeate was collected and 50 microliters from these were plated on the agar plates. The four agar plates were used; one for each type of membrane with two permeates, with 1/2 area of agar for *E.coli* and 1/2 for *S.aureus*. The membranes after the dead-end flow test were also placed at the top of agar plates to monitor the presence of any alive bacteria present at the surface. The plates were incubated for 18-20 hours at 37°C: providing the conditions suitable for bacterial growth.

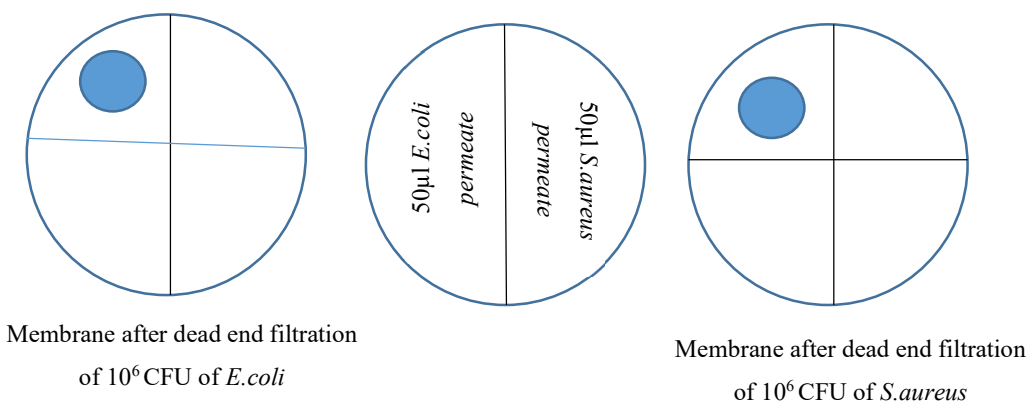


Figure 5.3: Antimicrobial TEST III: Agar plating of permeate and membrane after dead-end filtration.

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APPENDICES

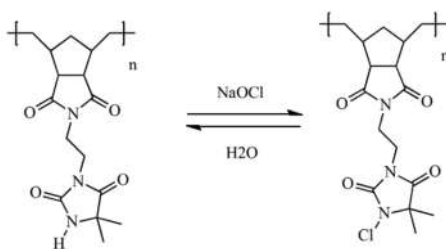
A. CHLORINATION OF MEMBRANES AND CHLORINE RELEASE TESTS

Reagents

- **Sodium thiosulphate** (Batch-I 0.1 and Batch-II 0.09 Molar) standardized against 6M HCl.
- **Starch indicator** (1g starch per 200 ml distilled water boiled, cooled and filtered)
- **15% NaOCl Stock Solution 100-200ml** (adjusted to pH7 by using acetic acid; freshly prepared).

a. Determination of Maximum Chlorination time of membrane s-P(NDH).

Membranes were stirred in 35 ml of stock solution with time of 1,2,3 and 4 hours. In each 35 ml of stock solution (used separately for each membrane) = 0.614mg of Cl^+ was present, respectively.



After chlorination, the membrane was placed in 35 ml of distilled water stirred for 15 mins. After this 1g of KI was added, which turned the solution and membrane to light yellow to brown in colour. The addition of KI had stained the membrane due to

the presence of active chlorine. The contents of the flask were titrated against $\text{Na}_2\text{S}_2\text{O}_3$ (0.1 M) solution until light yellow. Few drops of starch indicator was added, giving the solution and membrane light to medium blue shade. The contents were again titrated until colour less and the final reading was noted (Table A-1). The procedure was repeated for the other three membranes parts as well.

$$\text{Active Chlorine} = \text{Cl}^+ = \left\{ \frac{[\text{Na}_2\text{S}_2\text{O}_3(\text{ml})] \left[\text{Molarity of Na}_2\text{S}_2\text{O}_3 \left(\frac{\text{mol}}{1000\text{ml}} \right) \right]}{2} \right\} \times 35.5 \left(\frac{\text{g}}{\text{mol}} \right)$$

Table A-1 : *Iodometric testing of membranes after first chlorination cycle*

Each 1/4 th of membrane part Time for stirring (hours)	Uptake Capacity of Cl^+ in membrane from the 35 ml solution having 0.614g Cl^+ available. (mg)	Theoretical assumption for whole membrane (mg)
1	0.12	0.48
2	0.36	1.44
3	0.51	2.04
4	0.51	2.04

The maximum limit of adsorption of active chlorine and bonded chlorine in membrane is three hours.

b. TEST II: Determination of Maximum chlorination in membrane.

Polymer s-P(NDH) fabricated membrane were vacuum dried at 30 °C for 1 h. Dried membrane was weighed (w_1) and stirred for 60 mins, 30 mins and 10 mins in 100 ml of 15% NaOCl solution adjusted to pH 7 using acetic acid. The chlorinated membrane was washed by dipping in water three times to remove the adsorbed chlorine. Chlorinated membrane was vacuum dried at 30°C for one hour and weighed (w_2). The membranes stirred for 10 mins, 30 mins and 60 mins showed different chlorine uptake, further the 60 mins stirring was chosen initially (Figure A-1).

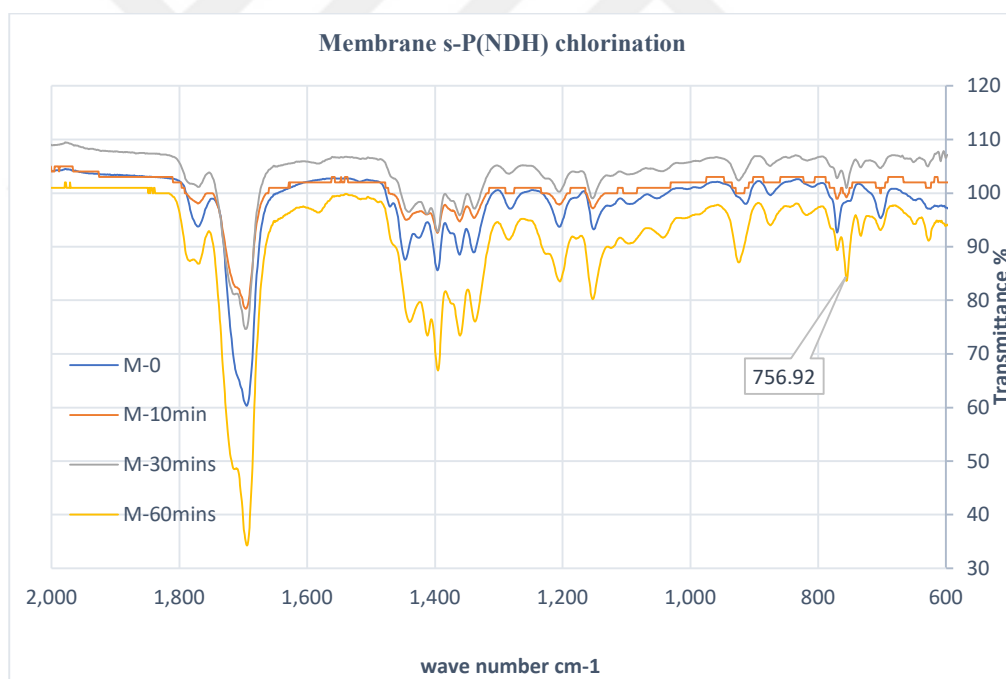


Figure A.1: ATR-IR of s-P(NDH) at variable times of chlorination

c. Chlorine release test (unstirred condition)

New piece of membrane dried in vacuum oven for 1 h (0.0250g). Then it was stirred in 100 ml of 15% NaOCl solution at pH 7. Membrane stirred for 60 mins, dip washed three time and dried in oven for 1 h. The dried chlorinated membrane (0.0254g) was soaked in 20 ml of water. The membrane was taken out and repeatedly soaked into next beaker with 20 ml of water. The solutions were collected are tabulated in Table A-2.

Table A-2 *Chlorine release test*

Beaker with 20 ml of water	Membrane soaking time Sec	*Addition of KI (0.4) g
1	30	-
2	30	
3	60	-
4	60	
5	120	-
6	600	
7	900	-
8	1800	-
9	3600	-

(* KI test for presence of active chlorine)

The static test was completed in time of two hour. Water analysis showed absence of chlorine when KI was added in the beakers after respective time of soaking of membrane.

d. Chlorine release test (stirring condition).

The same membrane was subjected to the dynamic test. The membrane was stirred in the 20 ml of water over the period of 2 hours by shifting membrane after calculated time and the sample water was observed using KI (0.4g) for presence of any chlorine Table A-3.

Table A-3: *Dynamic chlorine release test*

Beaker with 20 ml of water	Membrane stirring time (min)	Addition of KI (0.4) g	Beaker with 20 ml of water	Membrane stirring time (min)	Addition of KI (0.4) g
1	1	-	9	5	-
2	1	-	10	5	-
3	1	-	11	10	-
4	2	-	12	10	-
5	2	-	13	10	-
6	2	-	14	30	-
7	5	-	15	30	-
8	5	-			

Weight of membrane 0.0250g

Weight of membrane after chlorination = 0.0254g

Weight of membrane after release test (vacuum dried) = 0.025

The adsorbed chlorine was removed from the membrane after drying the chlorinated membrane in vacuum oven. The bonded chlorine did not leach into water even with maximum time of stirring. In the proceeding tests the vacuum drying of membrane

after chlorination was avoided to determine the correct time of chlorination and amount of adsorbed chlorine in membrane.

e. Dynamic test II Determination of active chlorine release rate from s-P(NDH) membrane (non-vacuum dried condition)

A fresh piece of membrane dried in oven for 1 h, was chlorinated in 100 ml of 15% NaOCl solution at pH 7 for 60 mins. The membrane was left to stir in distilled water for 5 days for chlorine release rate with periodic change of fresh water. The periodic assessment of water sample after stirring the same membrane to 128 h of dynamic testing while changing the water each day, on day 5th the water showed no sign of active chlorine. The membrane was not stained with KI, it was dried for one hour at ambient conditions and the ATR-IR was checked. It was noted that the chlorination peak was still at its position of 756 cm⁻¹. This peak intensity was compared with the chlorinated membranes of variable time (Figure A-2, A-3). It was seen that the membrane chlorinated for 10 mins had the same IR N-Cl bond intensity as the membrane subjected to chlorine release test (Table A-4).

Table A-4: Amount of Adsorbed chlorine in membrane of s-P(NDH)

Beakers with 20 ml of water	Membrane stirring time (h)	Addition of KI in water (0.4) g	Na ₂ S ₂ O ₃ used (x.ml)	Cl ⁺ = [(0.09mol/l) (x.ml) (35.5g/mol)]/2 (g)
1	56	+	0.2	0.00032
2	24	+	0.7	0.0011
3	24	+	0.4	0.0006
4	24	-	-	-

Amount of adsorbed chlorine in membrane after 60 mins of chlorination = 2.02mg

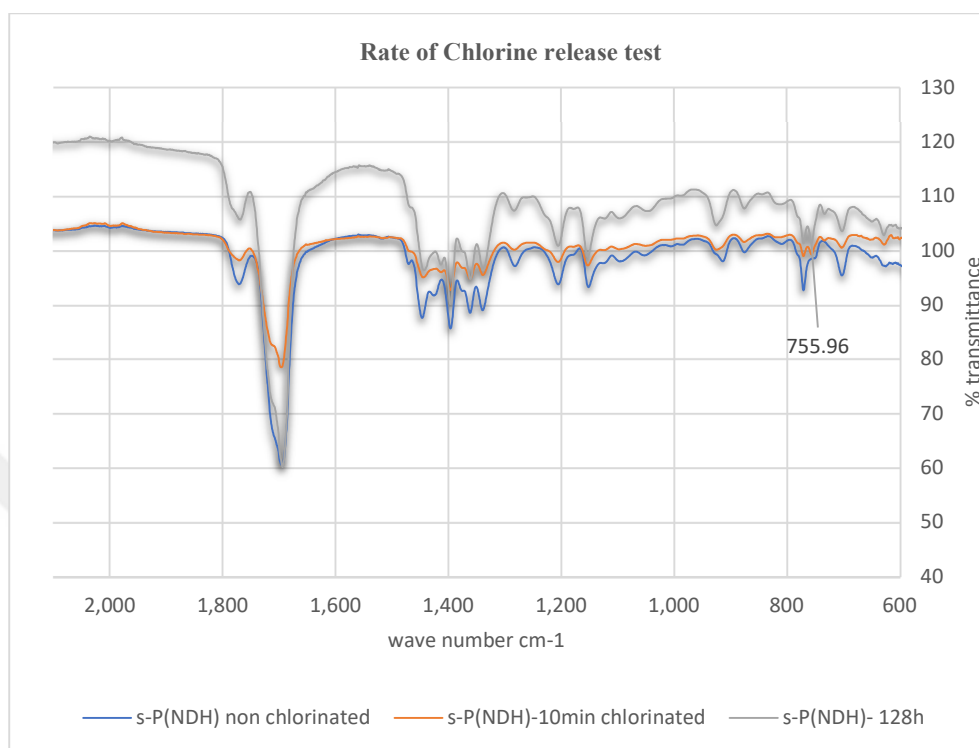


Figure A-2: Comparative ATR-IR of membrane after release of adsorbed chlorine

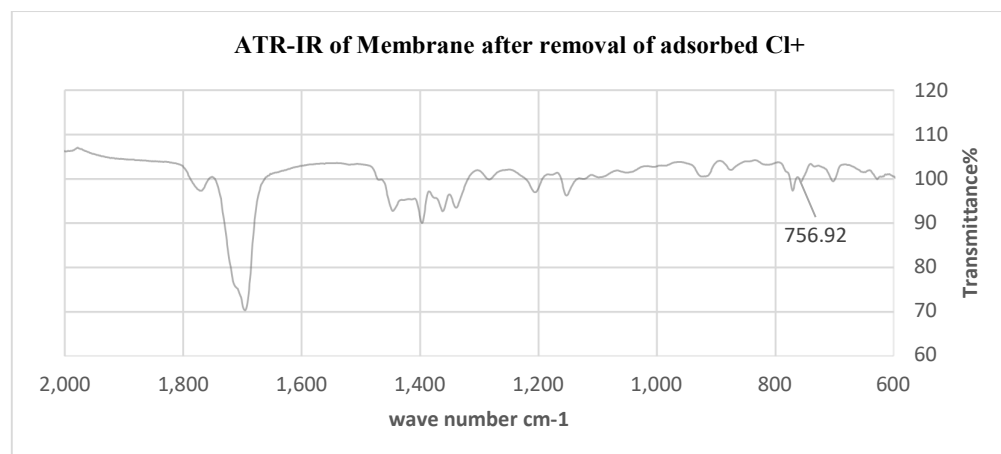


Figure A-3: ATR-IR of membrane s-P(NDH) of bonded chlorine

f. Determination of Adsorbed Chlorine in Ten Minutes stirring

New piece of s-P(NDH) membrane of 4.1 cm² was dried in vacuum oven at 30 °C for 1 hour. 15% NaOCl solution prepared in 100 ml of water adjusted to pH 7. The dried membrane was stirred in 35 ml of stock solution for 10 to 12 minutes. Membrane was immediately stirred in fresh water (distilled) for almost 30 mins while changing the water three times to remove the adsorbed chlorine (**Table A-5**). Further, membrane was dried at ambient conditions and ATR-IR was checked (**Figure A-4**).

Table A-5: Quantification of Adsorbed chlorine (Cl⁺) in membrane

Water beaker	KI test	Na ₂ S ₂ O ₃ used X ml	Cl ⁺ = [(0.09mol/1000ml) (x ml) (35.5)]/2 (mg)
1	+	0.2	0.32
2	+	0.1	0.16
3	-	-	-

Total Cl⁺ adsorbed in membrane after ten mins stirring time = 0.48mg

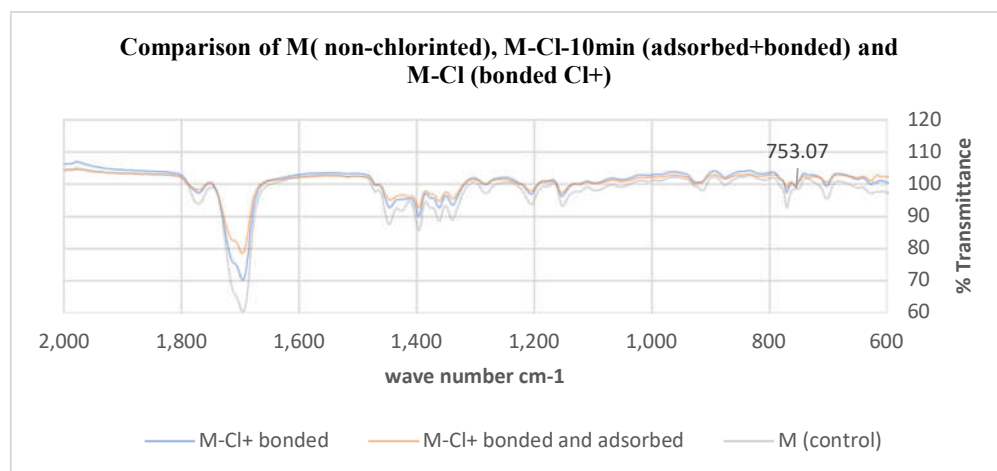


Figure A-4: ATR-IR of membrane after ten minutes of chlorination

g. Membrane Sacrificial iodometric test

In a small beaker membrane s-P(NDH) chlorinated and washed was added to 1 ml of DMF. After complete solubility KI (0.4 g) was added. With the addition of KI the contents of beaker turned yellow /brown in color which turned dark blue with the addition of 2 ml of Starch indicator. It was then Titrated with 0.09 molar $\text{Na}_2\text{S}_2\text{O}_3$ solution (**Table A-6**). It was seen that the polymer fully precipitated as white solid. It was filtered and dried for further use. This test was done only with membrane of s-P(NDH).

