



Production of membranes from nanofibrous polysaccharides

Master Thesis

Study programme:

N0723A270002 Textile Engineering

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Thesis Supervisors:

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Master Thesis Assignment Form

Production of membranes from nanofibrous polysaccharides

Name and surname: **Elçin Tören, B.Eng.**
Identification number: T19000379
Study programme: N0723A270002 Textile Engineering
Assigning department: Department of Nonwovens and Nanofibrous materials
Academic year: **2020/2021**

Rules for Elaboration:

1. Study of Literature regarding nanofiber technology, nanomembranes, pullulan materials and their properties, and polyvinyl alcohol.
2. Elaborate Theoretical Part includes; nanofibers and manufacturing techniques, properties of pullulan and usage areas, membrane technology membrane process.
3. Elaborate Experimental Part regarding theory.
4. Evaluation of Results.
5. Discussion and Conclusion.

Scope of Graphic Work:

Scope of Report: 40-60

Thesis Form: printed/electronic

Thesis Language: English



List of Specialised Literature:

1. RAMAWAT, KISHAN GOPAL and MÉRILLON, JEAN-MICHEL, Polysaccharides Bioactivity and Biotechnology. Springer International Publishing, 2015. ISBN-978-3-319-16297-3
2. BARHOUM, AHMED and BECHELANY MIKHAEL. Handbook of Nanofibers. Springer International Publishing, 2019. ISBN-978-3-319-53654-5
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Date of Thesis Assignment: November 1, 2020

Date of Thesis Submission: May 28, 2021

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ACKNOWLEDGEMENT

Firstly, I would like to thank my thesis supervisor Ing. Jiří Chvojka, Ph.D. of the Technical University of Liberec. Ing. Jiří Chvojka, Ph.D. office was continuously open whenever I had a matter concerning my research and writing. He helped me sincerely, dealing closely with every problem related to my thesis. Steered me in the right direction whenever he thought I required it.

I would also like to thank the people who helped me with this thesis: Prof. Ing. Jakub Wiener, Ph.D., Ing. Marie Kašparová, Ph.D., Ing. Adnan Ahmed Mazari, Ph.D. and Ing. Hana Musilová at the Technical University of Liberec.

Finally, I must specific my deep feeling to my parents who have continually supported me completely (Bülent Tören & Nevruz Tören), and to Ekin Tören, Ebru Benli, Murat Karababa, Yasemin Yeşilyurt, Özge Akdağ for providing me with unfailing support and continuous encouragement throughout my years of study and through the method of researching and writing this thesis. This accomplishment would not have been possible without them.

Thank you.

Author

Elçin Tören

Abstract

During a relatively long human existence on Earth, the human species passed through Stone, Iron, and the Bronze Age. These periods always brought new information and materials. The last few decades have been marked as the time of polymer materials. These materials are widely used in daily life. Nowadays, facilities come to miniaturisation, and society is undoubtedly going through a process that can be called nanotechnology time, and this development also creates several needs.

Nanotechnology has an important role in its wide usage area and increasing the quality of life. It offers a healthier and safer life with the advantages it adds to the field of medicine. The time spent for all these is taken under control, and it is ensured that the works are provided with the least cost loss. Thanks to nanotechnology, the speed of development of technology is increasing, and it is used more efficiently. The efficiency of technology benefits both labour and time.

The aim of this thesis is to use nanofibers obtained from pullulan polysaccharide in production for functional use. Pullulan is a natural compound, and there may be recent interest in the use of natural polymers in medical applications, for example, as proteins in biotechnological materials, as well as biocompatibility. Pullulan, the fungal yeast *Aureobasidium Pullulan* has recently been used in various biomedical applications such as medicines, tissue engineering, wound healing, and diagnostic imaging. When combined with the advantages of the electrospinning technique, this thesis is a polymer that can be formed from biologically sourced materials such as electrospinning, polysaccharides and has a wide range of uses. It has been used to create nanofibers. It finds a wide range of use with its controllable pore size advantages. In addition, in this thesis, a non-porous and water-insoluble nano film has been obtained with surface treatment, making it usable in other industrial areas such as food packaging.

Key Words: electrospinning, polysaccharides, membrane, nanofilm

Abstrakt

Během relativně dlouhé lidské existence na Zemi prošel lidský druh dobou kamennou, železnou a také dobou bronzovou. Tato období vždy přinášela nové informace a materiály. Posledních několik desetiletí bylo označeno jako doba polymerních materiálů. Tyto materiály jsou široce používány v každodenním životě. V dnešní době je jasné, že technologie dochází k miniaturizaci a společnost nepochybně prochází procesem, který lze nazvat časem nanotechnologií, a tento vývoj také vytváří řadu potřeb.

Nanotechnologie hraje důležitou roli v široké oblasti použití a zvyšování kvality života. Nabízí zdravější a bezpečnější život s výhodami, které přináší do oblasti medicíny. Čas, který se nad tím tráví, je pod kontrolou a je zajištěno, že práce bude poskytována s nejnižší ztrátou nákladů. Díky nanotechnologii se zvyšuje rychlost vývoje technologie a využívá se efektivněji. Efektivita technologie prospívá práci i času.

Cílem této práce je použít nanovlákná získaná z pullulan polysacharidu ve výrobě pro funkční využití. Pullulan je přírodní sloučenina a v poslední době může být zájem o použití přírodních polymerů v lékařských činnostech, jako například proteinů v biotechnologických materiálech, stejně jako o biokompatibilitu. Pullulan, plísňové kvasinky *Aureobasidium Pullulan*, se v poslední době používají v různých biomedicínských činnostech, jako jsou léky, tkáňové inženýrství, hojení ran a diagnostické zobrazování. V kombinaci s výhodami techniky elektrostatického zvlákňování je tato práce polymerem, který lze vytvořit z biologických materiálů, jako je elektrostatické zvlákňování, polysacharidy, a má širokou škálu použití. Používá se k vytváření nanovláken. Najde široké spektrum použití s výhodami řízené velikosti pórů. Kromě toho byl v této práci získán neporézní a ve vodě nerozpustný nano film s povrchovou úpravou, díky čemuž je použitelný v jiných průmyslových oblastech, jako je balení potravin.

Klíčová slova: elektrostatické zvlákňování, polysacharidy, membrána, nano film

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LIST OF SYMBOLS

Symbols	Long Name	Units
ρ	density	g/cm ³
μm	micrometre	μm
$\text{\AA}$	pore radius	$\text{\AA}$
BET	Brunauer–Emmett–Teller	
DNA	deoxyribonucleic acid	
DSC	differential scanning calorimetry	
ECM	extracellular matrix	
g	mass	g
kV	kilo voltage	V
Ma	molecular mass	g/mol
M _w	measure of molecular weight	Da
NaCl	sodium chloride	
nm	nanometre	nm
Pa	Pascal	
pH	power of hydrogen	
PVA	polyvinyl alcohol	
R.H	relative humidity	%
rpm	revolutions per minute	rpm

SD	standard deviation	
SEM	scanning electron microscopy	
Temp	temperature	C°
Tg	glass transition temperature	C°
wt.	weight percent	%
h	hour	h

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1.INTRODUCTION

Nanotechnology is the name given to the whole of science, engineering and technology studies in sizes ranging from 1 to 100 nanometres; We can say that it is a whole of studies done on an exceedingly small scale. The word nano is employed to explain a selected size resembling millimetres or centimetres; a millimicron may be a unit of life capable of one billionth of a meter or a simple fraction of a millimetre. Nanoparticle refers to one substance particle of one to one hundred nanometres in size. They cannot be visible with the naked eye, but the exciting factor is that they're too small to be seen with conventional microscopes.

Nanotechnology: It is widely used in computer technologies, scientific studies, electrical electronics, chemistry, biology, and physics. One of the most significant usage areas is medicine. Nano plays an essential role in technological developments, especially against viruses that affect the whole world and cause fatal damage, such as the Corona Virus that has emerged recently. Thanks to the products achieved with the help of nanotechnology, drugs, medical materials, and machines can be produced according to ČSN P CEN ISO/TS 80004-1 (012003) [1].

Recent advances in electrospinning techniques have enabled the production of continuous fibres with dimensions on the size of nm [2].