Table A-6: Sacrificial Membrane test for quantification of bonded Cl^+ in membrane.

	$\text{Na}_2\text{S}_2\text{O}_3$ used X ml	$\text{Cl}^+ = [(0.09\text{mol}/1000\text{ml}) (\text{x ml}) (35.5)]/2$ (mg)
1.	0.7ml	1.12mg

Total adsorbed Cl^+ in membrane= 0.48mg

Bonded Cl^+ to membrane= 1.12mg.

h. Determination of Adsorbed Chlorine in Blend Membranes

For blend membranes same ten minutes chlorination time and 15-20 minutes washing time was followed. The KI and starch indicator were added to the sample before titrating it against $\text{Na}_2\text{S}_2\text{O}_3$. The active chlorine was calculated from average of sodium thiosulphate used during each membrane test, that was determined from three data points respectively (Table A-7). The IR was checked after removal of adsorbed chlorine.

Table A-7: Adsorbed Chlorine in Blend Membranes

Blend Membrane	Average $\text{Na}_2\text{S}_2\text{O}_3$ used (ml) (y)	$Cl = \frac{(y)(0.09)(35.5)}{2 \times 1000}$ (g)	Adsorbed Cl^+ (mg)
PES:s-P(ONDH)	2.7	4.3×10^{-3}	4.31
PES:s-P(NDH)	0.5	7.9×10^{-4}	0.79
PES:PESmCA	1.1	1.75×10^{-3}	1.75

B. Antimicrobial Test Preparation and Sterile Methods

The reagents used during antimicrobial tests are LB, LB-agar and HEPES buffer. The experimental preparations are presented in table B-1 and method of experiment in Table B-2 respectively.

B.1 Reagent 1: Lysogeny Broth (LB)

Ingredients*

Yeast 1g

NaCl 1g

Peptone 0.5g

In sterile deionized water the ingredient quantities are assembled. Further in the test tubes 5 ml of LB is poured and autoclaved before preparing the bacterial inoculum. (*for 100 ml deionized water, using required sterilized dried glass ware).

LB = Sterilize and keep at ambient conditions.

pH=7.4 using NaOH 0.5 molar solution.

B.2 Reagent 2: Lysogeny Broth Agar (LB-A)

Ingredients*

Yeast 1g

NaCl 1g

Peptone 0.5g

Agar 1.5g

In sterile deionized water the ingredient quantities are assembled and autoclaved. Further it is heating and homogenizing at 200-220°C. (*for 100 ml deionized water, using required sterilized dried glass ware).

pH=7.4 using NaOH 0.5 molar solution

LB-A= is plated in petri dishes right after complete solubility of agar.

B.3 Reagent 3: Hepes Buffer (HB)

NaCl 15mmol

Hepes 10 mmol

pH 7.4 (using NaOH pellet)

In sterile deionized water the ingredient quantities are assembled and autoclaved before use.

Table B-I: *Pre-Experimental Preparations*

S.no	Experiment Title	Instrument	Procedure	Operation completion time
1	Sterilization (micro pipettes, forceps, spatula, glass bottles of required volume, Eppendorf tubes, glass tubes, glass beads)	Autoclave	The required materials are place in the Autoclave. The chamber base is filled with distilled water one inch above the base. The lid of autoclave is closed until the indicator light turns off. The System temperature is set to the temp of 120 °C for time of 20 mins. It is a cyclic operation. After 3 hours the autoclave chamber is turned off and the lid is safe to open when the temperature reaches to ~80 °C.	3 h + x h (x h = time of cooling down of chamber)
2	Drying	Drying oven	The sterilized materials are then placed in drying oven for overnight. The temperature is maintained at 65°C.	10 -12 h

S.no	Experiment Title	Instrument	Procedure	Operation completion time
3	Reagents assembling		<u>Reagent 1: Lysogeny Broth (LB)</u> Use: Inoculum of bacteria (inoculum-1: I-1) <u>Reagent 2: Lysogeny Agar (LA)</u> Use: solid growth surface Inoculum of bacteria: inoculum 2 (I-2) <u>Reagent 3: HEPES Buffer (HB)</u> Use: in preparation of I-2 [All the reagents after assembling are autoclaved before use.]	1 day
4.	Platting of Agar plates	Vertical Laminar Flow Hood	<u>Protocol 1</u> (Sterilize hands with 70% ethanol). Place the LB-A bottle in the hood. Open the batch of petri dishes and place them in the hood. Take one plate; open 1/3 of the lid, pour the LB-A to the 1/2 of plate, shake slightly in circle to remove any bubble and to even the level. Place the 1/3 opened petri dish towards the safe/farthest side in hood until it solidifies. After it solidifies in max. 20 mins, cover the lid and place the agar plate (AP) in fridge for further use. This operation is repeated consecutively for the number of AP of interest.	

Table B-II: *Experimental Sequence*

Day	Experimental Title	Procedure	Equipment/ instrument	Operation time
1	Inoculum-1(I-1)	<u>Protocol 2</u> (Sterilize hands and working area with 70% ethanol and open Bunsen burner laminar flame for **sterilized operation in lab). **(~23 cm radii of sterile surface from the Bunsen flame.) Prepare inoculum from the required bacterial strain stock kept at -80 °C in glycerin. Take a small scoop of bacteria using inoculating loop and carefully place in the test tube having 5 ml of LB while dipping it without touching the sides of test tube. Prepare the required test tubes with the same procedure. Cover the lids of test tube. (all of this operation is done in the **sterile zone of laminar flame). Place the test tube/s in the shaking incubator at 37 °C at 200 rpm for 17-18 hours. Place the I-1 in fridge (4 °C for 20-30 mins) before harvesting of bacteria.	Sterilized apparatus. Shaking incubator	Y hours + 17-18h (Y h = time to prepare sample for I-1)

Day	Experimental Title	Procedure	Equipment/ instrument	Operation time
2	Concentrating the bacteria of I-1 (I-1 _c)	<Observe Protocol 2> Take Eppendorf tube and add 2ml of I-1, placing the lids back immediately. Centrifuge the eppendorf for 2mins at 3000 rpm. Discard the supernatant while small white pallet of bacteria will stay stick in the cone of eppendorf tube. Add again 2 ml of I-1 and repeat the procedure, finally labelled as I-1_c. <u>Protocol 3</u> (Glass bottle sprayed with 70% ethanol and used as trash can for all the supernatant and micropipettes used during the operation).	Required materials and Centrifuge instrument.	
Same day	Washing and sample preparation of concentrated Inoculum-1 (I-1 _c)	<Observe Protocol 2> In eppendorf tube of I-1_c add H.B 1ml and do pipetting(mixing). Cover the lid of eppendorf and wait 3-4 mins. This procedure is to remove the dead bacteria. Centrifuge the bacteria at 3000 rpm for 2 mins. Discard the supernatant. Repeat the procedure 3 times. Add 1ml H.B. to final palette of bacteria in eppendorf tube, now termed as Inoculum-1 washed (I-1_w) <Observe Protocol 3>	Required Materials. Centrifuge.	

Day	Experimental Title	Procedure	Equipment/instrument	Operation time
Same day	Measurement of Optical Density	Take clean UV cuvettes, pour 2ml of H.B in two cuvettes and take reference absorbance to auto zero, where the UV was already adjusted to 600nm. From the sample cuvette remove 100 μl of H.B and add 100 μl of I-1_w. Place the cuvette in sample holder in the instrument and note the absorbance. Example Calculation: Absorbance=0.080 for 100 μl Hence OD of 2000 μl of I-1_w = 1.6 <u>Experimental optimized value of <i>E.coli</i> Top 10 bacterial strain at 600 nm is 0.125 having 5×10^8 CFU/ml. and for <i>S.aureus</i> 25923 is 0.4 having 5×10^8 CFU/ml used to adjust the dilutions in this work.</u> <u>Sample 1 Inoculum-2</u> with 5- or 10-times Dilution adjusted with following formula. $OD_{\text{optimized}} \times 5 = OD_{\text{sample}} \times V_1$ Further the required dilutions of 5×10^8, 10^8, 10^7, 10^6, 10^5, 10^4 and 10^3 are prepared for control experiments and in same manner for the antimicrobial tests as well. <Observe Protocol 3>	Required Materials. Centrifuge. UV.	

Day	Experimental Title	Procedure	Equipment/ instrument	Operation time
Same day	Inoculum-2 (I-2)	<observe Protocol 2> Labelling the required number of agar plates. Putting 7 glass beads in each plate. Sample preparation in new eppendorf using the I-2 and the antimicrobial /test material with required concentration. Or putting the required test sample after placing the 50 µl of I-2 from 5 times dilution sample and 10 times dilution sample in separate plates and helping the even absorption of I-2 by slow circular motions of glass beads. The procedure is repeated until required no' of control, blank and antimicrobial sample is placed in the agar plate. The deposited volume is kept to 50 µl in each. After this step the beads are removed out of the agar plate in separate bottle sprayed with 70% ethanol (to wash and reuse). The plates are inverted, bringing agar base to the top side and incubating the plates at 37 °C for 17-18 hours in oven for inoculum of bacteria at solid surface. If membranes are placed at the top of bacteria plated agar plates, then the plates are not inverted. They are incubated as it is. <Observe Protocol 3>	Heating oven.	

Day	Experimental Title	Procedure	Equipment/instrument	Operation time
3.	Bacteria Count	Bacteria colonies are counted and noted.		
4.	Washing procedure	If agar plates are disposable, they are poured with bleach solution and dispatched off in plastic bags. If agar plates are glass petri dishes, they are poured in with bleach solution and left for 30 mins. After that the agar with seeded bacteria are removed with spatula into plastic waste disposal bags and seal locked. While the plates are washed with warm soap solution, rinsed with water, and distilled water. Plates are dried at ambient condition and wrapped in aluminum foil for autoclaving and reusing.		

B- 3 Calculations of bacteria estimation and percent kill.

Average bacteria colonies under one membrane = 830 CFU

Formula:

(Area of agar plate / area of membrane) x Average bacteria colonies under one membrane = $(78.5\text{cm}^2/4.1\text{cm}^2) \times 830 \text{ CFU} \approx 15,770 \text{ CFU}$

1. percentage of colonies found in *E.coli* filtrate of PES:s-P(ONDH) = $17/15770=0.11\%$

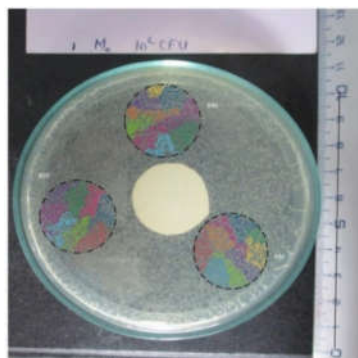
✓ Kill % = 99.89

2. percentage of colonies found in *S. aureus* filtrate of PES:s-P(NDH) = $14/15770 = 0.09\%$

✓ kill % = 99.91

3. percentage of colonies found in *S. aureus* filtrate of PES:PESmCA = $2/15770=0.01\%$

✓ kill % = 99.99



(from the following photograph, *E.coli* 10^6 CFU, assumption for *S.aureus* is also taken from the same as *E.coli* is smaller in size from *S.aureus*)

C. SUPPORTING INFORMATION

C-1 ATR-IR of *p*-toluenesulfonyl chloride (TsCl) and *p*-toluenesulphonyl hydrazide (TSH)

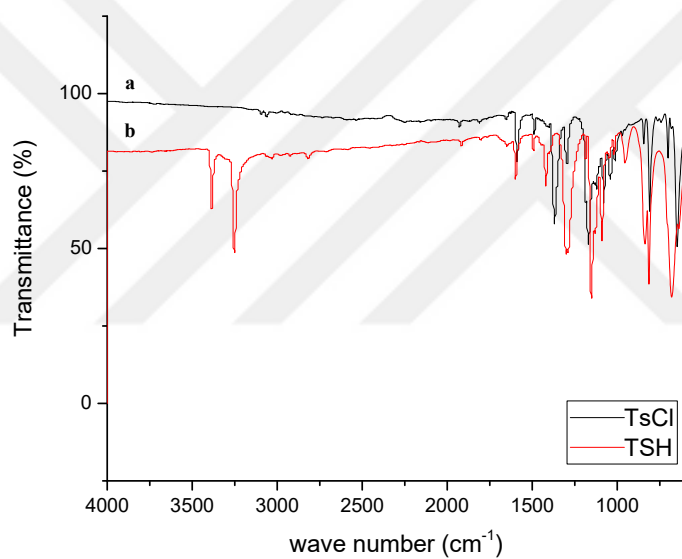


Figure C-1: IR of a) *p*-toluenesulphonyl chloride; b) *p*-toluenesulphonyl hydrazide.

C-2 XRD of compound 15 and 16

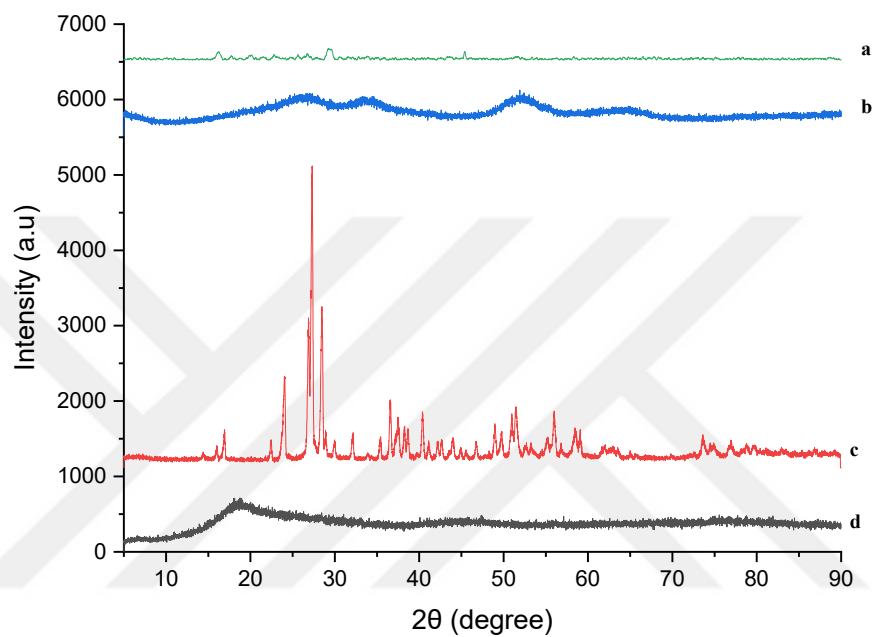
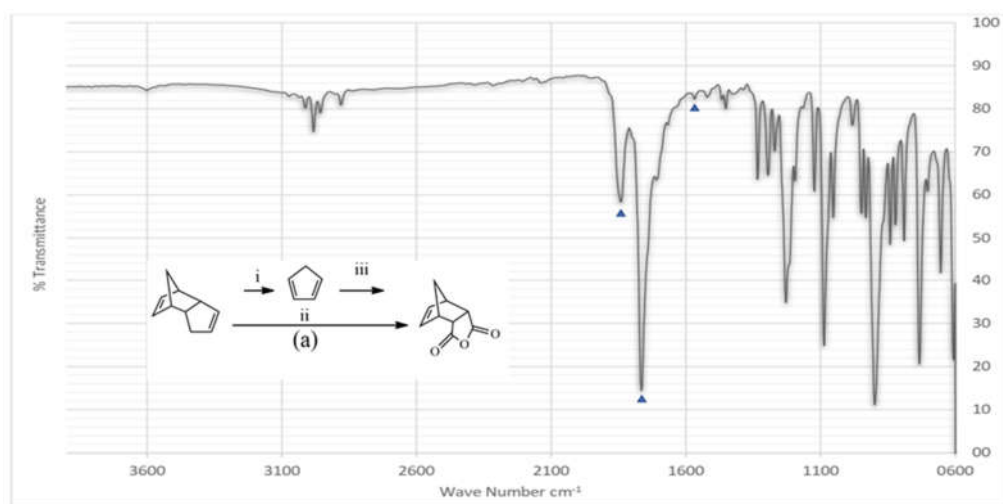


Figure: C-2: a) Compound 16; b) compound 15; c) Cyanuric chloride; d) PES.

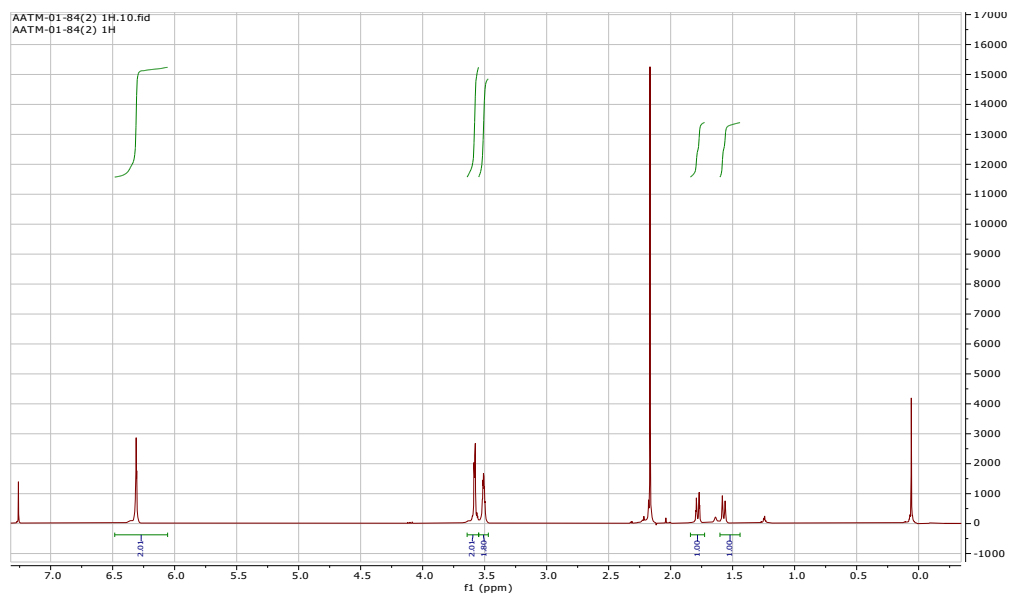
C-3 Characterizations during synthesis of P(NDH) and s-P(NDH)