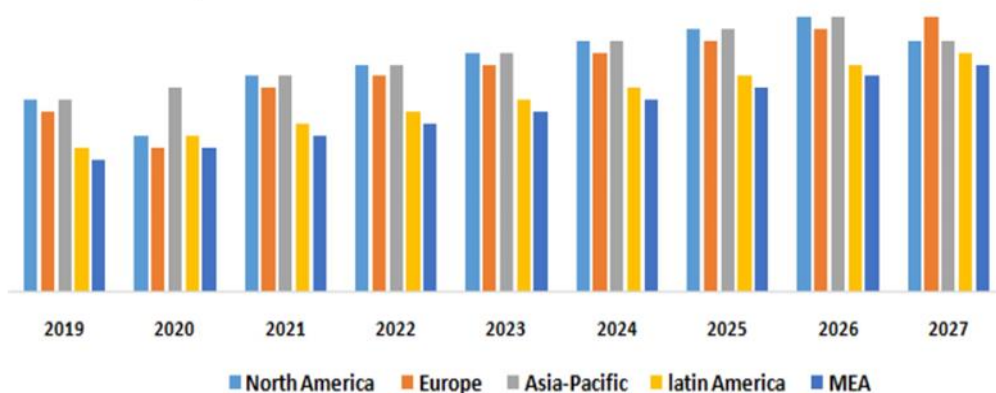


Fig.1.1. Nanofiber market by region [1].

The report includes a special analysis of the impact of the COVID-19 internment on the financial gain of market leaders, followers, and disruptors. Since protection is applied otherwise in different regions and countries, the result of an equivalent effect is different per regions and segments. The report mentioned this short and long impact on the market and can also facilitate decision-makers to define short-term and long-term methods for companies by region [1,2].

1.2. Areas of Use of Nanofibers

Electro spun nanofibers are potential for many applications such as in high performance air filters, protective textiles sensors advanced composites, photovoltaic cells, medicine, scaffolds in tissue engineering and membranes in separation technology [1].

1.2.1. Water Purification Application

Water pollution due to contaminant from various disposal sites mainly from industrial by products such as plating, ceramics, glass, mining, and battery manufacturing etc. Purification of water is required badly for drinking and alternative household purpose. There are many water purification methods such as filtration (size separation), adsorption, chemical coagulation, photo degradation, biodegradation, distillation, active sludge etc, but filtration is the most versatile chemical-free method and it is cost effective, time and energy saving. Therefore, filtration systems have significantly improved in the past decade. The filtration processes are categorized mainly by the average size of the pores in the membranes. These include microfiltration, ultra-filtration, nano filtration, and reverse osmosis etc. Nanofibers have many novel properties such as high porosity, good flexibility, large surface area per unit volume, and interconnected open pore structure. These characteristics make them attractive for ultra-filtration applications. Several nanofibers based on cellulose are more suitable for ultra-purification of water [1].

1.2.2. Air Filtration Applications

Nanofibers possess very large surface area to volume ratio, low weight, high permeability, that create them applicable for a wide range of air filtration applications such as industrial gas purification, filtration for combustion engines, fuel cells and cabin air filtration etc. An interesting application of nanofiber-based cigarette filters which efficiently remove tar and other harmful materials. The normal cellulose acetate-based filters are unable to adsorb wide range of harmful components [1].

1.2.3. Biomedical Applications

Polymeric nanofibers are helpful for a range of medical specialty applications, such as filtration devices, medical prostheses, scaffolds for tissue engineering, wound dressing, controlled drug delivery systems, cosmetic skin masks, protecting clothing etc. These could be fabricated from a variety of polymers including nondegradable polymers such as polyurethan, nylon, polycarbonate, and biodegradable polymers such as polyhydroxy acids, polyanhydrides as well as from blends and copolymers [1].

1.2.3.1. Wound dressing

Nanofibers can be used for the treatment of wounds or burns of a human skin. Fine fibres of biodegradable polymers can be directly sprayed/spun onto the injured location of skin to form a fibrous mat dressing. This helps the formation of normal skin on the wounds and eliminates the formation of scar tissue which would occur in a traditional treatment. Wound dressing materials provide Helps the formation of normal skin growth, allows air exchange, prevent scar tissue formation, prevent bacterial colonization, and can be drug delivery system [1].

1.2.3.2. Drug delivery

Drug delivery using normal syringe (average diameter 0.2 – 0.5 mm) is painful. Drug-friendly physical immobilization at the surface is also finished through the usage of surface-modified prefabricated nanofibrous meshes that have a particularly high surface area to quantity ratio, resulting in better drug loading quantity per unit mass than another device. The immediate release of medication from the nanofiber surface can enable facile dosage control of some therapeutic agents, suitable for a few specific applications such as prevention of bacterial infection occurring within few hours after surgery. Use of hollow nanofiber syringes could avoid this problem because of their fineness. In addition, very small amount of drug can also be delivered with the help of nano pumping system. Therefore, a miniaturized drug delivery system consisting of a sensor to measure the level of blood sugar in case of a diabetic patient, a hollow nanofiber-based needle, and a suitable micro/nano pump could be the ideal solution. It could be attached to the body of the patient conveniently to ensure the supply of the insulin as and when required depending upon the level of blood sugar [1].

1.2.3.3. Tissue engineering

Surgery by tissue engineering requires an appropriate cell source, optimal culture conditions and a biodegradable scaffold as the basic elements. Scaffold is a highly porous material with interconnected pores having large surface area desirable for high-density cell and tissue cultures the scaffolds with nanoscale architectures have bigger surface area for absorbing proteins and present more binding sites to cell membrane receptors. The adsorbable proteins further can modification the conformations, exposing additional binding sites, expected to produce an edge over microscale architectures for tissue generation applications. [1].

1.2.4. Protective Clothing

Currently, protecting covering used by soldiers is charcoal based. It has many limitations such as in terms of water/air permeability, heavy weight etc. Therefore, a lightweight and breathable fabric, which is permeable to both air and water vapor, and highly reactive with deadly chemical agents, is desirable. Protective clothing made of nanofibers that have a protective property against chemicals and is permeable to both air and water vapor can be given as an example [1].

1.2.5. Nanofibers as Sensor Material

Generally, the temperature program for a DSC analysis is supposed such the sample holder temperature can increase linearly as a function of time. Electro spun ceramic nanofibers, of various semiconducting oxide materials such as titanium dioxide (TiO_2), iron oxide (Fe_2O_3), etc. are considered very good for sensing of gases like carbon monoxide (CO), nitrogen dioxide NO_2 , etc. Titanium dioxide nanofibers act as good substrate for supporting other sensing materials such as noble metal nanoparticles [1].

Polysaccharides are homopolymers or copolymers of monosaccharides. In nature, polysaccharides are often found in several organisms, as well as algae-derived polysaccharides (e.g., alginate), plant origin (e.g., cellulose and starch), microbial origin (e.g., dextran) and animal origin (e.g., chitosan and hyaluronic acid). They additionally diversify their chemical structure, chemical composition, mass, and ionic character, all of that contribute to their practicality and biological activities. Various producing ways are developed for nanofibers as well as drawing, templet synthesis, section separation, self-assembly, and electrospinning. Among them, the electrospinning method has become the foremost enticing because it is efficient, extremely productive and might be applied to a spread of polysaccharides. It has been used in many studies to date using polysaccharides and their derivatives to produce electro spun nanofibers that could potentially be useful in regenerative medicine [3,4].

Electro spun nanofibers obtained from polysaccharides it is widely used in regenerative medicine, including tissue engineering applications. Tissue engineering aims to provide artificial tissue or organs to patients affected by the loss or failure of a tissue or organ. Tissues and organs are typically designed using a combination of a patient's cells and polymer skeletons. The compound scaffold utilized in this approach is designed to mimic several of the roles of extracellular matrices (ECMs) of tissues in the body. These roles include proper regulation of cells, controlling tissue structure, regulating the function of cells, and allowing diffusion of nutrients, metabolites, and soluble factors [5].

Nanofiber matrices have recently become attractive in tissue engineering mechanical properties due to their large surface area, high porosity, controllability, and ability to interact with cells to mimic natural ECMs. This review includes a important place within the recent advancement of the many nanofiber matrices from polysaccharides and their medical specialty applications, together with tissue engineering scaffolds, wound dressings, controlled drug unleash systems and immobilization substrates of biocatalysts and enzymes [6].