Compound 1

ATR-IR

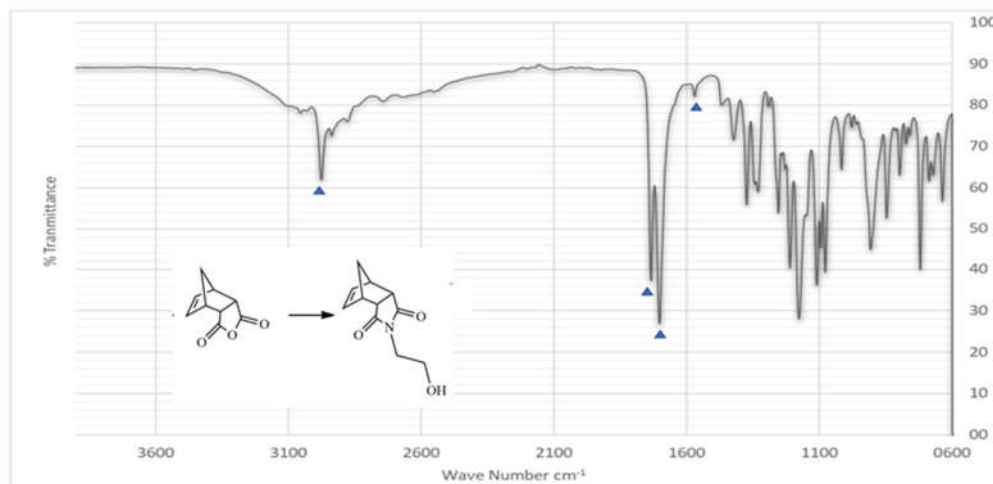


¹H NMR

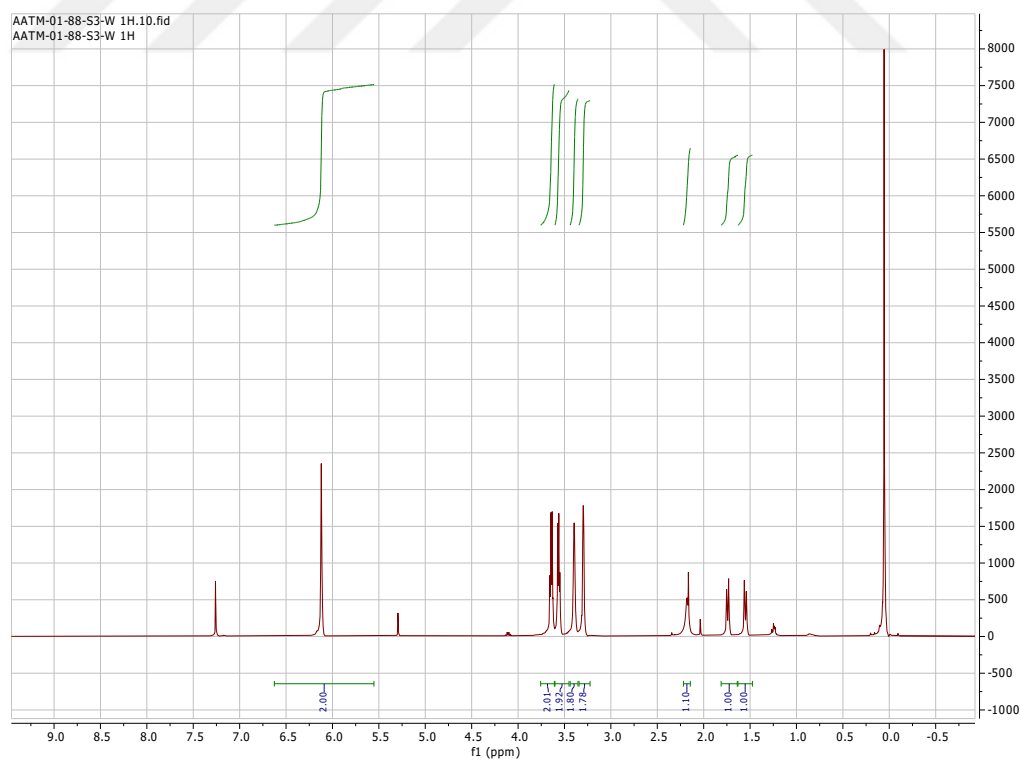


Compound 2

ATR-IR

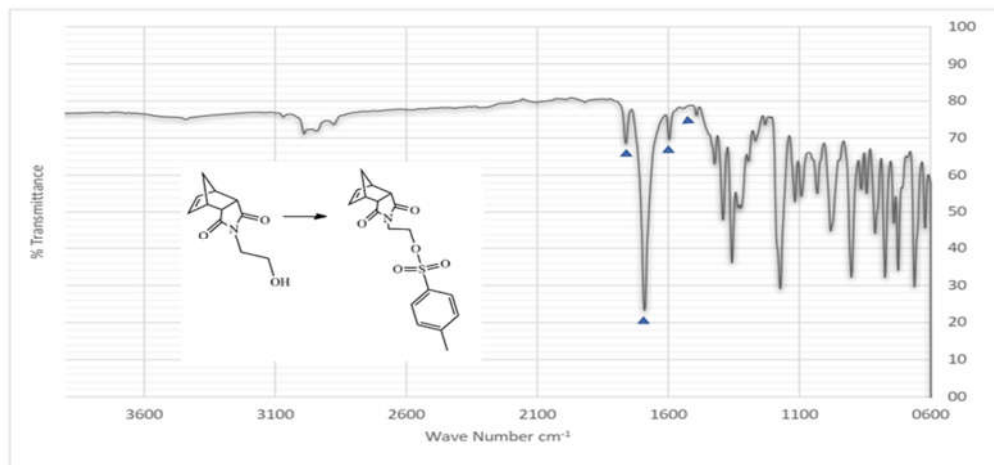


^1H NMR

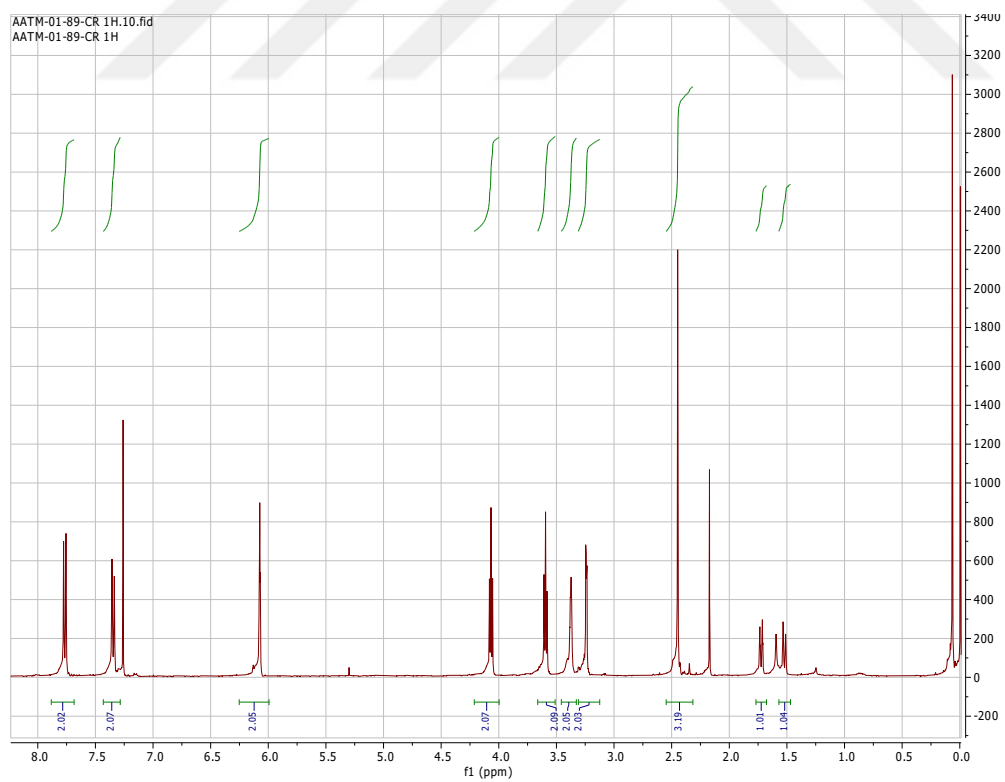


Compound 3

ATR-IR

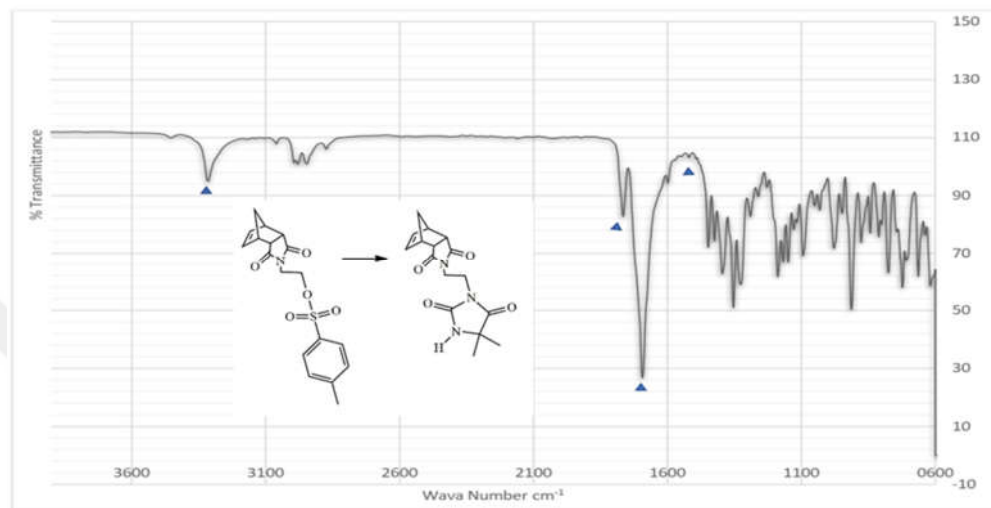


^1H NMR

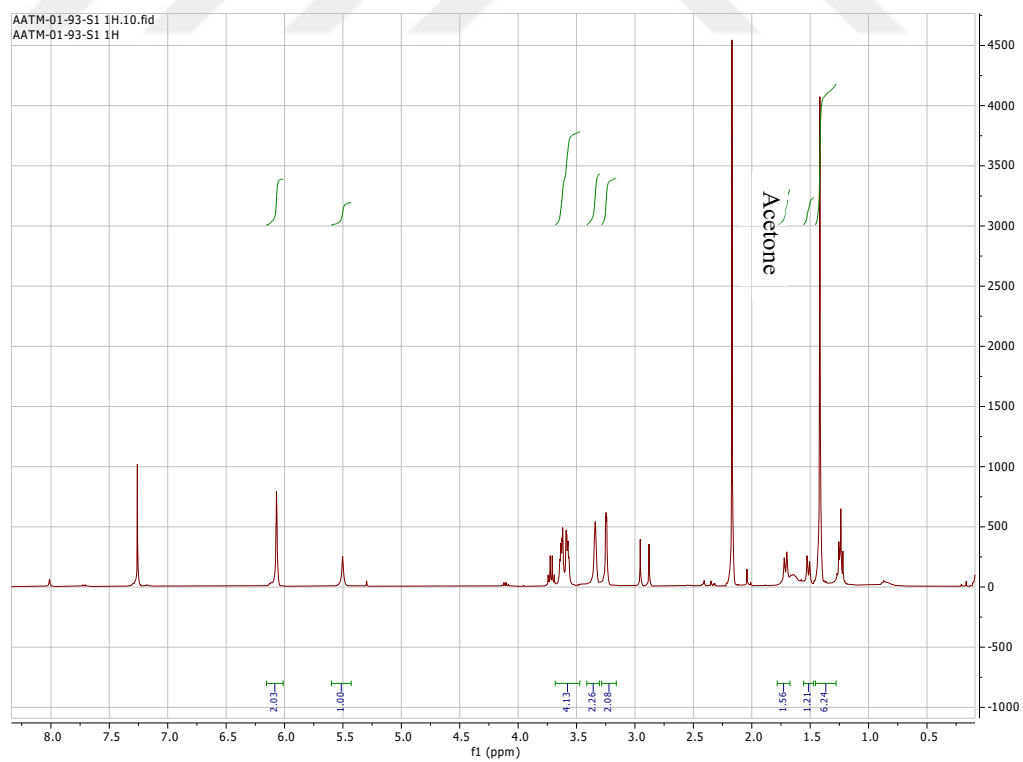


Compound 4

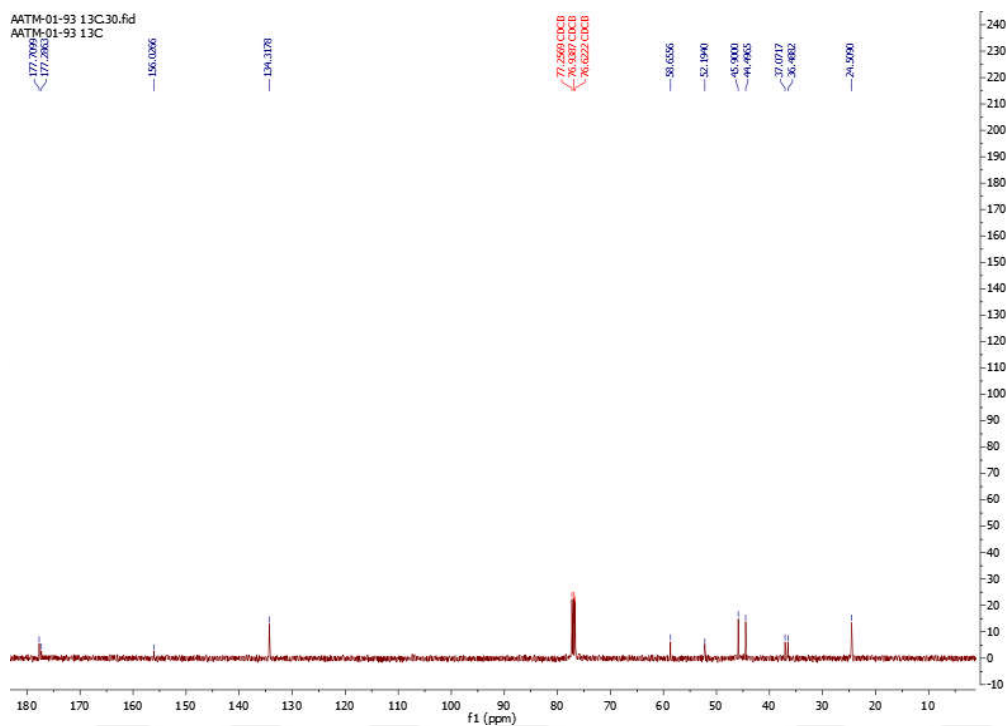
ATR-IR



¹H NMR

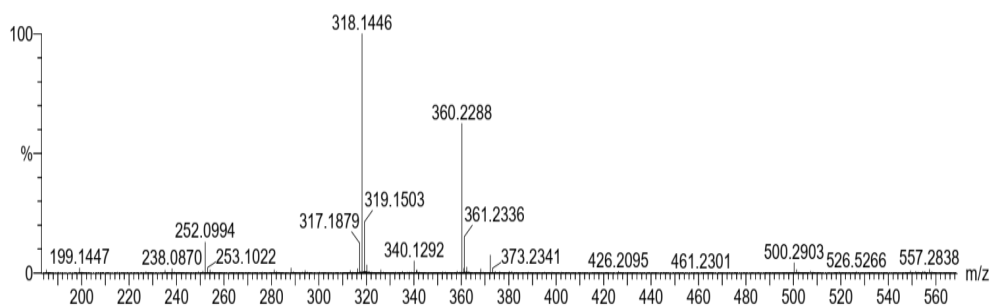


¹³C NMR



HRMS

C₁₆H₁₉N₃O₄ + H⁺ Mol.weight 318.1446 g/mol

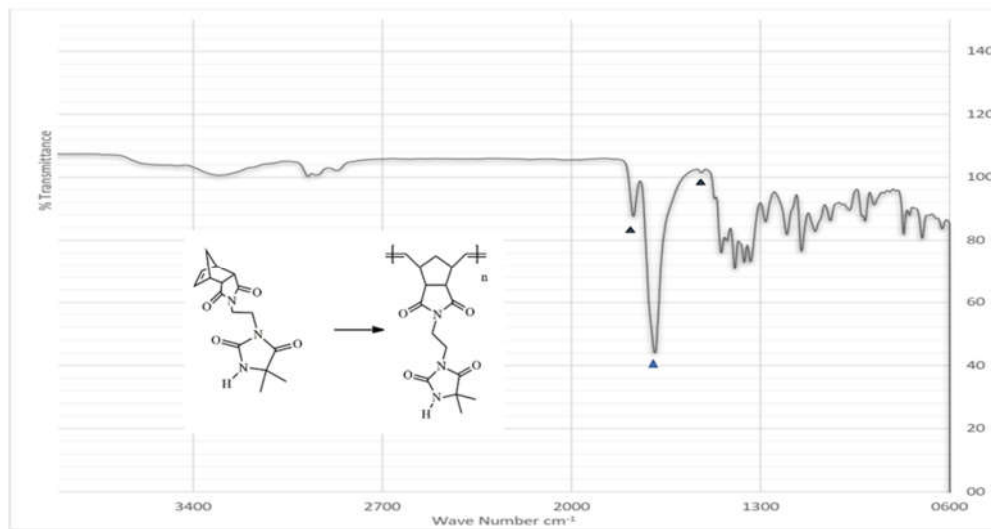


Minimum: -5.5
Maximum: 1000.0 5000.0 1000.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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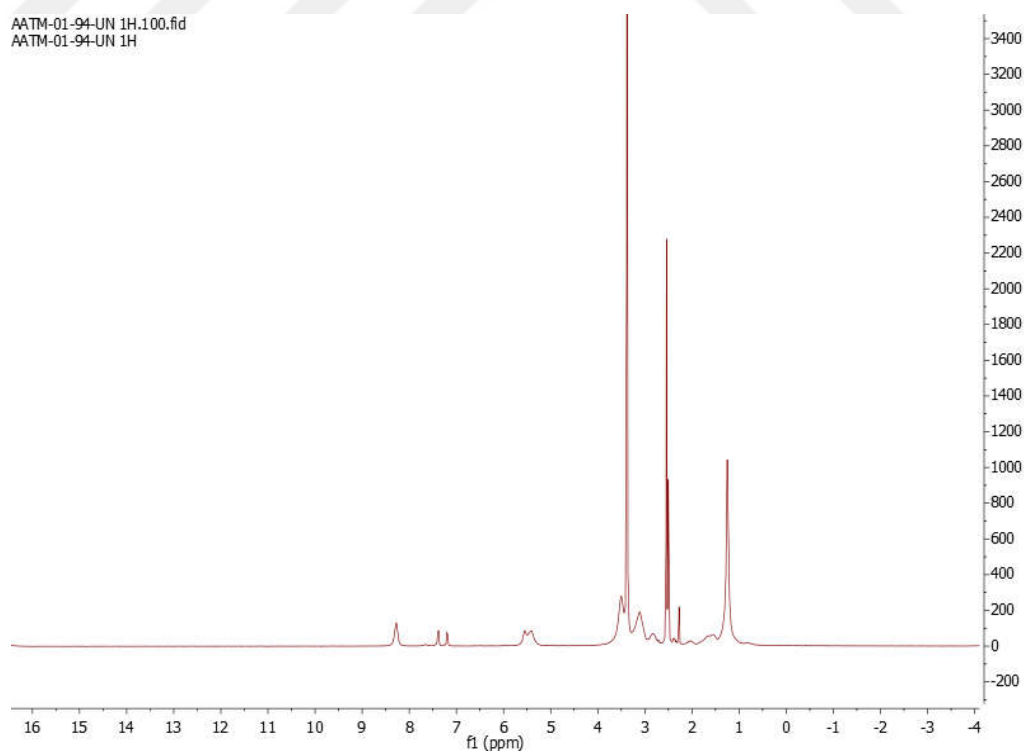
Compound 5

ATR-IR

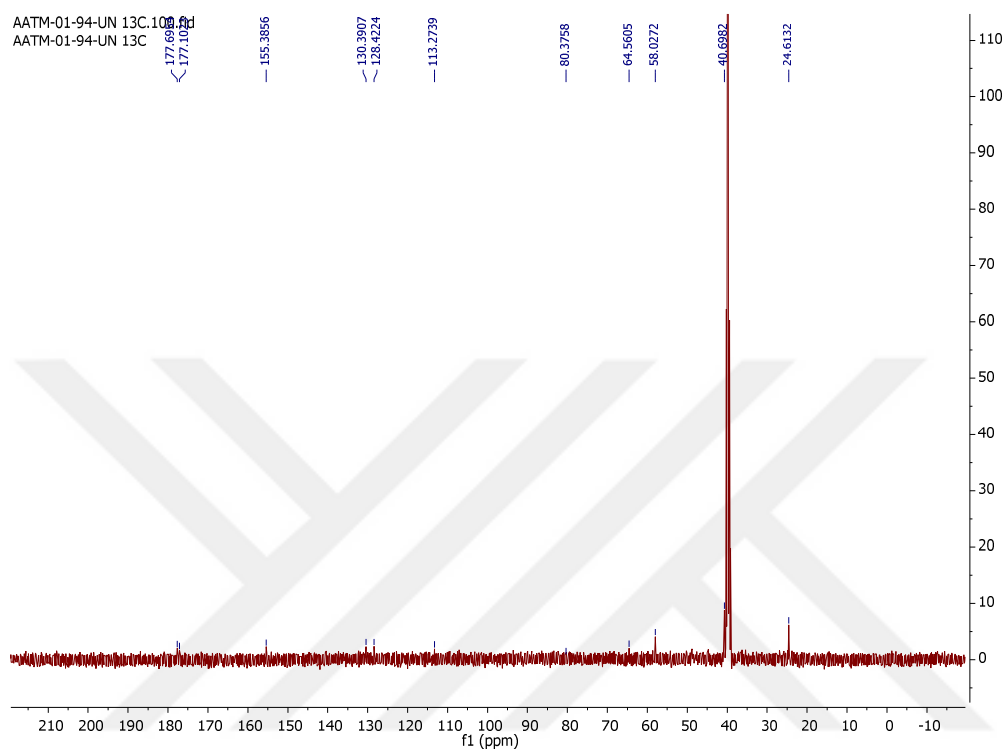


¹H NMR

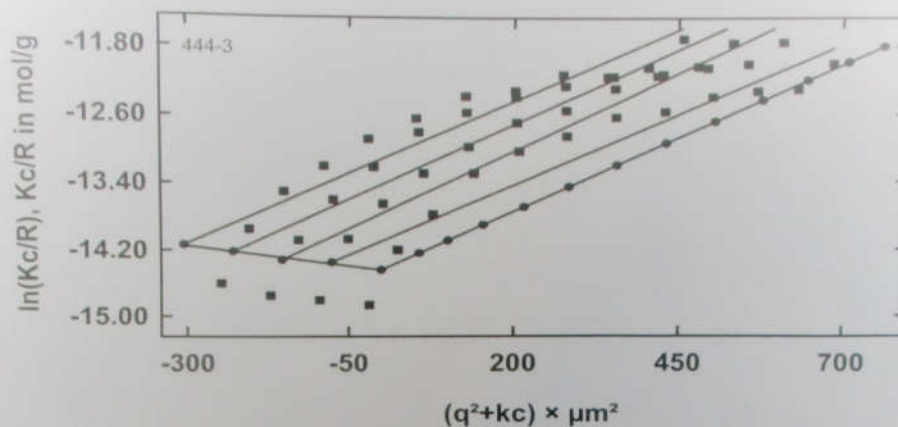
AATM-01-94-UN 1H.100.fid
AATM-01-94-UN 1H



¹³C NMR



SLS



Mw(c): 1.869e+06 g/mol Mw(q²): 1.869e+06 g/mol
A2: 2.617e-09 mol dm³/g² Rg: 1.020e+02 nm
C:\Program Files\ALV_V3.0\21082020_30242\444-3_1.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-3_2.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-3_3.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-3_4.sta

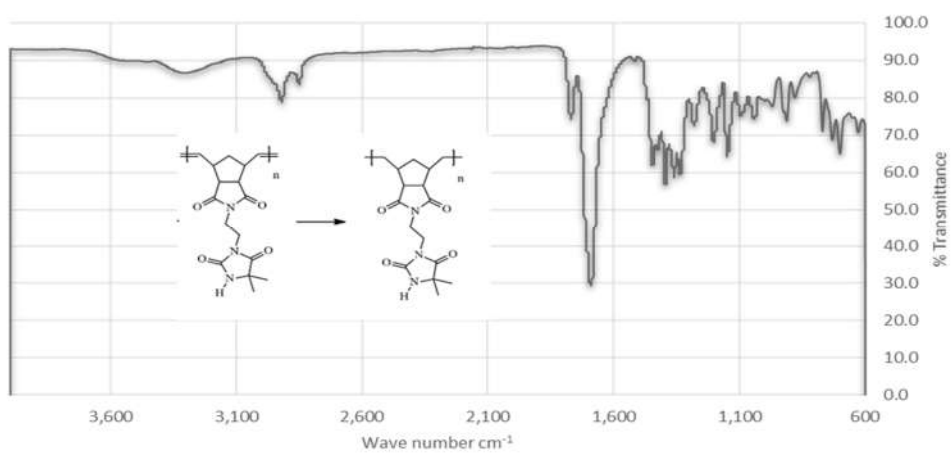
444-3
Static Light Scattering, results of Guinier-Plot, q²-dep.: 1, c-dep.: 1 smoothed

File	Conc/(g/dm ³)	Mw(app)/(g/mol)	<S ² >(app)/μm ²	Rg(app)/nm
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444-3_2.sta	22.8000	1.494e6	1.054e-2	102.643
444-3_3.sta	15.2000	1.656e6	1.099e-2	104.842
444-3_4.sta	7.6000	1.704e6	9.962e-3	99.809

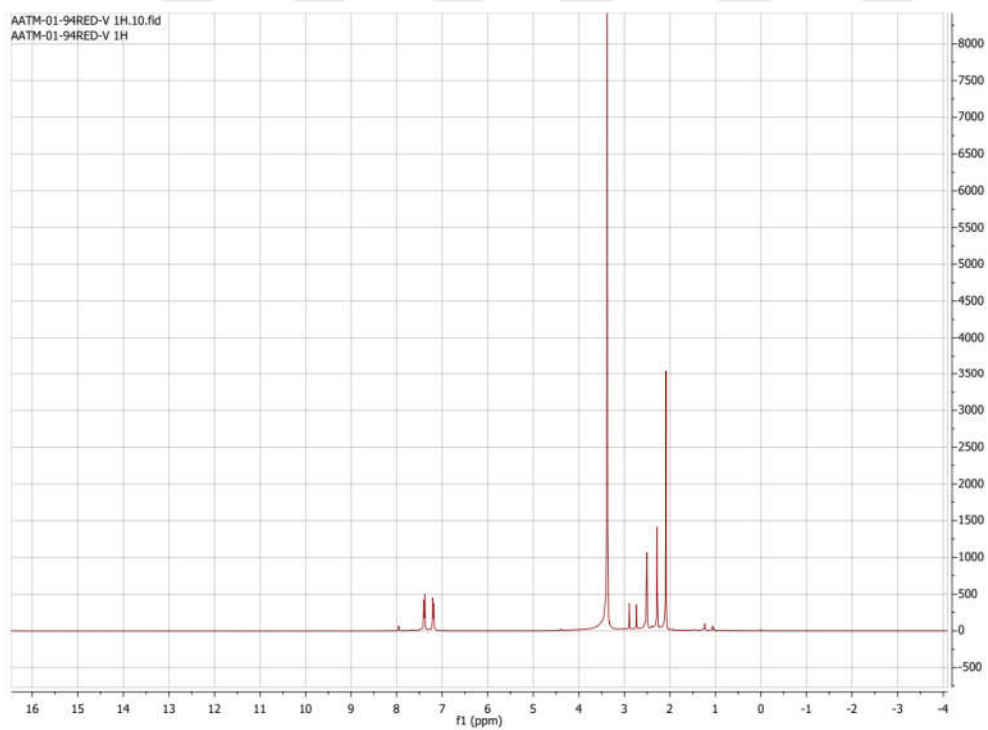
Mw(c): 1.869e+06 g/mol (± 2.99 %) Mw(q²): 1.869e+06 g/mol (± 5.3e-13 %)
A2: 2.617e-09 mol dm³/g² (± 14.8 %) Rg: 1.020e+02 nm (± 1.5e-13 %)

Compound 6

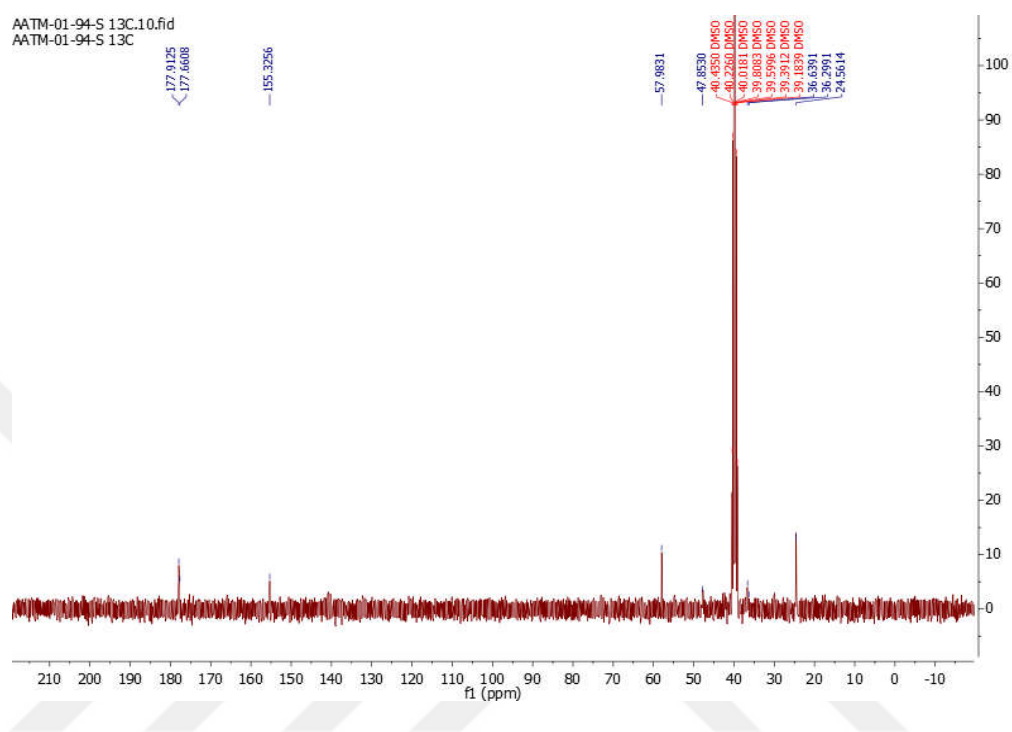
ATR-IR



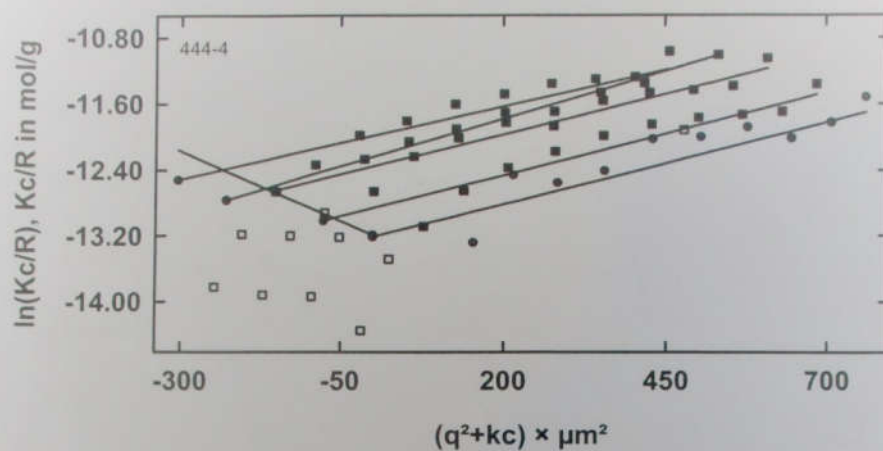
^1H NMR



¹³C NMR



SLS



Mw(c): 5.415e+05 g/mol Mw(q²): 5.539e+05 g/mol
A2: 3.112e-08 mol dm³/g² Rg: 7.733e+01 nm
C:\Program Files\ALV_V3.0\21082020_30242\444-4_1a.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-4_2.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-4_3.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-4_4.sta

444-4
Static Light Scattering, results of Guinier-Plot, q²-dep.: 1, c-dep.: 1

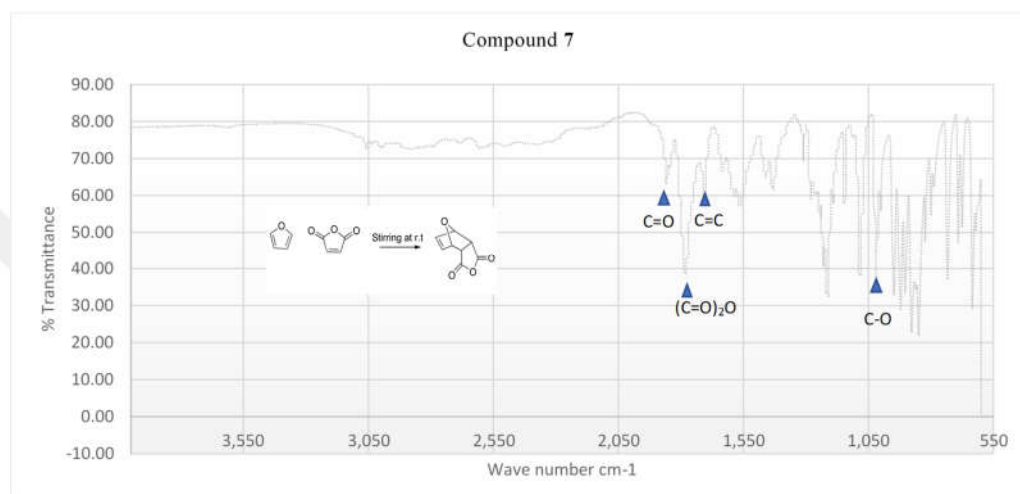
File	Conc/(g/dm³)	Mw(app)/(g/mol)	<S²>(app)/μm²	Rg(app)/nm
Conc.=0	0.0000	5.539e5	5.980e-3	77.329
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444-4_2.sta	23.2100	3.515e5	6.914e-3	83.151
444-4_3.sta	15.4700	3.142e5	5.838e-3	76.406
444-4_4.sta	7.7400	4.548e5	6.045e-3	77.747

Mw(c): 5.415e+05 g/mol (± 5.48 %) Mw(q²): 5.539e+05 g/mol (± 5.96 %)
A2: 3.112e-08 mol dm³/g² (± 11.8 %) Rg: 7.733e+01 nm (± 2.21 %)

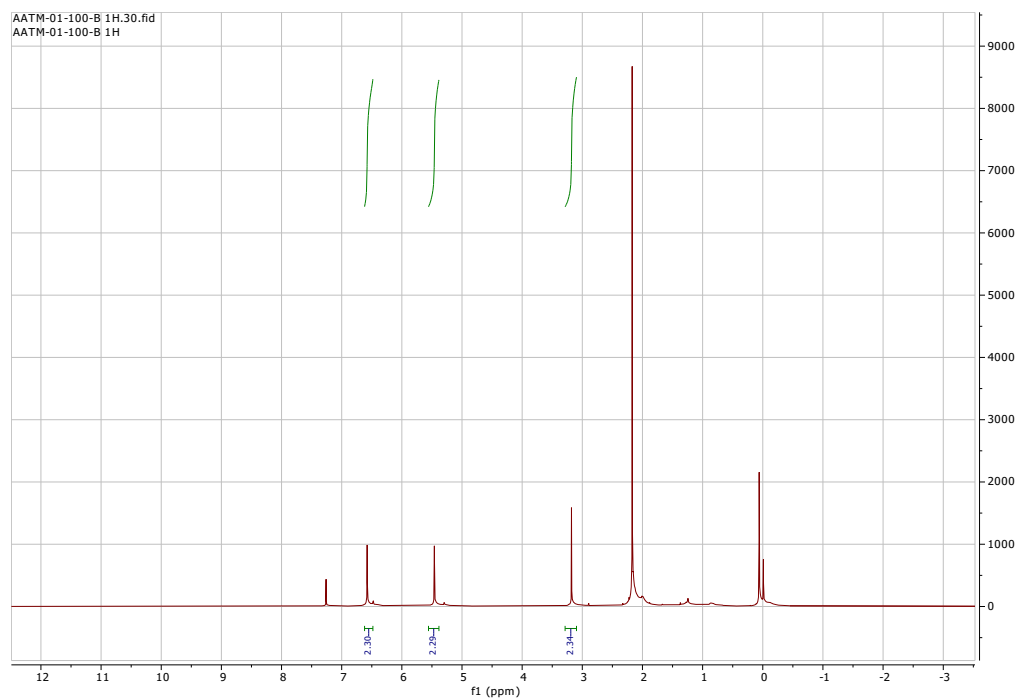
C-3 Characterizations during synthesis of P(ONDH) and s-P(ONDH)