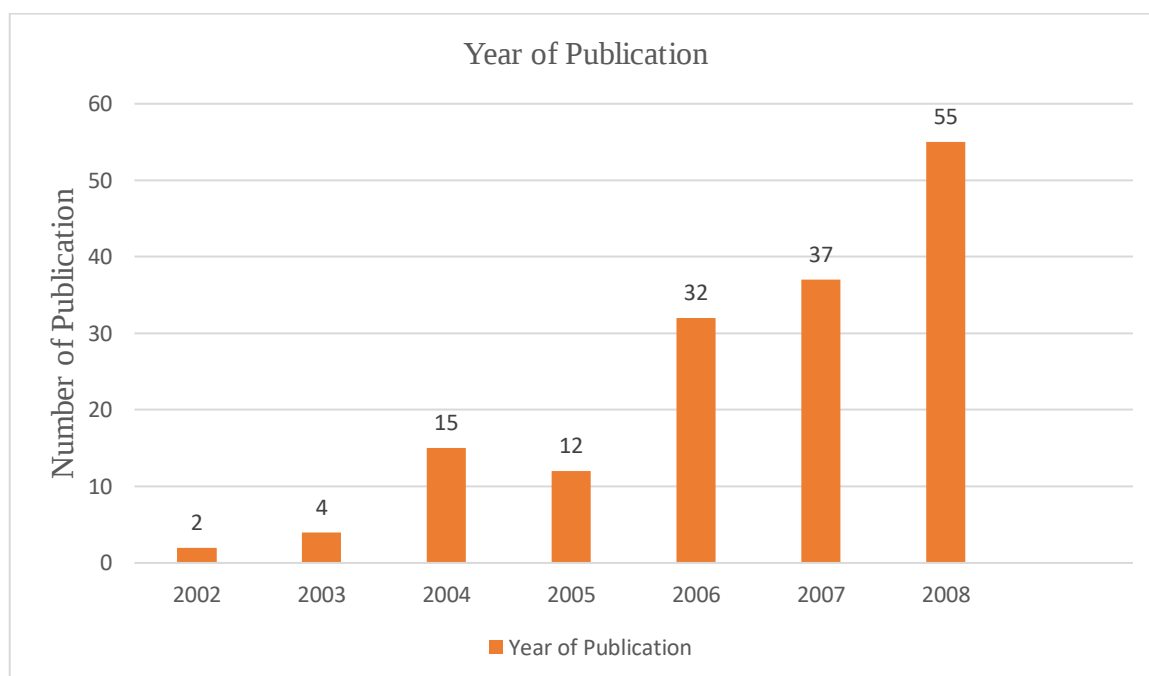


Fig.1.2. Scientific publications on electro spun polysaccharide nanofibers found in the SciFinder Scholar search system [6].

Membranes obtained by using Nano Technology have a wide market area today.

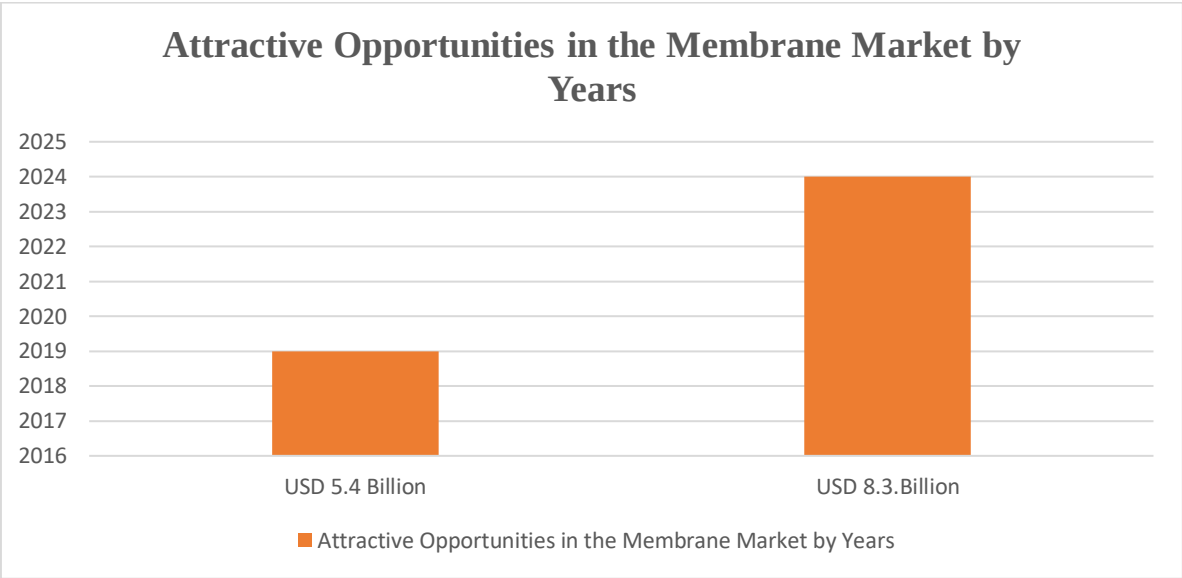


Fig.1.3. Attractive opportunities in the membrane market by years [6].

As seen in Figure 1.3, the membrane market was estimated at 5,4 billion dollars in 2019, and the market size is predicted to increase from 5.4 billion in 2019 to 8,3 billion dollars by 2024. India, China, Brazil, and Saudi Arabia are expected to see high membrane usage by 2024 due to population growth and rapid industrialization [6].

In addition to Fig.1.3, the functional membrane market by country is shown in Fig.1.4.

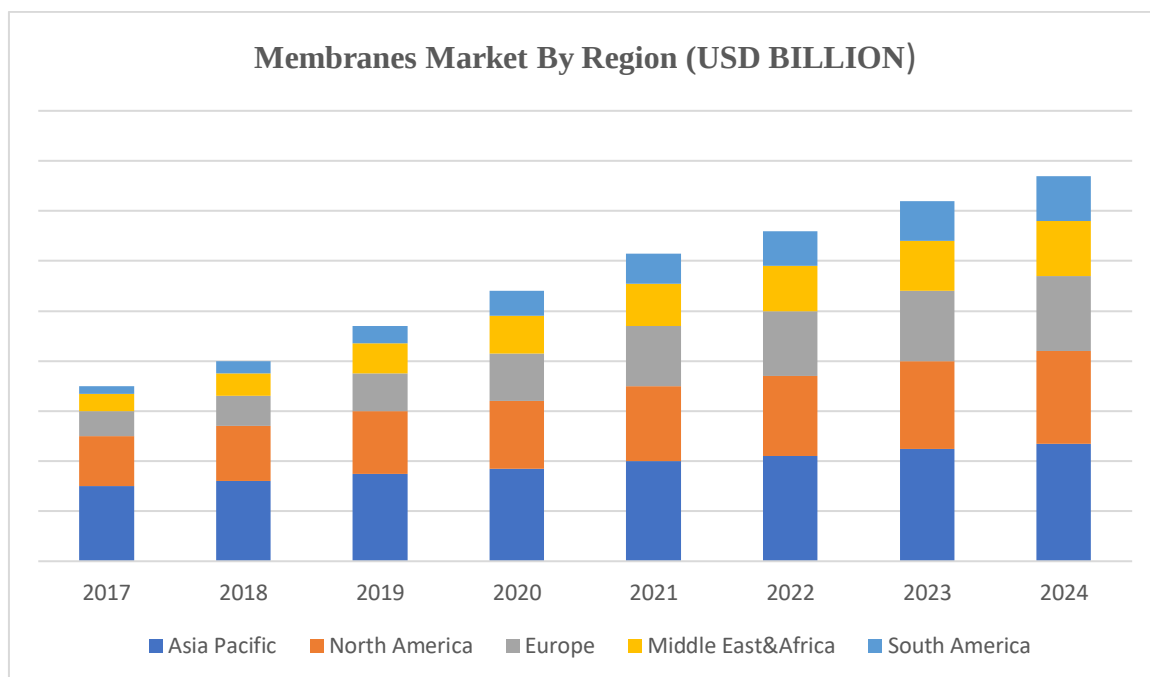


Fig.1.4. Membranes Market by Region (USD BILLION) [5].

As shown in Figure 1.4, functional membranes have a considerable market area [5].

This thesis, it is aimed to produce functional multi-purpose materials by producing nano-polysaccharide-based membranes by making use of the above-mentioned properties of nanotechnology and nanofibers [7].

Polymer mixing constitutes a beneficial methodology for the improvement or modification of the chemical properties of chemical compound materials; an essential property of a compound mix is the miscibility of its parts because it affects the mechanical properties, the morphology, and therefore the permeability and degradation. Compound blends are physical mixtures of structurally completely different polymers or copolymers that act with secondary forces with no covalent bonding, resembling element bonding, dipole-dipole forces, and charge-transfer complexes for homopolymer mixtures. An example of these mechanical properties is the tensile strength table of Pullulan / PVA nanofibers, shown in Table 1.1. Tensile strength data were obtained by atomic force microscopy. [7].

Table 1.1. Tensile strength of Pullulan/PVA blends nanofibers with various blend ratios by weight [7].

Blend ratio by wt. (Pullulan/PVA)	Tensile strength (MPa)
100/0	5.70 ± 0.10
80/20	6.10 ± 0.10
60/40	6.60 ± 0.10

Poly (vinyl alcohol) (PVA) may be a semi-crystalline hydrophilic compound with sensible chemical and thermal stability. It is a highly biocompatible and non-toxic polymer, and it will be processed simply and has high water permeability. PVA will form physical gels in numerous sorts of solvents that result in the utilization of PVA during a wide selection of applications in the medical, cosmetic, food, pharmaceutical and packaging industries. Its flexibility and toughness are good; therefore, it's a typical artificial compound that is accustomed improve the physical properties through the mix with alternative materials that have fewer physical properties [7].