Compound 7

ATR-IR

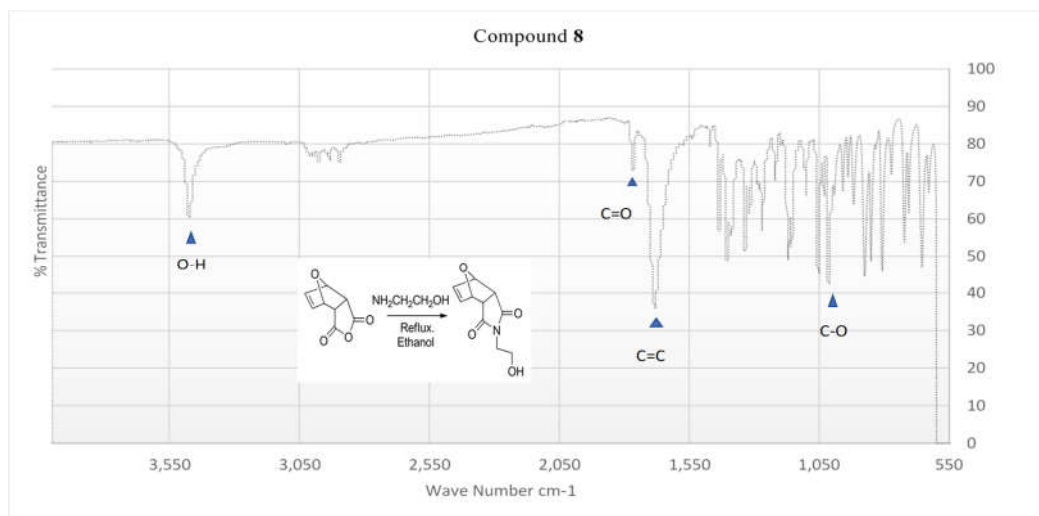


^1H NMR

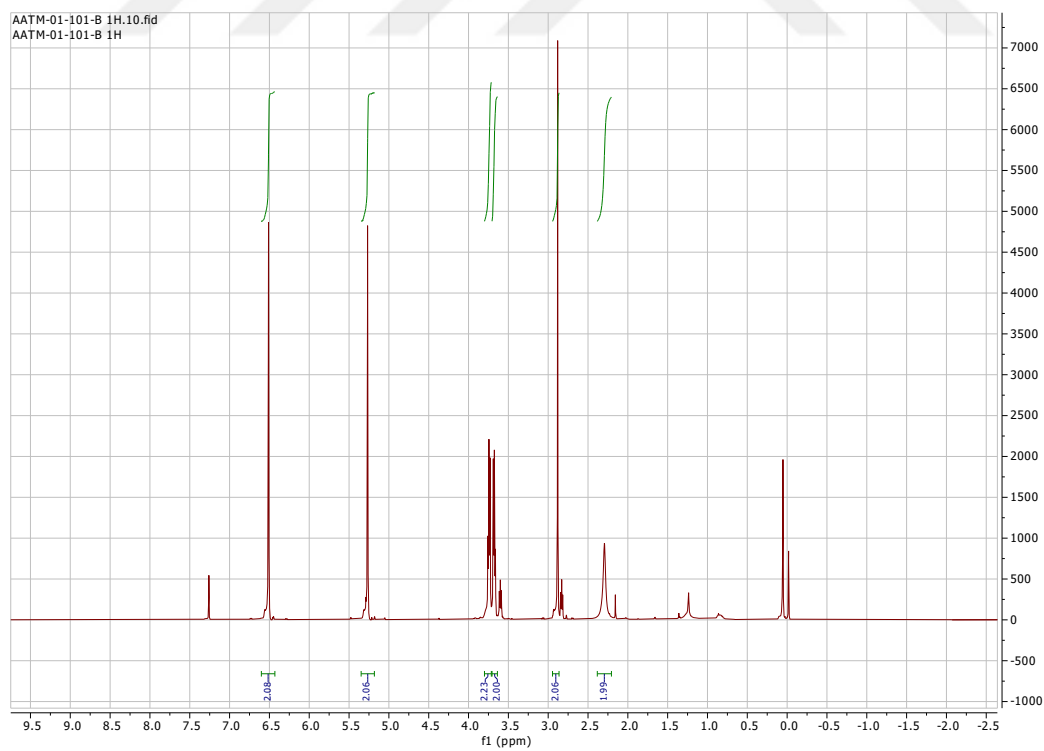


Compound 8

ATR-IR

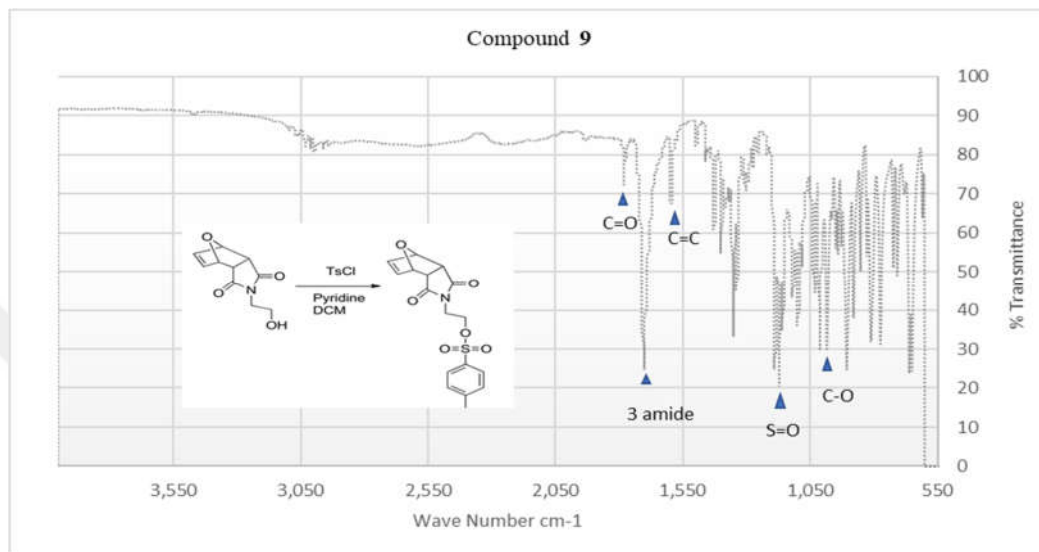


¹H NMR

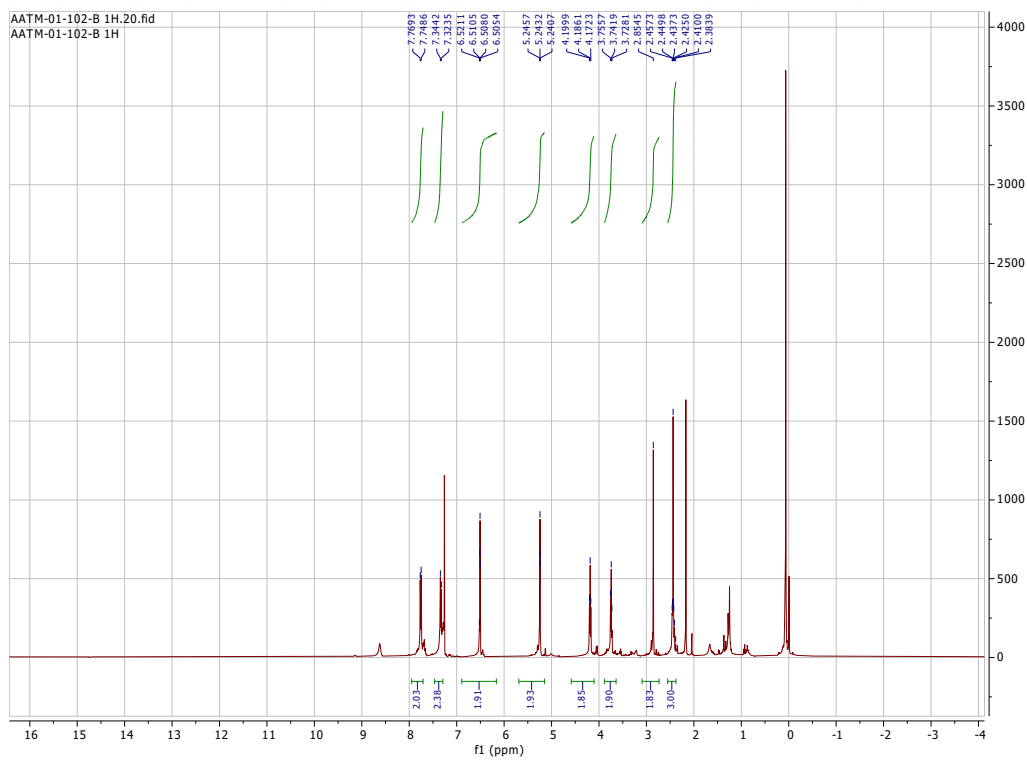


Compound 9

ATR-IR

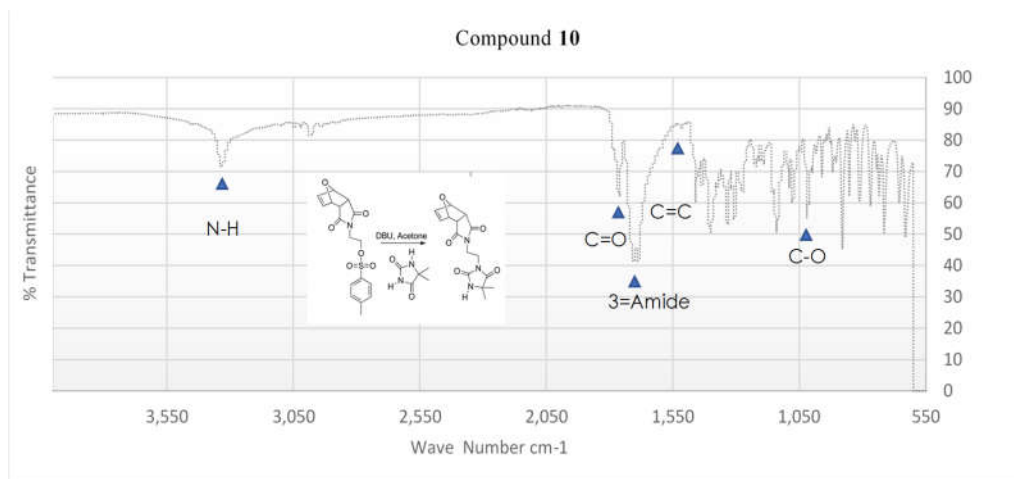


¹H NMR

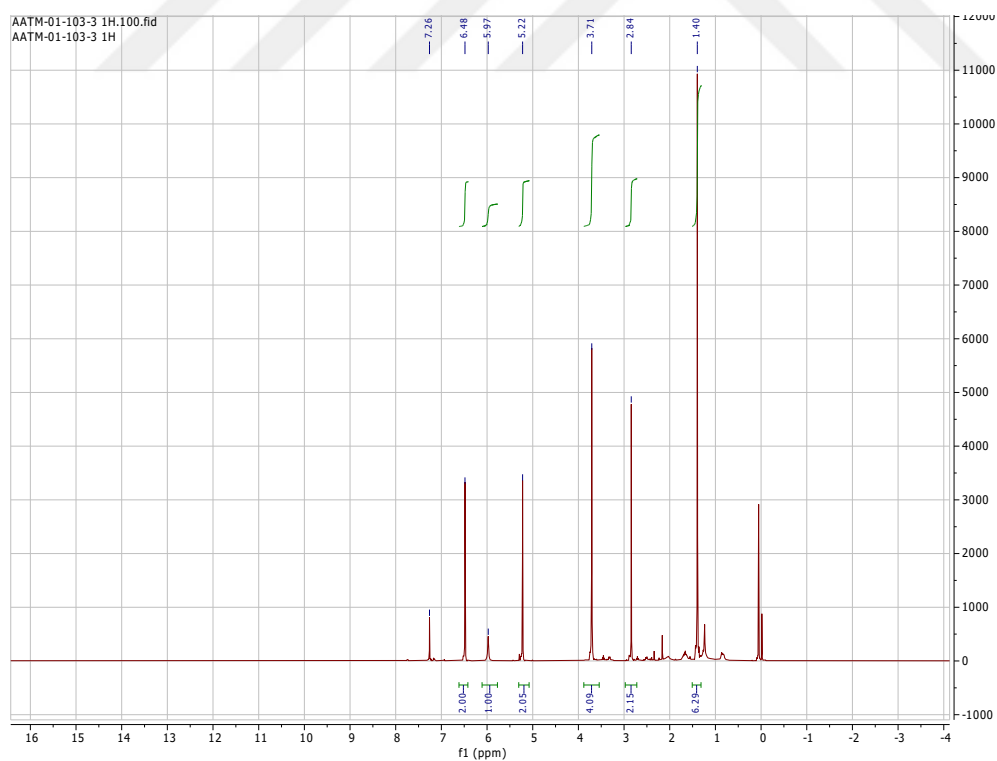


Compound 10

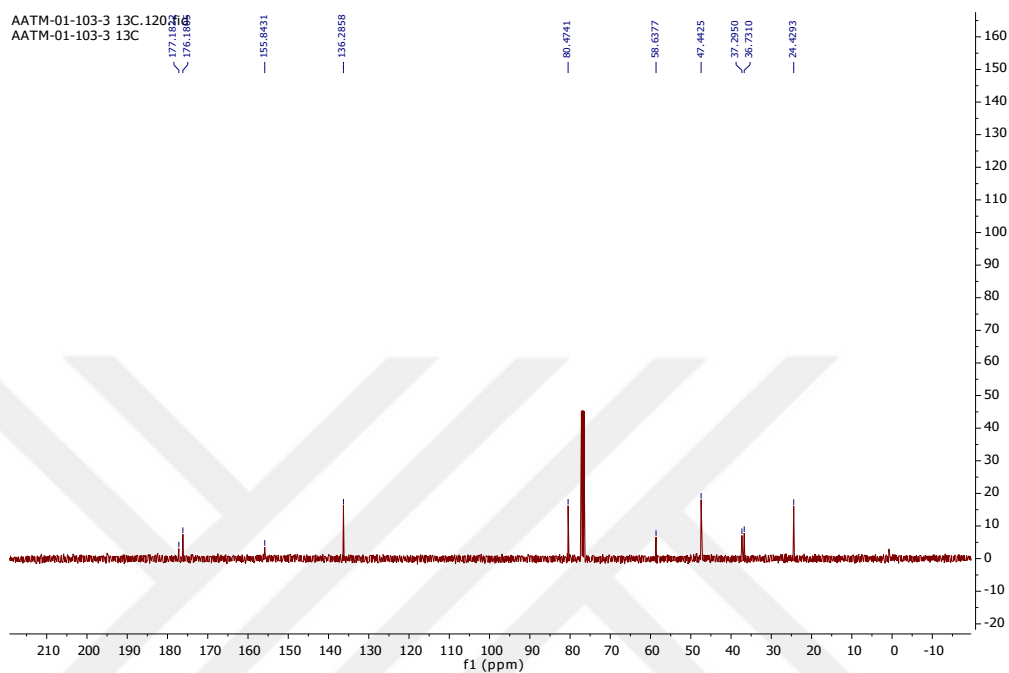
ATR-IR



¹H NMR

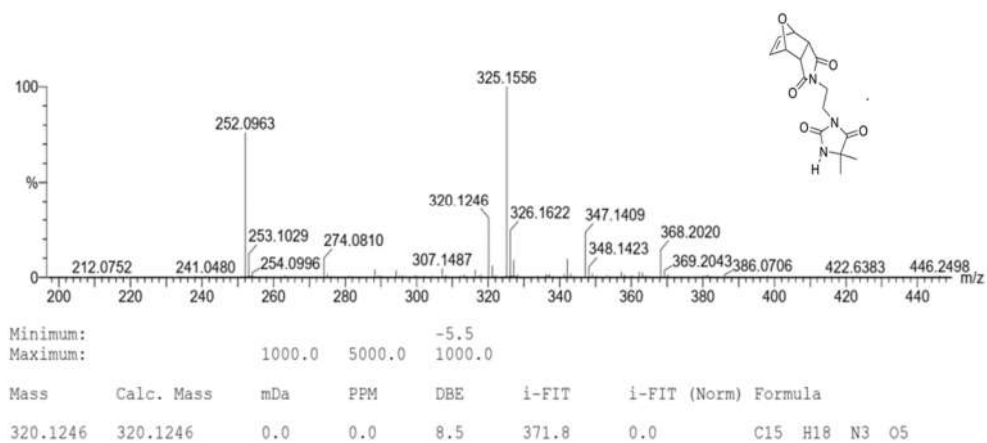


¹³C NMR



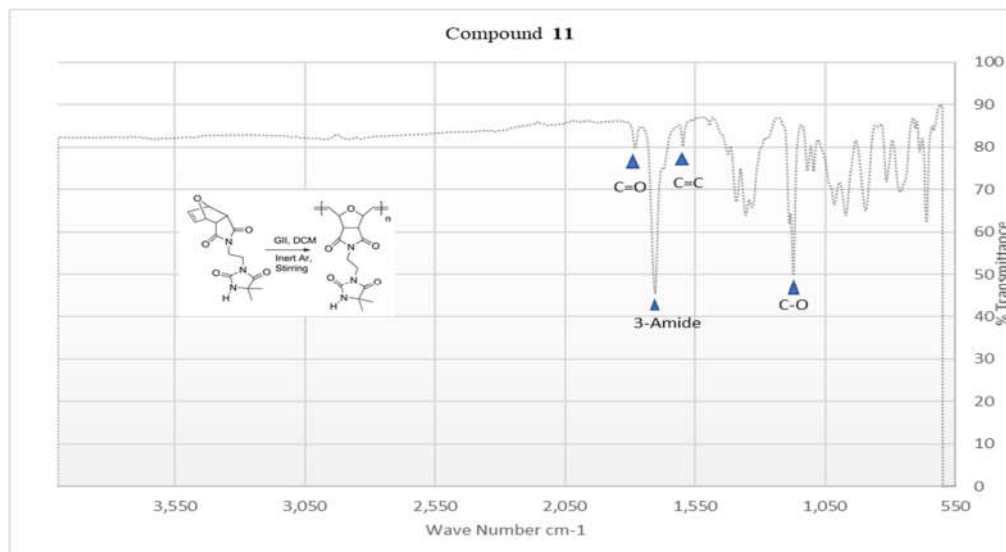
HRMS

Monomer 2: C₁₅H₁₇N₃O₅ + H⁺ Mol.weight = 320.1246 g/mol



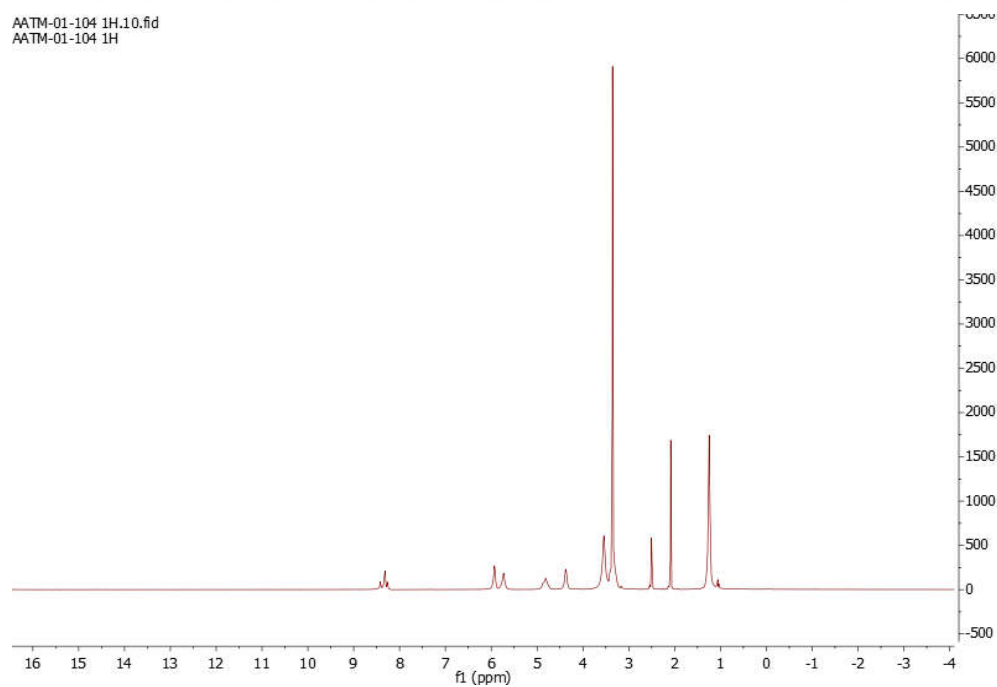
Compound 11

ATR-IR

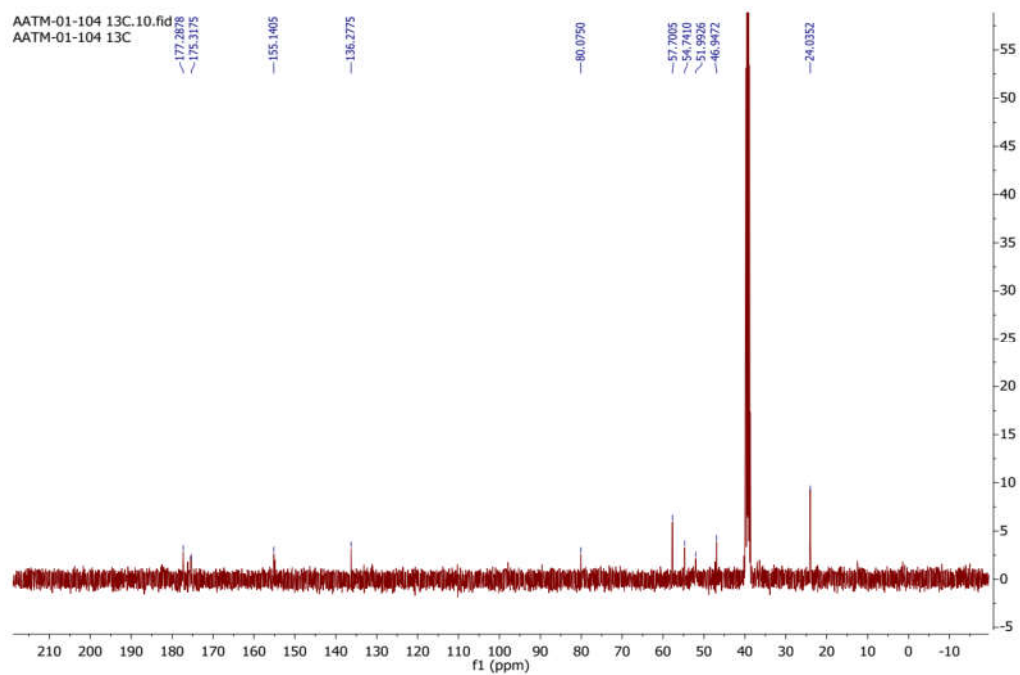


¹H NMR

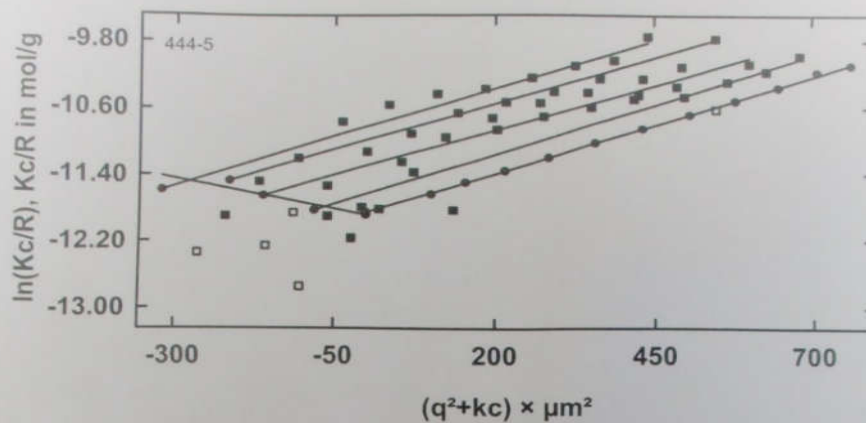
AATM-01-104 1H.10.fid
AATM-01-104 1H



^{13}C NMR



SLS



Mw(c): 1.482e+05 g/mol Mw(q²): 1.438e+05 g/mol
A2: 2.177e-07 mol dm³/g² Rg: 8.422e+01 nm
C:\Program Files\ALV_V3.0\21082020_30242\444-5_1.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-5_2a.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-5_3.sta; C:\Program Files\ALV_V3.0\21082020_30242\444-5_4.sta

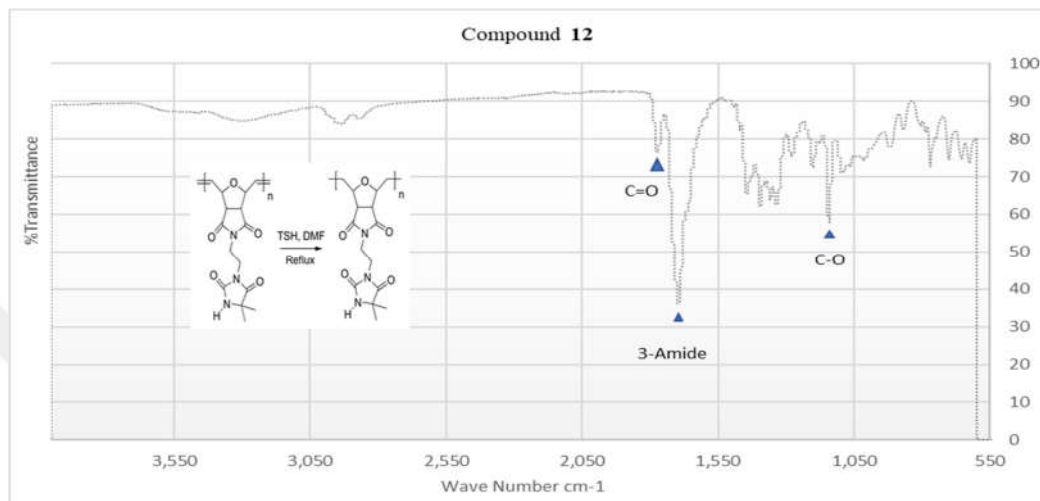
444-5
Static Light Scattering, results of Guinier-Plot, q²-dep.: 1, c-dep.: 1 smoothed

File	Conc/(g/dm³)	Mw(app)/(g/mol)	<S²>(app)/μm²	Rg(app)/nm
Conc.=0	0.0000	1.438e5	7.094e-3	84.223
444-5_1.sta	7.6250	1.073e5	6.993e-3	83.627
444-5_2a.sta	5.0800	9.679e4	6.728e-3	82.022
444-5_3.sta	3.8100	1.166e5	6.578e-3	81.105
444-5_4.sta	1.9100	1.398e5	7.252e-3	85.161

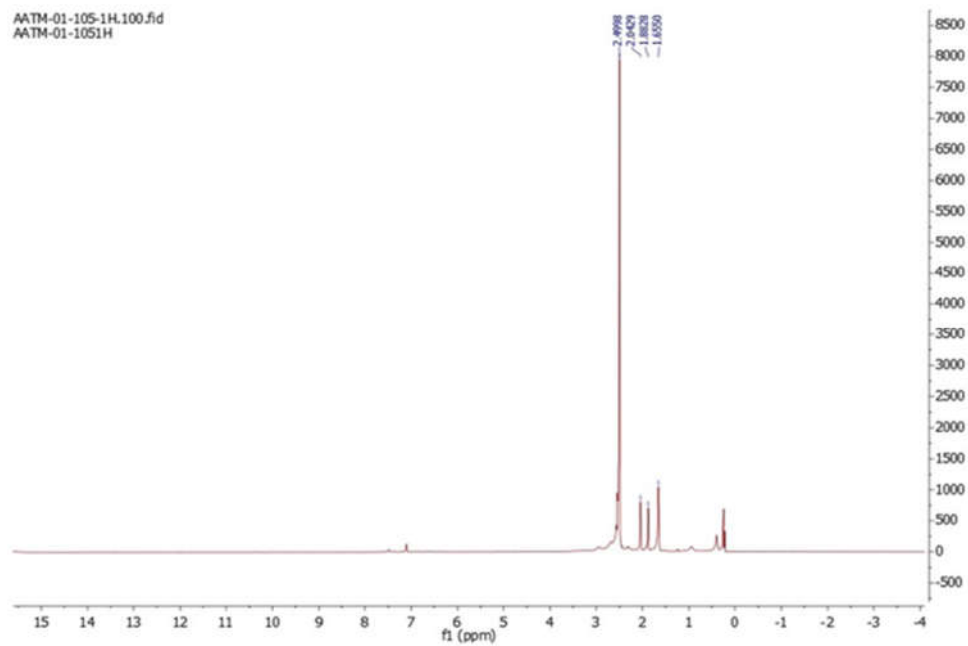
Mw(c): 1.482e+05 g/mol (± 9.68 %) Mw(q²): 1.438e+05 g/mol (± 15.5 %)
A2: 2.177e-07 mol dm³/g² (± 32.8 %) Rg: 8.422e+01 nm (± 4.73 %)