PVA with functional teams is helpful in practical investigations of functional polymers owing to its easy preparation as a bulk material, films, associate degreed fibers. Pullulan may be a natural compound and recent interest within the use of natural polymers, for example, as proteins in biotechnological materials and medical specialty applications furthermore as their biocompatibility. Pullulan is an extracellular microorganism polyose created by the fungus-like yeast, *Aureobasidium Pullulans*. Due to its properties, Pullulan is employed as a low-calorie ingredient in foods, gelling agent, coating associate degreed packaging material for food and drugs, binder for fertilizers, and as an oxidation-prevention agent for tablets. Alternative applications embrace contact lenses manufacturing, biodegradable foil, plywood, water solubility enhancer, and increased oil recovery. Pullulans water-soluble, insoluble in organic solvents, and non-hygroscopic. It is additionally non-toxic. Due to these features usage rate is increasing [7].

2.THEORETICAL PART

2. 1. Nano Fibres

Nanotechnology is that science involved the study of the phenomena and functions of matters at intervals the dimensional vary of 0.1-100 nm. Nanomaterial's analysis takes a materials science to engineering science. Investment advances in materials scientific discipline and the synthesis that is developed in support of microfabrication analysis. Herein, there are some examples to imagine merely but little that's the nanoscale: the thickness of a sheet of paper 100,000 nm, the breadth of a person's hair 80,000 nm and conjointly the diameter of human double-strand deoxyribonucleic acid a combine of nm [1,2].

Nanofiber enables use in various application areas equivalent to batteries and fuel cells, capacitors, transistors and diodes, systems for energy transfer, composites for zone structures, drug delivery and tissue engineering [2].

A "fibre" is defined geometrically as an elongated, thread-like structure. The term "nano" technically refers to the one-billionth of a unit scale. Generally, the nanofiber is a term used for fibres with a diameter between 50 and 300 nanometres [1].

2.2. Manufacturing Techniques of Nano Fibres

Many ways are often used for nanofiber production: bicomponent extrusion, phase separation, template synthesis, drawing, melt blowing, electrospinning, and centrifugal spinning. Bicomponent fibres can be defined as extruding two polymers together in the same fibre from the same spinneret; some examples of bicomponent fibres include sheath-core,

eccentric, islands-in-the-sea, etc. Islands-in-the-sea form fibres are also called matrix-filament fibres because, in cross-section, they appear as one polymer is inserted into a matrix of a second polymer. Islands-in-the-sea fibres may have a uniform or non-uniform diameter of the island portion.

Nanofiber materials have a range of uses in these areas; energy transfer devices, composites for zone structures, drug delivery and tissue engineering appreciate batteries and fuel cells, condensers, transistors, and diodes [2].

Nanofibers offer a decent material system that will improve the electrical, optical, thermal, and mechanical properties of the many styles of bulk materials. By electrospinning and non-electrospinning, various materials (metal, metal oxides, ceramics, polymers, and carbon) are developed into nanofibers to date.

Therefore, several non-electrospinning techniques have been developed to increase the yield of nanofibers in processing. A variety of those techniques embody resolution processing or air-jet, spinning, drawing techniques, for example, synthesis, centrifugal spinning, half inversion/separation, and freeze/drying synthesis [1,8].

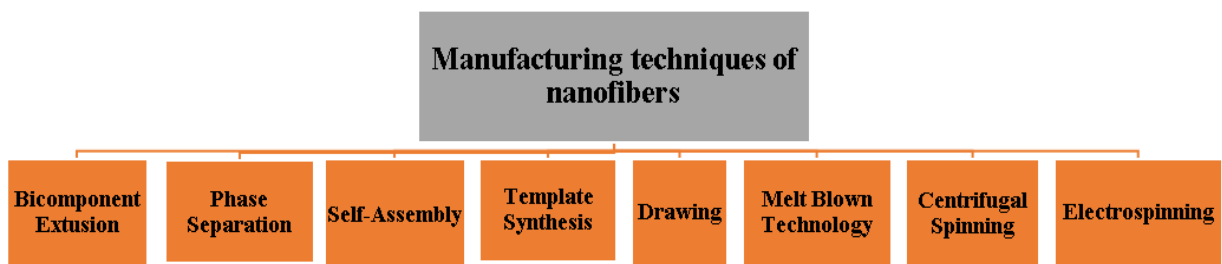


Fig.1.5. Manufacturing techniques for nanofibers [8].

2.2.1. Bicomponent Extrusion

Bicomponent fibres will be outlined as extruding two polymers along within the same fibre from the constant spinneret. Some types of bicomponent fibres, as seen in Figure 1.1[8], include sheath-core, eccentric, islands-in-the-sea and segmented pie fibre.

Bicomponent fibres will be outlined as extruding two polymers along within the same fibre from the constant spinneret. Some types of bicomponent fibres include sheath-core, eccentric, islands-in-the-sea and segmented pie fibre.

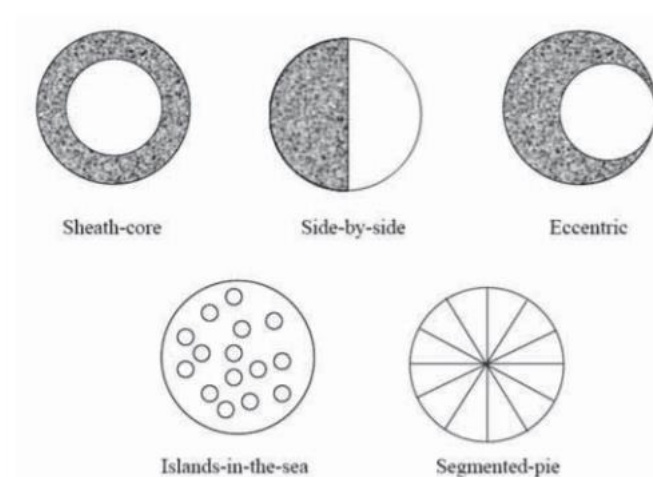


Fig.2.1. Cross-sections of bicomponent fibres [8].

Essentially, these filaments are spun from the mix of 2 polymers within the needed magnitude relation, wherever one chemical compound is suspended as drops in the second's melt. Fast cooling of the fibre beneath the spinneret holes is an important feature in fibres production. The variations in suitability between the two polymers would nearly hinder the suitability of its mix, except lower mixtures concentration (20%). Using heat, solvent, or chemical, one of the

components of the fibre may be removed; or using mechanical equipment. Two polymers are supplied in bicomponent extrusion to a clear spinneret cavity, split by a blade edge of the septum, which feeds the two segments into side-by-side arrangements [8].

2.2.2. Phase Separation

In phase separation, a chemical compound is initially blended with a solvent before suffering gelation. The most important mechanism during this system is that the separation of parts thanks to physical inconsistency. The solvent phase is then extracted, leaving the other residual phase [8].

2.2.3. Self-Assembly

Self-assembly is when each element forms a regular structure with non-covalent bond. The self-assembly technique is used to get peptide nanofibers and peptide amphiphiles. The method was galvanised by the natural folding method of organic compound residues to create proteins with unique three-dimensional structures. The self-assembly process of peptide nanofibers involves various driving forces such as hydrophobic interactions, electricity forces, H bonding and van der Waals forces and is influenced by external conditions such as ionic strength and pH scale. Another problem is that its production is not easy due to the complicated producing process and low productivity [8].

2.2.4. Template Synthesis

The synthesis of templates requires the use of a template or assembly to provide a performance-enhanced substance or form. Therefore, casting technique and DNA reproduction can be believed to be a template-based synthesis where the mould refers to a metal oxide membrane containing nanoscale diameter pores. Using water pressure in conjunction with the porous membrane management causes extrusion of the chemical

compound that, by contacting with a solidification answer, provides a nanofiber whose diameters are managed by the pores [8].

2.2.5. Drawing

It is the application of the dry spinning method at the molecular level. A micrometre with a diameter of a few micrometres touches the droplet, and the nanofiber is obtained by retracting the micropipette. The micropipette is then far away from the nanofiber to draw at a speed of roughly $1 \times 10^{-4} \text{ ms}^{-1}$ from the nanofiber. The extracted fibre is poured onto the surface by touching it with the micropipette tip. Material body at the sting of the driblet inflated with evaporation. So, draw a fibre contains an elastic material that may tolerate robust deformations even with sample cohesive to retain stress throughout pull [8].

2.2.6. Melt Blown Technology

The diameter of the fibres obtained in the classical melt-blown method is between 2-5 μm , and the structure of the plate and the collection system, which includes nozzles to produce small-diameter fibres in large quantities, has been specially designed. This technique provides the use of thermoplastic polymers, as seen in Figure 1.2 during an economic spinning operation [9].