Compound 12

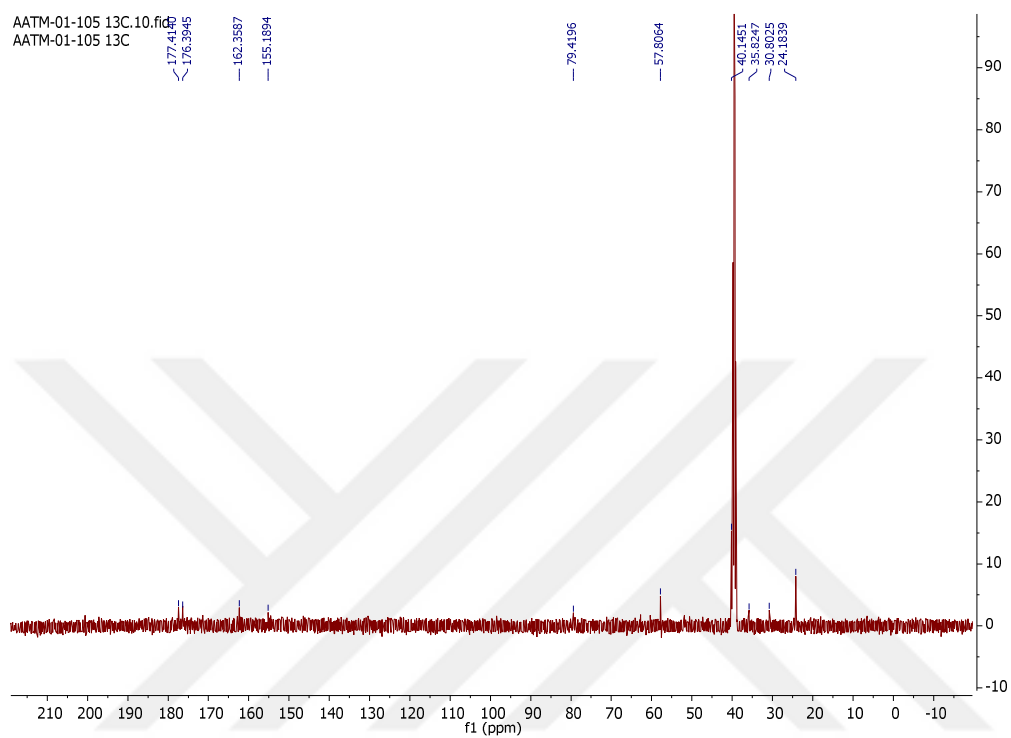
ATR-IR



^1H NMR

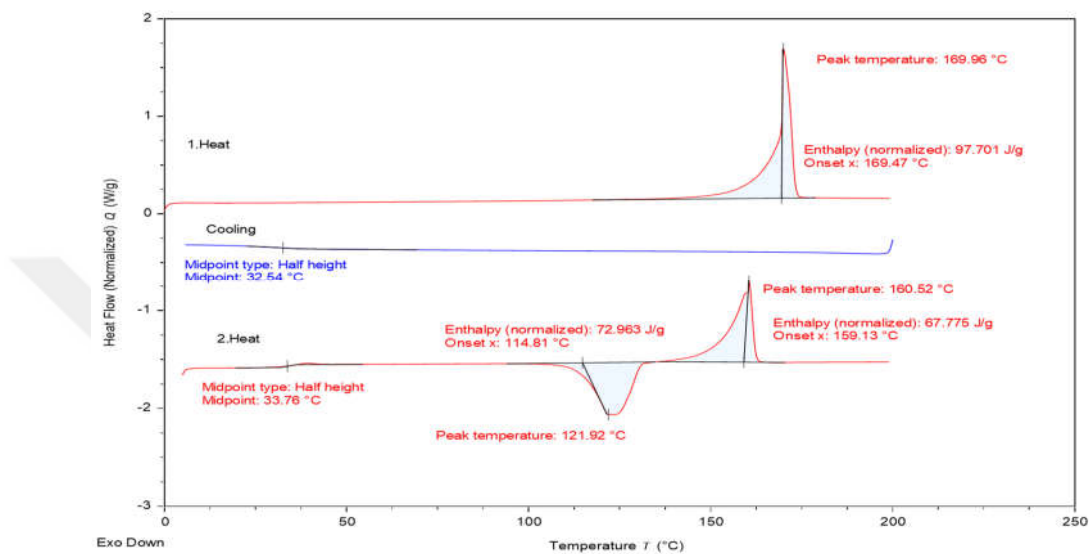


^{13}C NMR

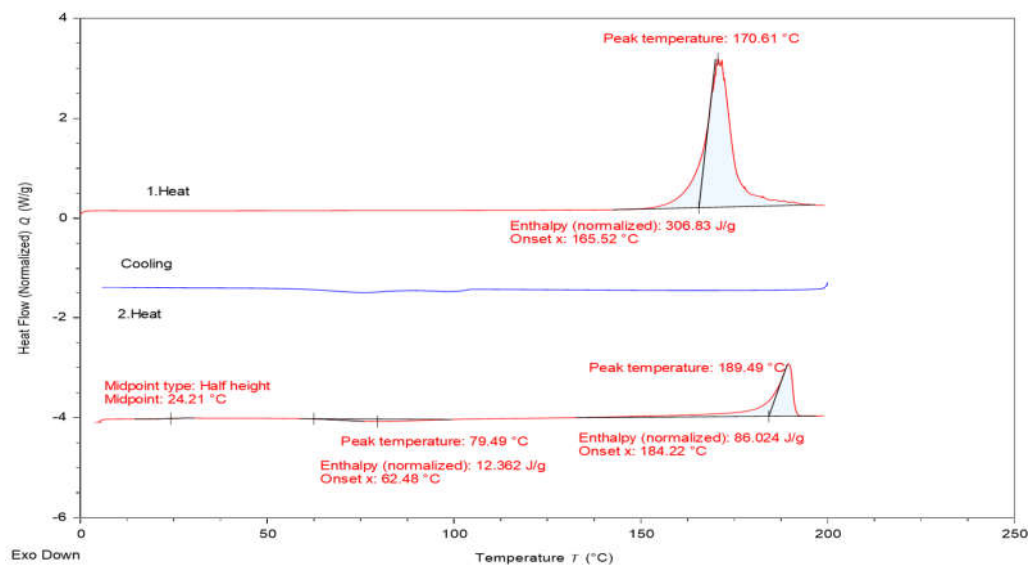


C-4 DSC and TG/DTA of monomers 4 and 10

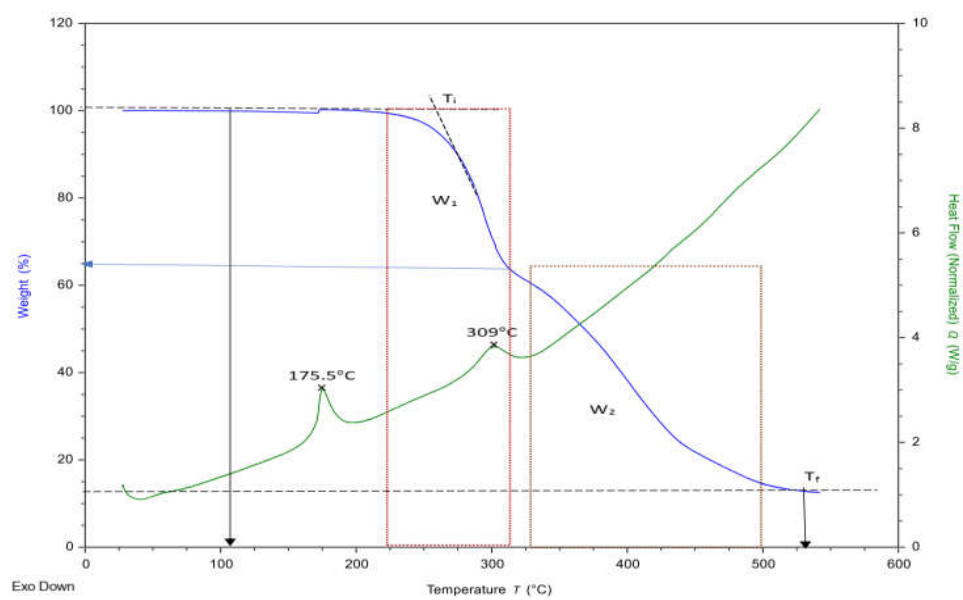
DSC of 4 m-NDH at 5 °C/min



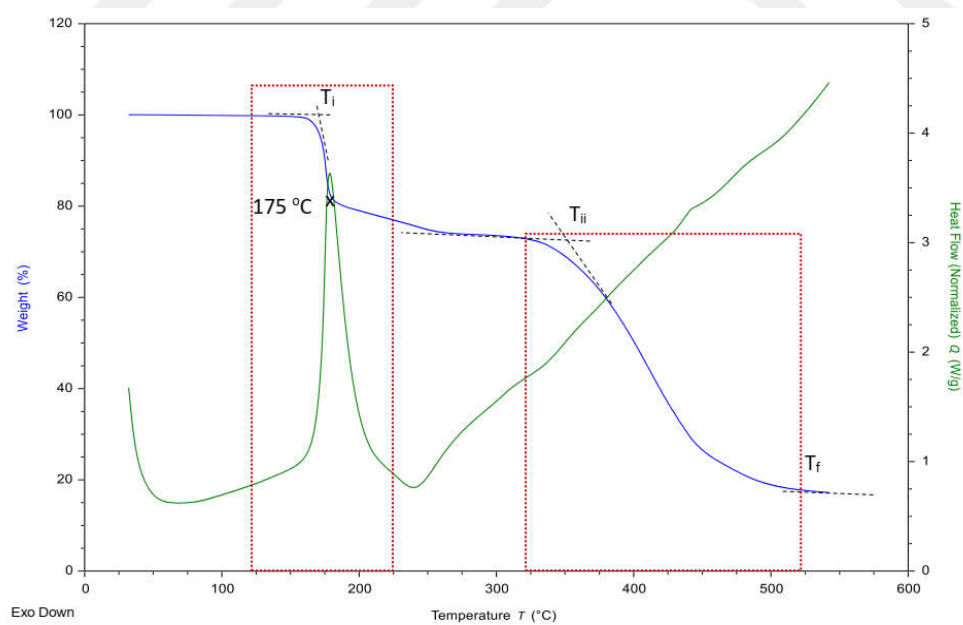
DSC of 10 m-ONDH at 5 °C/min



TG/DTA of 4 m-NDH

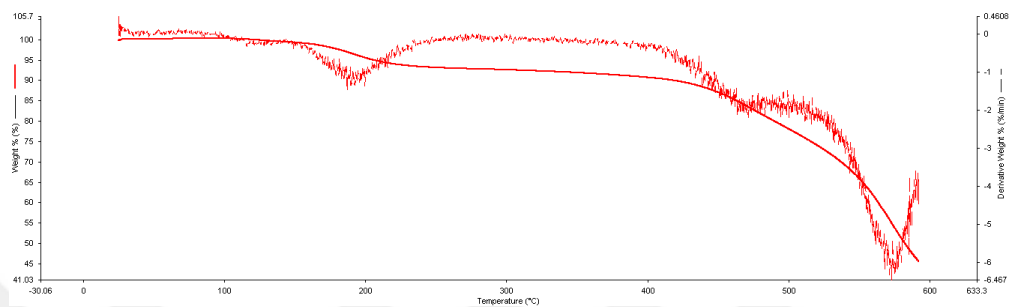


TG/DTA of 10 m-ONDH

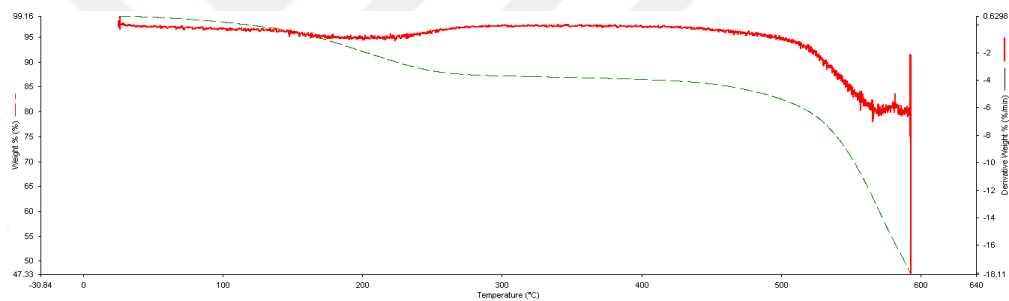


C-5 TGA/DTG of Blend polymer membranes

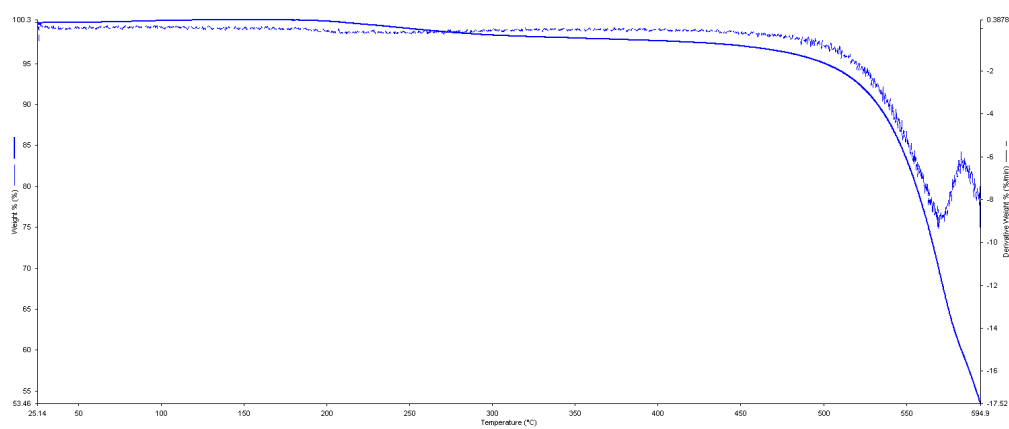
PES:s-P(NDH)



PES:s-P(ONDH)