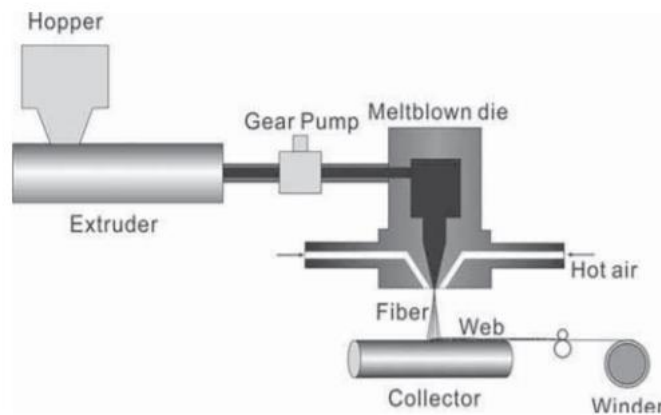


Fig.2.2. Detailed schematic of the melt blowing process [9].

2.2.7. Centrifugal Spinning

The polymer solution prepared with a suitable solvent, the electrode in contact with this solution, the high voltage direct current power supply and a grounded collector plate is the main components of the electrode spinning process. Polymer solution uses in this method. It is taken into a suitable syringe, and the solution flows out of the syringe with the help of a pump. In the meantime, a high voltage potential is applied between the collector plate and the tip of the syringe. The polymer solution in the metal syringe tip is up to a certain voltage value. It is in the form of a spherical droplet. When the critical voltage value is reached, the surface tension of the polymer solution and electrostatic forces are equalised, and the polymer drop takes the form of a cone (Taylor cone). After this point, continuous fibres are formed from the metal end of the syringe towards the collector plate with the slightest increase in voltage. The solvent in the polymer solution evaporates rapidly in the meantime [8].

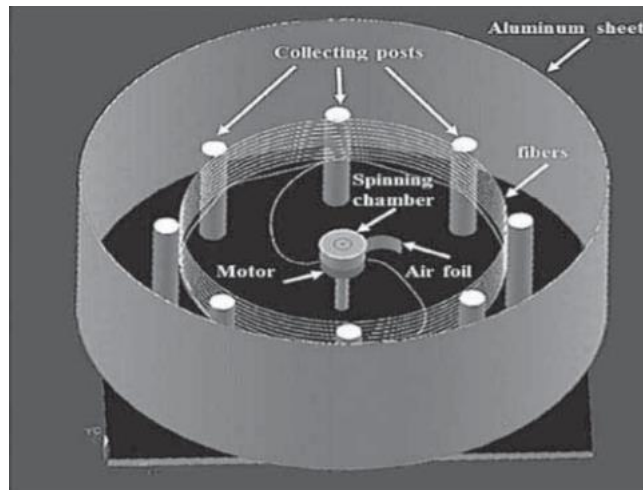


Fig 2.3. Schematic drawing of the centrifugal spinning system [8].

2.2.8. Electrospinning

The polymer solution prepared with a suitable solvent, the electrode in contact with this solution, the high voltage direct current power supply and a grounded collector plate is the main components of the electrode spinning process. Polymer solution used in electrospinning method. It is taken into a suitable syringe, and the solution flows out of the syringe with the help of a pump. In the meantime, a high voltage potential is applied between the collector plate and the tip of the syringe. The polymer solution in the metal syringe tip is up to a certain voltage value. It is in the form of a spherical droplet. When the critical voltage value is reached, the surface tension of the polymer solution and electrostatic forces are equalised, and the polymer drop takes the form of a cone (Taylor cone). After this point, continuous fibres are formed from the metal end of the syringe towards the collector plate with the slightest increase in voltage. The solvent in the polymer solution evaporates rapidly in the meantime. The Electrospinning manufacturing technique is shown in figure 1.4 [1,10].

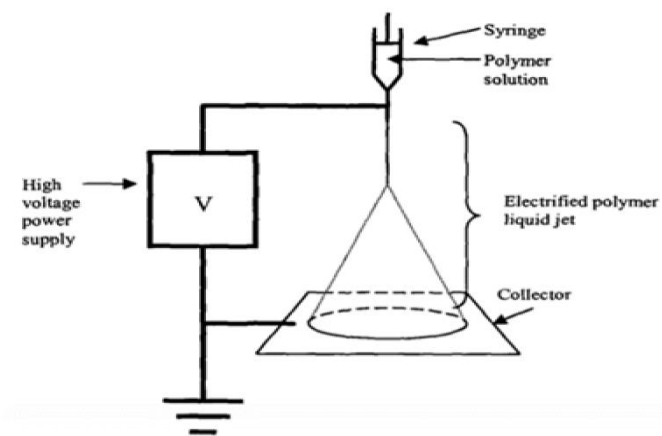


Fig.2.4. Schematic diagram of the electrospinning process [11].

The electrospinning technique has some advantages; these are [10,11]:

- A suitable solvent ought to be obtainable for dissolving the chemical compound.

- The pressure of the solvent ought to be appropriate so that it evaporates quickly enough for the fibre to take care of its integrity when it reaches the target; however, not too quickly to permit the fibre to harden before it reaches the metric linear unit vary.
- The body and physical phenomenon of the solvent should neither be too large to forestall the jet from forming nor be too little to allow the chemical compound answer to empty freely from the pipet.
- The facility offer ought to be adequate to overcome the body and physical phenomenon of the chemical compound answer to create and sustain the jet from the pipet.

2.3. POLYSACCHARIDES

In nature, principally, the carbohydrates exist as polymers instead of monomers. During this context, polysaccharides are believed to be the foremost thick compound supermolecule. These polymers accommodate ten or additional sugar units and composed of thousands or many thousands of easy sugars that are guaranteed along by glycosidic bonds, thus have molecular plenty as high as a hundred million mass units. The most performed polysaccharides are decided by the monomers. Contains or the style during which they are coupled along. Polysaccharides gift in plants, animals, and microorganisms are majorly liable for their structural and functional roles. The foremost common polysaccharides are polysaccharide in plants and animal starch in animals. They act as storage polysaccharides, whereas bound others give structural support and are referred to as structural polysaccharides. Structural polysaccharides include polysaccharide, mostly in invertebrates [4].

Microbial sourced polysaccharides produced based on developments in biotechnology are widely used in various industrial applications thanks to their superior properties. One of these polysaccharides is pullulan.

2.3.1. Pullulan

Pullulan is a complex sugar (polysaccharide) made by a yeast-like fungus *Aureobasidium pullulans*. In the USA, it is in the status of “Generally regarded as safe (GRAS) components. Pullulan is produced only by the *Aureobasidium pullulans* strain of the fungi using ammonium sulphate as a nitrogen source. Pullulan is an intracellularly synthesised biopolymer at the cell wall membrane [13].

The reasons for choosing Pullulan in this thesis; Pullulan is a non-mutagenic, non-toxic, tasteless, unscented, and edible polysaccharide. The fact that Pullulan films have transparent, colourless, and edible properties also attracts great interest in their use as a reliable coating material. It is also used in cosmetic products, lotions, and shampoos. In addition to these applications, it has recently been used in various biomedical applications such as medicines, tissue engineering, wound healing, and diagnostic imaging [13]. Pullulan’s physical properties are shown in table 2.1.

Table 2.1. Physical properties of Pullulan [13].

Property	Effect
Solubility	Insoluble in organic solvents other than Pullulan, dimethylformamide and dimethyl sulfoxide, which are highly soluble in water.
Stability	Thanks to the bonds in carbons 1, 4 and 6, Pullulan is relatively stable. It decomposes and chars similarly to starch at 250-280 °C.
Viscosity	It dissolves in water by forming a stable viscous structure. It is less viscous than other polysaccharides but does not lose its viscous properties. The viscosity is proportional to its

	molecular weight (M_A). Surface tension is close to water (74 dynes / cm^2)
Ability to create a film	Aqueous solutions of 5-25% can be formed in edible films. It has low oxygen permeability (0,5 cc / m^2 / 24 hours: 60% R.H. and 23 °C), thermal stability, anti-static and preserving its elasticity after being frozen.
Adhesion and binding	It has intense adhesive properties. It is a good auxiliary element in spraying processes.
Moisture retention	It has a moisture content of 10-15% at R.H. <70%. It is not hygroscopic and sticky. It could be used as a moisturiser and binder.
Biodegradability	It is a slow digestion polysaccharide and can be degraded by microbial enzymes such as pullulanase and iso pullulanase.

2.3.2. Industrial Uses of Pullulan

The dried associated powdered crude pullulan culture concentrate has potential industrial use as an adhesive, dispersant, coagulant, or flocculant. Refined Pullulan is very soluble, non hygroscopic, non-toxic, tasteless, odourless, perishable, rubberised to atomic number 8, and proof against oil and grease and changes. It is many potential applications in numerous industries that embrace packaging or coating material for foodstuffs to forestall oxidation. Pullulan also can be side to foods to decrease their caloric price or improve their texture, thickness, or wetness retention. Pullulan or pullulan derivatives may additionally have the price as adhesives, films, and moulded products and as a coating agent for products appreciate fertilisers, prescribed drugs, paper, tobacco, shatter-proof glass, and planography plates. Pullulan shows healthful potential in immunising agent production and as a plasma extender [4,14].

2.3.2.1. Drug Delivery

Pullulan nano gels, nanoparticles and microspheres will act as economic drug delivery systems with increased porosity and retention result. The medicine conjugated with these forms tends to accumulate in tumour/diseased tissue or cells rather more than they are doing in traditional tissues and cut back the toxicity of the drug towards normal cells. These forms will bear cell acquisition with the assistance of cell adhesion receptor integrin, e.g., lectin-like receptors on liver cells end in the upper biological affinity of Pullulan towards the liver [15,16].

The high relative molecular mass pullulan has a short half-life amount in blood circulation, and it accumulates within the liver pullulan will conjugate antiviral drug and this advanced help in dominant viral hepatitis virus malady by directly targeting the liver with none facet effects. It also can manage the dose demand for liver targeting once injected intravenously. The addition of cyclodextrin to pullulan microspheres improves the soundness of the drug (diclofenac), its dissolution rate and bioavailability. Varied pH-sensitive and temperature-dependent pullulan drug delivery systems may be ready by the addition of needed oligomers pendants to neutral Pullulan [17,18]. The pullulan microspheres oligomer pendants act as temperature-dependent non-toxic drug delivery systems. Similarly, pH-sensitive pullulan drug delivery systems work on the premise of a pH distinction in traditional cells and cancer cells. This delivery system is safe and conjointly has higher drug retention in tumour cells with low cardiotoxicity [19].

2.3.2.2. Gene Delivery

Pullulan and pullulan derivatives will act as a potential carrier of genes or macromolecule's nanoparticles of pullulan with deliquescent core facilitate in targeted sequence delivery while not exhibiting any toxicity to conventional cells. Pullulan nanoparticles will bind the specified ligands with their surface and defend them from DNA degradation. Pullulan hydrogels encapsulate with high loading potency and possess sustained unharness of DNA

[15]. It has a high affinity with cationised pullulan DNA compared to neutral Pullulan. Tubular pullulan hydrogels may be used for sequence transfer to tube-shaped structure swish muscle cells or native arteries. These hydrogels have sensible ion properties that ease the sequence targeting to the specified web site. These cannular forms may be formed into 3D matrices for defines of sequences from DNA I degradation and for important gene transfer. Pullulan may be used for sequence medical care by delivery of tiny busy ribonucleic acid (siRNA) to arteries and additionally facilitates gene silencing in tube-shaped structure cells [20,21,22].

2.3.2.3. Tissue Engineering

Tissue engineering could be a method to reinforce the self-healing potential of the broken tissues or organs by making a cell suitable environment mistreatment an acceptable artificial three-D (3D) scaffold. 3D scaffold structure varies with sort of scleroprotein fibres in living thing matrix (ECM) of abraded tissues variety of biopolymers that have properties same as natural tissues will be moulded into varied forms like scaffolds, hydrogels, micro-beads, and nanoparticles for tissue engineering application. The applying of those biopolymers in tissue engineering chiefly involves surface modification practices. The surface properties of Pullulan are often increased by substitution of desired chemical moieties on its group teams. Pullulan has excellent mechanical properties, high association capability and wonderful biocompatibility. Because of these properties, Pullulan primarily based scaffolds have a promising role in facilitating cell-based dermal replacement, tissue engineering of tube cells and bone regeneration [15].

Pullulan colloidal gel scaffolds facilitate building cell-laden microtissue complicated to encapsulate abraded cells for his or her regeneration and proliferation. Hydrogels have nonheritable hefty attention as wound dressings similarly. They need similar physical characteristics to natural soft tissues and may absorb an outsized amount of liquid colloidal gel wound dressings will offer dampish surroundings to the wound and defend it from microorganism infection. These hydrogels conjointly act as carriers for antimicrobial agents with no loss in their bioactivity. Pullulan-collagen composite hydrogels have consistent

consistency and may replicate the dermal design and, with success, incorporate stem cells for early wound healing. Porous nanofibrous pullulan/poly (vinyl acetate) membranes considerably enhance cell proliferation rate and may be used for the expansion of fibroblasts. As shown in Figure 2.5, the properties of pullulan-based scaffolds have a promising role in cell-based dermal replacement, tissue engineering of vascular cells and facilitating bone regeneration [23,24].

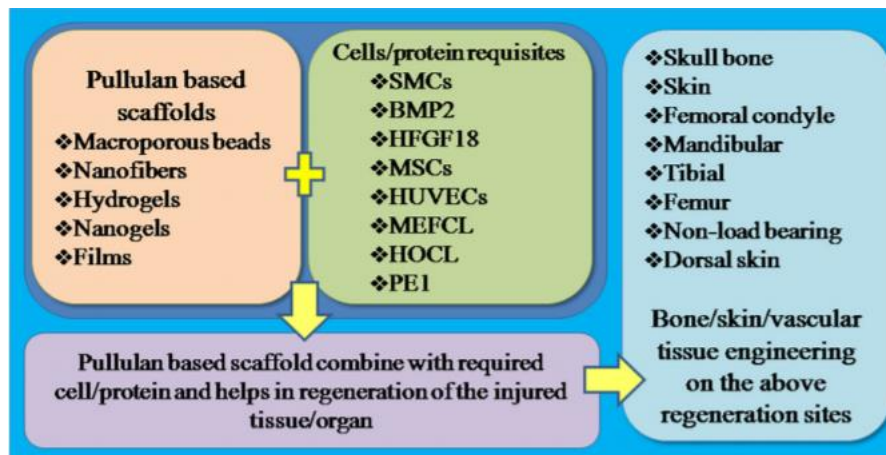


Fig. 2.5. Role of pullulan-primarily based scaffolds totally in tissue engineering-primarily based regeneration therapy totally [15].

SMCs: Smooth Muscle Cells; BMP2: Bone Morphogenetic Protein 2; HFGF18: Human Fibroblast Growth Factor 18; MSCs: Mesenchymal Stem Cells; HUVECs: Human Umbilical Vein Endothelial Cells; MEFCL: Mouse Embryonic Fibroblast Cell Line; HOCL: Human Osteoprogenitor Cell Line; PE1: Prostaglandin E1

2.3.2.4. Vaccination

The figure is created from differing kinds of cells, like blood cells, brain cells, and skin cells. These genes signal that cells manufacture proteins to be healthy functioning and formation of tissues. Cancer begins with a mutation in sure genes of healthy cells conjointly the practicality of proteins and causes the abnormal growth of cells. Cancer invades close tissues, metastasise, and might be fatal if left untreated. It includes the acceptable immunologic response of matter peptides. Nanogel-based nasal immunisation might contain

high levels of antigens. It will adhere to nasal epithelial tissue so transferred to membrane nerve fibre cells. The vaccinium helps in causation protein responses while not the addition of any membrane adjuvant [15,25].

2.3.2.5. Film Forming Agent

Several biopolymers are currently employed in several different areas, corresponding to within the food, pharmaceutical, and medical speciality fields, thanks to their unique physicochemical properties, particularly in comparison with their oil-derived counterparts (e.g., plastics). Applications embody their use as emulsifiers, stabilisers, binders, gelling agents, coagulants, lubricants, film formers, thickening agents, and suspending agents, just to provide a couple of examples.

The pharmaceutical dosage forms suggest that by that drug molecules are delivered to the location of action inside the body. These dose forms ought to be of fine quality, therefore as scale back the environmental result on the drug. Within the Nineteen Seventies, chop-chop dissolving dose forms were introduced as another to traditional tablets. The film coating will be ready by compression, extrusion and evaporation of mixture containing varied polymers, e.g., gelatine, pullulan, pullulan organic compound, polyvinyl alcohol, amylase, etc. The coating helps in bodily function simple capsule, covers the unpleasant style of the drug, and improves patient compliance. The coating improves the tablet's look and splintering resistance, together with targeted drug unharness to the actual website and helps in reducing pill interval. The shelf life and strength of the capsule will be augmented by coating with a sugar resolution containing pullulan derivatives, i.e., pullulan esters and/or pullulan ether created by substitution reactions exploitation radicals. Soft capsules will be film-coated with a mix of Pullulan and different binding substrates, e.g., gelatine, agar, etc. [15]. These Pullulan coated capsules exhibit sensible strength and better solubility.