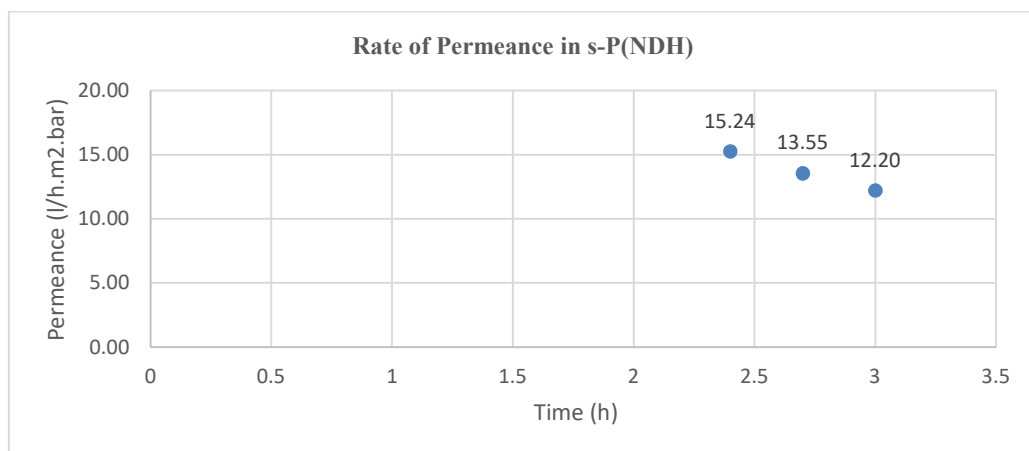
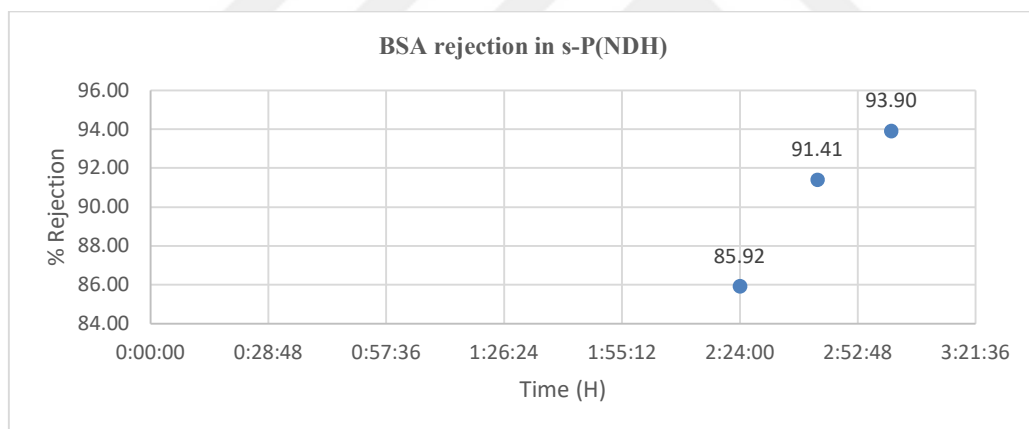
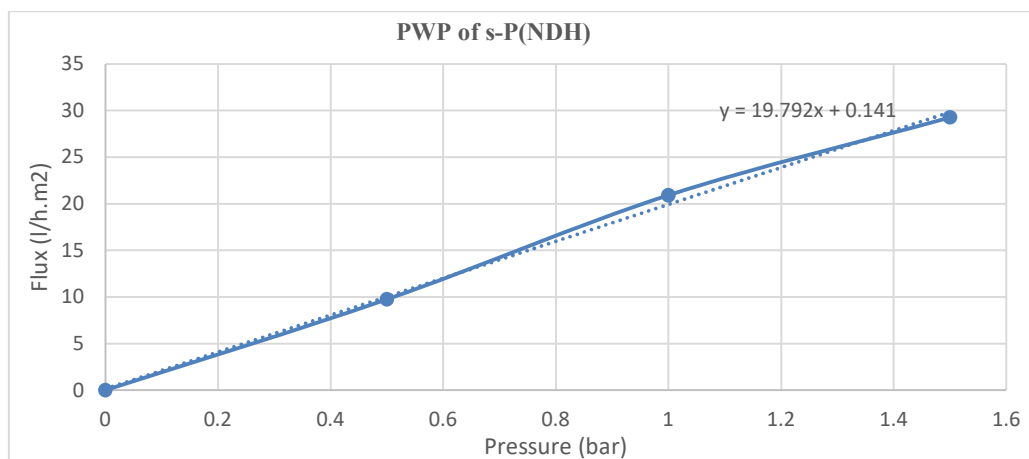


PES:PESmCA

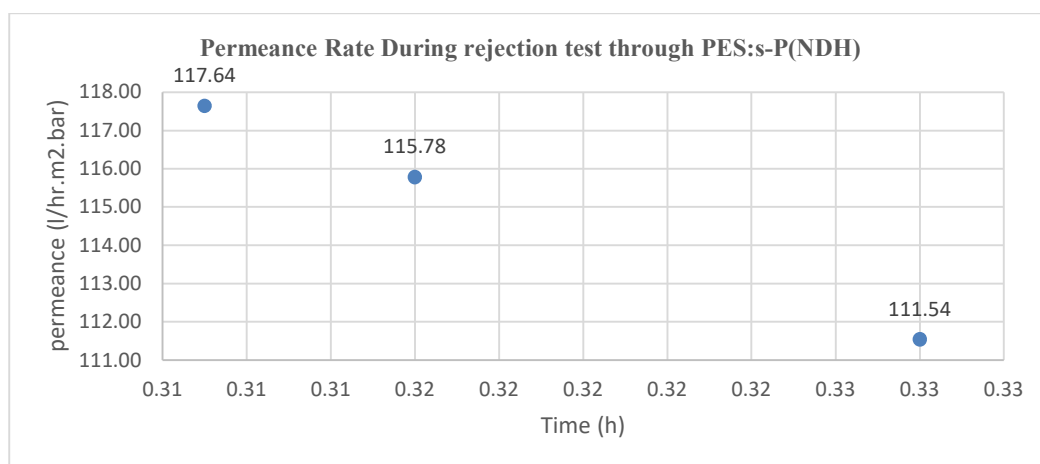
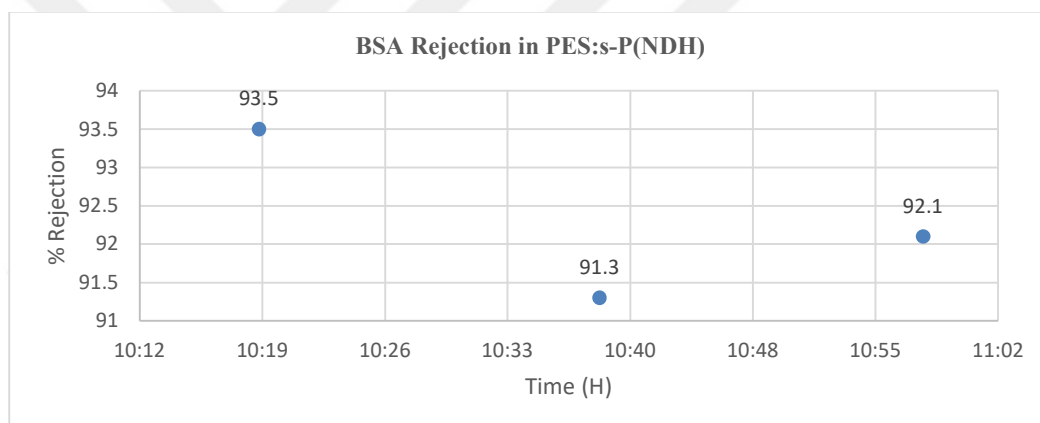
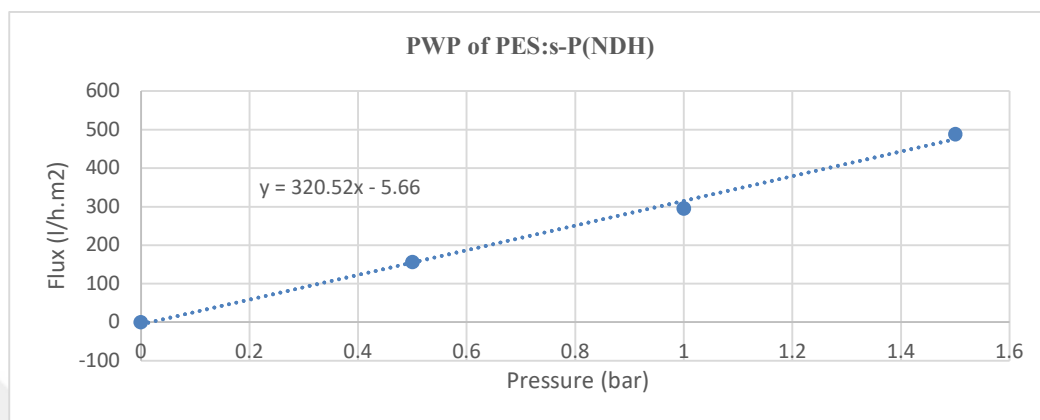


C-6 Pure water permeance of Pure and Blend membranes

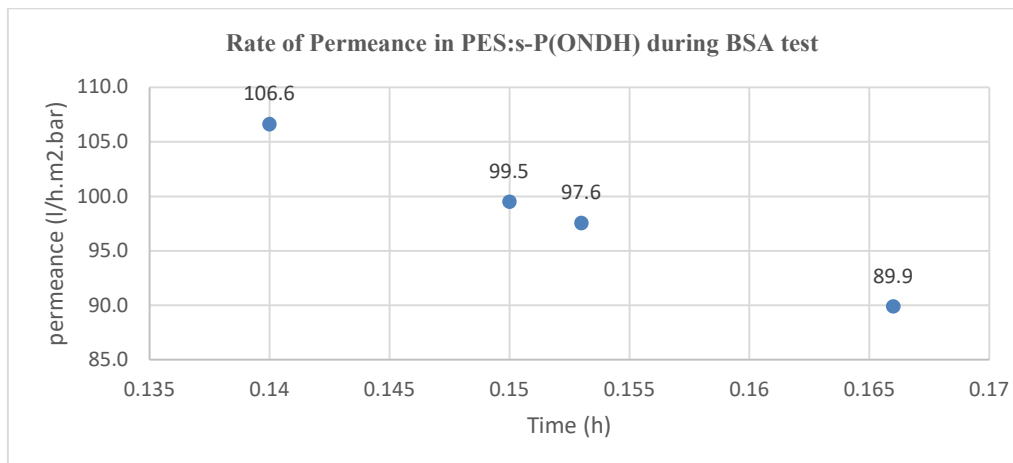
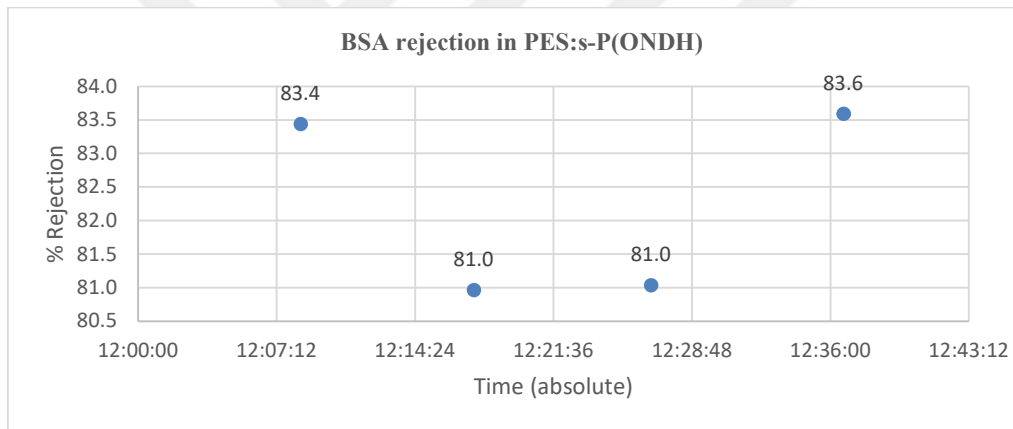
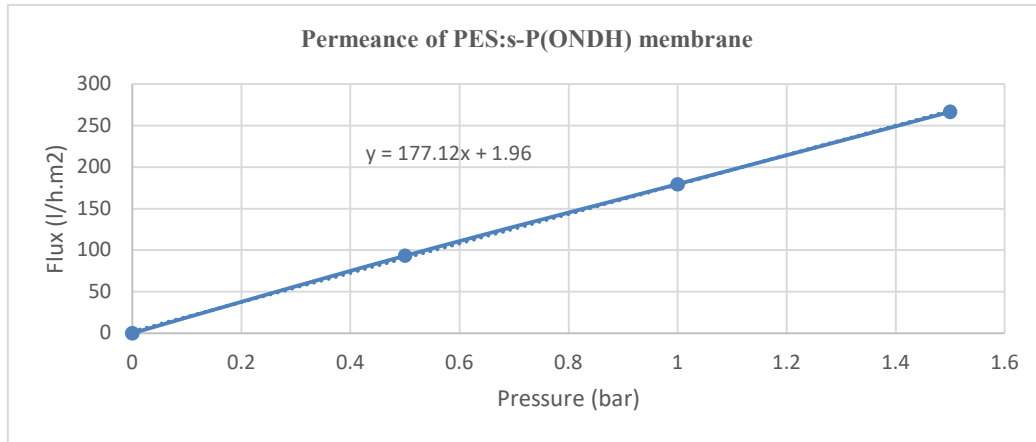
20% s-P(NDH) membrane



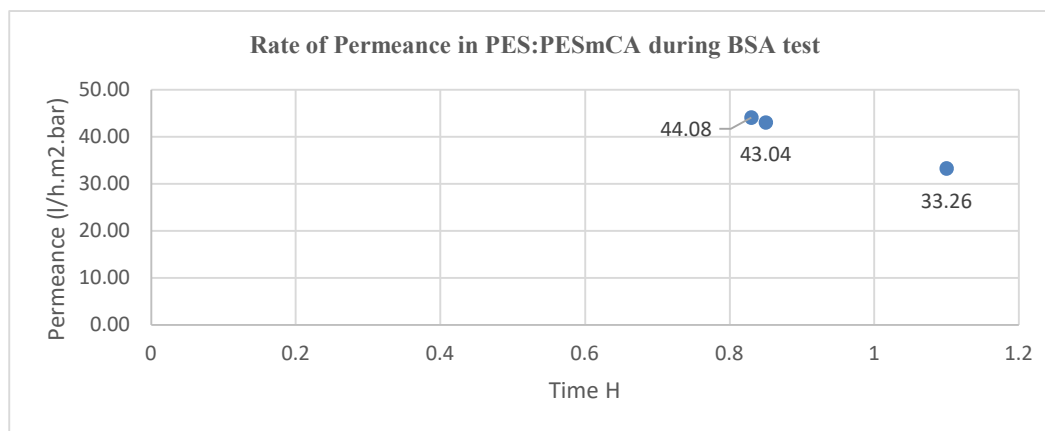
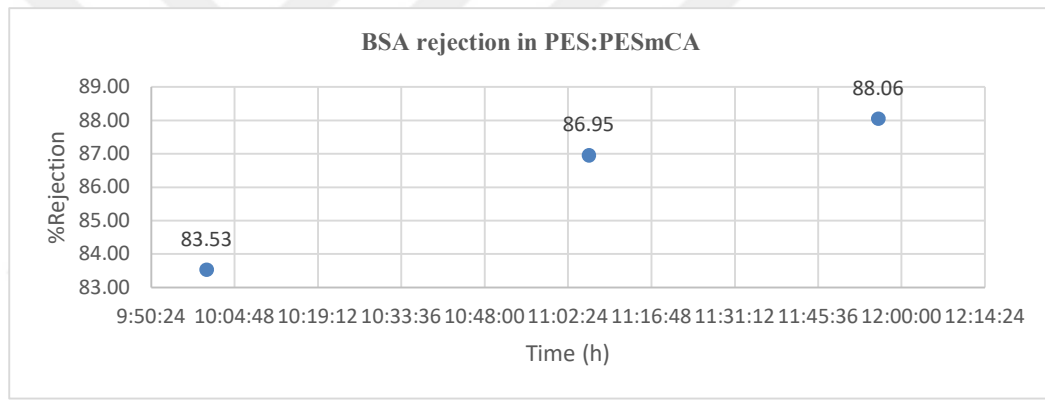
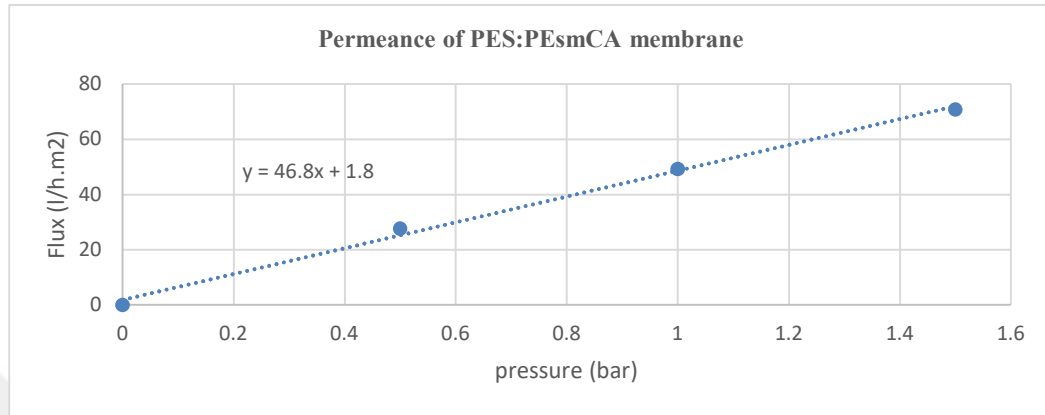
20% PES:s-P(NDH) membrane



20% PES:s-P(ONDH) membrane



20% PES:PEsmCA membrane



**D Membrane Module Design from conclusions of this Research Work for
dead end filtration of contaminated water.**

Sieve 1: chlorinated membrane of s-P(NDH), PES:s-P(NDH), PES:s-P(ONDH) or PES:PESmCA.

Initially the hypothetical mixed suspension of microorganism and proteins pass through the membranes. Only microorganisms will be killed (concluded from Bacterial challenge TEST **I**, **II** and **III**). No biofilm formation at the surface of the membrane, concluded from Test III. All the four membranes will be efficient during filtration providing bacteria free filtrate I.

Sieve 2: Membranes of s-P(NDH), PES:s-P(NDH), PES:s-P(ONDH) or PES:PESmCA.

Filtrate I pass through sieve II having any one of the above membranes to provide 85-95 % rejection of BSA protein. All the membranes are providing the molecular weight cut off of 65 kDa, but membrane s-P(NDH) will provide better rejections as compared to the other three.