Capsule coating will be through with soluble neutral Pullulan that forms solid low element semipermeable film on drying. The mixture consists of pullulan resolution, surfactants and setting systems. These coatings facilitate the targeted delivery of the encapsulated drug to the specified sites proprietary technique for coating tablets via liquid diffusion method

employing a mixture of hydrophilic Pullulan and insoluble alkyl radical acrylate-methacrylate advanced. Pullulan coating on pill surface together with some lubricants (polyethylene glycol suspending agent (Talc) and colourants will scale back the brown amendment in the colour of the tablet with time. The coating reduces the disintegration of the pill and improves the looks of the tablet to a completely white colour or desired colour.

The addition of Pullulan increases the steadiness and bioavailability of the drug by increasing its solubility. More involvement of chitosan reduces the flushing action of secretion over medicine, and along these polymers will lower the wetting time and disintegration time of tablets [26].

The Pullulan coated tablets facilitate straightforward execution of specific molecules to remote sites by obtaining instantly dissolved in water. These tablets stay stable at temperature for an extended amount and facilitate direct shipping and storage of bioassay molecules. The hardness of tablets augments with the rise within the concentration of Pullulan. Pullulan and its derivatives will be wrought into physiologically acceptable and orally expendable films. The oral films incorporate secretion stimulating agents that facilitate the disintegration of Pullulan on the tongue or in the cavity. Varied medicine will bind with these oral films and free to circulation. [26].

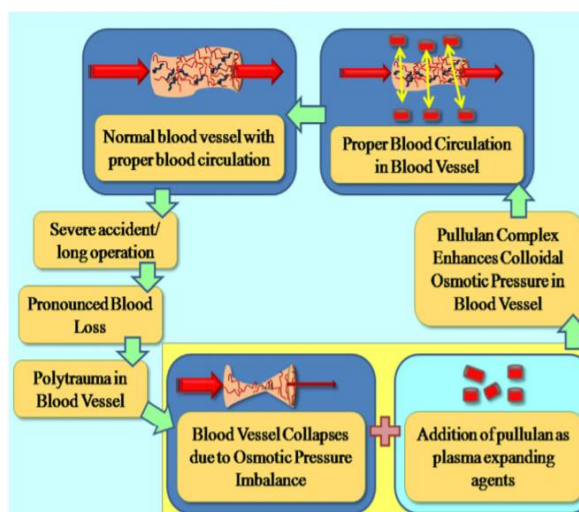


Fig.2.6. The functionality of Pullulan as a blood plasma expander [15].

These pullulan films will be changed by the addition of antimicrobial agents, e.g., essential oils thymic acid, eucalyptol, methanol, salicylate, etc. secretion stimulants and deodorising agents to cure lepidopteran issues like bacterial plaque, periodontal disease, and dangerous breath. Style masking agents (ambulate) will be further to Pullulan edible films to extend their compliance with patients. Pullulan is also used in film form in the food package feature recently [15,27].

Especially epoxy-based resins give some advantage, equivalent to developing a protecting layer setting apart food and drink from the metal accustomed make the cans. Without epoxies, the metal may be corroded, and ultimately microorganism could penetrate the cans, harming the protection in their contents, however additionally, their freshness, nutritional properties, and organoleptic homes such as taste and smell [15,27].

Glass packaging additionally relies on epoxy resins to defend lids from corrosion in bottles and jars, with a view to observe the European Union stringent law on meals packaging [15,27].

2.3.2.6. *Biomedical applications*

The use of natural compounds in medical applications has gained considerable interest because they are simply available, non-toxic, and biocompatible. Additionally, the low value and functionalization of their properties by chemical modification of functional groups make them tunable to the desired property. Such a biocompatible polymer is pullulan which finds its use in various biomedical applications. Pullulan is extensively investigated for its potential as an agent for liver targeting due to its specificity to bind to asialoglycoprotein receptor, which is overexpressed in human hepatic carcinoma cells. Pullulan has a greater probability of accumulating in tumour cells, and this property has additionally been applied within the field of imaging to boost the signal for economical visualization. Pullulan has also been applied as a biocompatible compound in varied different fields [28].

2.4. MEMBRANE TECHNOLOGY

Membrane technology encompasses connected science and engineering. Membrane technology is employed to describe the processes of mechanical separation to separate gas or liquid streams. Some essential steps in the advancement of membrane technology are described in Figure 3.1 [29].

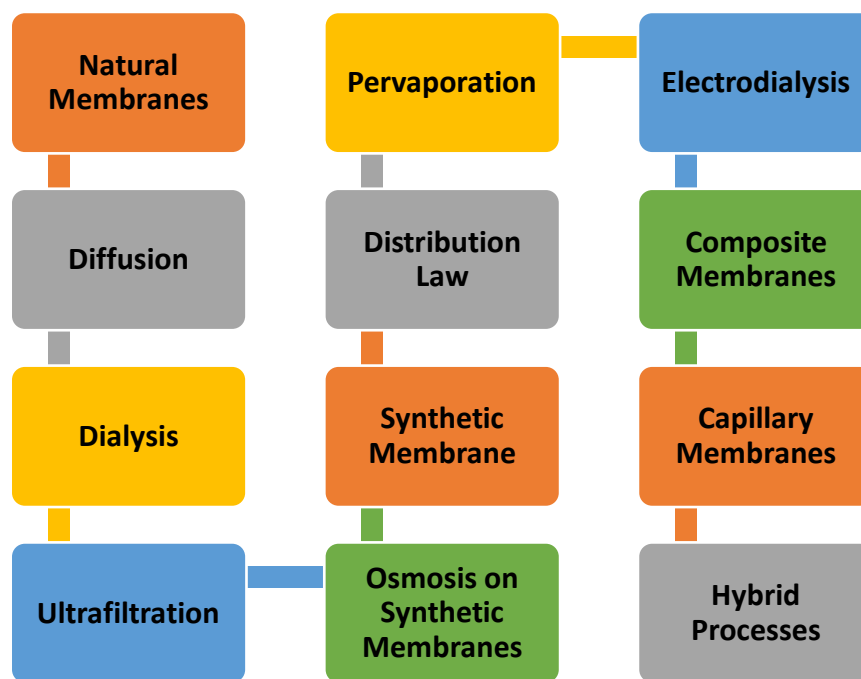


Fig.2.3.1. Steps in the development of membrane technology [28].

Because of its multidisciplinary character, membrane technology is employed in many industries, as well as water treatment for a domestic and industrial facility, chemical, pharmaceutical, biotechnological, beverages, food, metallurgy, and different separation processes. A schematic illustration of the applications of membrane processes is represented in Fig.3.2 [29].

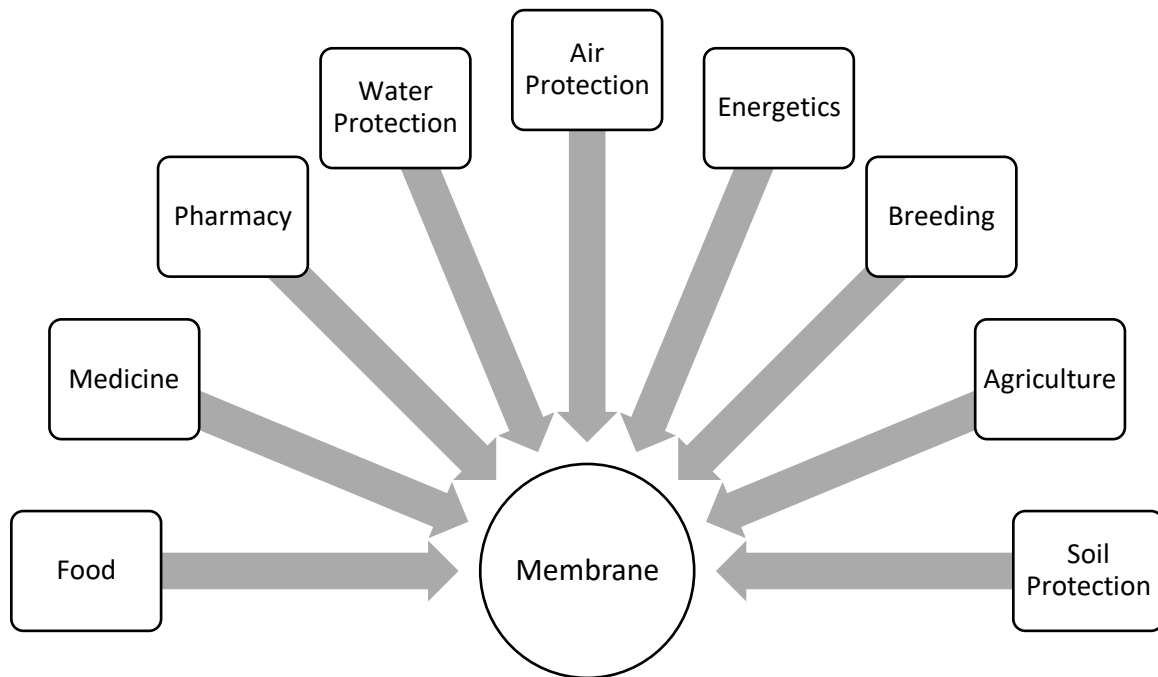


Fig.2.3.2. Schematic illustration of the applications of membrane processes is represented [29].

As with every technology, membrane technology has some advantages and disadvantages.

If we talk about the advantages, these are:

- Continuous separation under mild conditions.
- Membrane properties can be adjusted.
- Up-scaling is easy.
- Hybrid processing is possible etc.

Drawbacks:

- Low membrane lifetime
- Low selectivity and flux
- Upscaling is linear.
- Concentration polarisation membrane fouling

2.4.1. Membrane Processes

The main purpose altogether processes different for various compounds. It is that the uniform application of propulsion across the membrane that may be a semipermeable barrier with the property. Making specific even distribution of propulsion across the membrane and prevailing hydraulic conditions may be a vital and sophisticated method in module development and style of membrane processes. Microfiltration, ultrafiltration, nanofiltration, reverse diffusion, advanced diffusion, membrane distillation, electrodialysis, electrode ionisation, concentration and membrane contactor General info regarding the processes is bestowed [29].

2.4.1.1. Microfiltration process

Microfiltration membranes with pores are starting from 0,05-2 μm . It is employed to get rid of all substances larger than their pore diameter, particularly particles, viruses, and microorganisms, from solutions with their diameter. The separation mechanism in microfiltration membranes relies on the principle of physical sieving. In different words, though there are numerous electrical charge and sorption effects on the particles, the separation method is principally supported by pore sizes. Substances larger than the pore diameter are unbroken on the membrane surface [29,30]

Table 2.4.1. Industrial applications of microfiltration membranes [29].

Industry	Applications
Metal processing Industry	Used in metal processing. recovery of emulsions
Food industry	From fermentation products removal of microorganisms Removal of fat molecules In the dairy industry, like casein colloidal particles, serum protein molecules and milk/fat separation

	From starch and yeast processing residues gaining valuable content
Paper industry	Wastewater treatment
Medicine industry	Solutions or water sterile Filtration
Water sector	Pre-treatment before nanofiltration and reverse osmosis Use for direct water treatment. Organic matter removal

2.4.1.2. Ultrafiltration process

Ultrafiltration membranes with 1--100 nm pore diameters macromolecules, colloids, suspended solids, bacteria are used to separate viruses and substances with molecular weights greater than 1000 g/mol. Even though these substances create osmotic pressure, this is only in the order of a few bars, and the separation process in the ultrafiltration membrane process is provided with a hydrostatic pressure difference varying between 1-10 bar. The transport mechanism is molecular separation and diffusion, and the nature of the feed stream is important. The selectivity of the ultrafiltration membrane to leave It depends on the size difference of the desired material, the surface charge of the components, the properties of the membrane as well as the hydrodynamic conditions [29,30].

Table 2.4.2. Industrial applications of ultrafiltration membranes [30].

Industry	Applications
Automotive and home appliances	Electrophoretic paints rinsing Leaving the waters
Metal processing	Used in metal processing. Recovery of emulsions

Textile industry	Wastewater treatment
Food industry	Proteins from whey recovery From starch and yeast processing residues substance acquisition, Dietary milk production, Concentrating egg whites, Sterilising beverages and the defuzzification
Paper industry	Wastewater treatment
Medicine industry	Sterile filtration of solutions or water, Biologically active substances Isolation, impurity and Concentration (enzymes, viruses, nucleic acids, specific proteins) and blood fractionation
Water sector	Pre-treatment before nanofiltration and reverse osmosis, use for direct water treatment and removal of organic matter

2.4.1.3. Nanofiltration membrane

Nanofiltration membranes are produced to correspond to a molecular weight inhibition limit of approximately 1-10 nm and are generally used for the separation of organic solutions with low molecular weight, such as 200-1000 g/mol. Since they are effective in removing multivalent salts, they are also known as the partial demineralisation process [31].

They can retain dissolved salts, low molecular weight organic substances and organic dyes that their membranes cannot. However, the removal efficiency is under reverse osmosis membranes. Nanofiltration membranes work with both sieving and diffusion transport mechanisms at the porous and non-porous membrane interface, unlike reverse osmosis

membranes, which have a non-porous structure and solution-diffusion transport mechanism. Therefore, nanofiltration membranes find their place in an intersection between “loose” reverse osmosis (non-porous, diffusion) and “tight” ultrafiltration (porous, sieving). It enables it to be operated with higher water fluxes (water recovery). This situation causes significant energy savings. Besides, most nanofiber Membranes are surface charged, so that electrical interactions are also. It contributes to the transport and selectivity properties of the membranes [31].

Since nanofiltration membranes have tiny pores and can retain 200 g/mol weight molecules and above, their removal efficiency is high. It is an important fact that nanofiltration membranes have more selectivity for divalent ions, high solvent permeability and lower energy consumption. However, operating nanofiltration membranes at high pressures can increase energy consumption in cases where water flux decreases. Nanofiltration membranes are generally applied in the form of spiral wound modules.

Nanofiltration membranes are used for hardness removal from surface waters, organic matter removal, dissolved solids, and nitrate removal from well water, as well as pesticide and micro-contaminant removal. Especially in surface waters with low osmotic pressure, it is superior to reverse osmosis systems due to its low operating pressure.

Table 2.4.3. Industrial applications of membranes [31].

Industry	Application
Energy sector	Boiler feed water and cooling tower water recovery
Chemical process industry	Process water production and recovery
	Wastewater treatment and water recovery
	Water / organic liquid separation and organic liquid mixtures distinction
Metal and metal processing	Treatment of mining waste, recovery of coating rinsing water and recovery of metals

Food processing	Milk processing, lactose separation, sweetener concentrating, fruit juice and beverage processing and wastewater treatment
Textile	Dyeing and finishing, chemical recovery and water recovery
Paper Industry	Wastewater treatment and water recovery
Heavy metal removal	Removal of heavy metals that pollute the environment from surface and groundwater

2.4.1.4. Reverse osmosis process

The reverse osmosis process is used for the separation of salt and small organic molecules using membranes with dense active surfaces. The transport mechanism is dissolution/diffusion. Due to the high active surface density, operating pressures are higher than microfiltration and ultrafiltration processes. The reverse osmosis membrane consists of a thin film selectively permeable layer supported by a porous substrate. The thickness of this thin film selectively permeable layer is less than one micrometre and directly affects the flux and selectivity. The reverse osmosis process is mainly used for seawater and saltwater. It is used for desalination. Besides, it is widely utilised in industry to get ultrapure water. These membranes are used in kitchen type treatment systems used in homes [32].

Table 2.4.4. Industrial applications of reverse osmosis membranes [32].

Industry	Application
Desalination	Usable water production, seawater treatment, brine treatment and domestic wastewater recovery

Ultra-pure water	The pharmaceutical industry and medical applications
Energy sector	Boiler feed water and cooling tower water recovery
Chemical process industry	Process water production and recovery, wastewater treatment and water recovery, water/ organic liquid separation and separation of organic liquid mixtures
Textile	Dyeing and finishing, chemical recovery and water recovery

2.4.1.5. Membrane distillation process

With this process, especially high solubility in one step concentration can be reached, and purer water can be produced. In this technology, a microporous hydrophobic membrane separates two fluids with different temperatures and selective mass transfer is carried out across the membrane. This process takes place at a temperature lower than the boiling point of the solutions and under atmospheric pressure. Membrane hydrophobicity prevents the passage of the liquid phase through the pores, and the water vapour condenses on the cold surface by shifting from the warm side to the colder side. Here, the driving force is the vapour pressure difference that occurs at the membrane interface [33].

2.4.1.6. Electrodialysis process

In electrodialysis, ionic compounds in aqueous solutions are used as ion exchangers as the driving force of the electric field with the help of membranes. It is removing by using. The membrane is selective and semi permeable and allows only anions or cations to pass through. Separation takes place with the help of electrical charge rather than molecular size [33].

3.OBJECTIVE

The electrospinning process has gained immediate attention in recent years due to its potential application in medical fields such as drug delivery and tissue engineering. Electrospinning is the technique used to create polymer nanofibers that can be formed from bio-sourced materials, for example, polysaccharides, and has a wide range of uses.

The advantages of nanofibers produced with nanotechnology make this technology advantageous.

The benefits of nanofibers can be listed as follows:

- Large surface area.
- At the nano level, fibre diameter 50-1000 nm.
- High porosity
- The surface can be modified by antibacterial agents, infective drugs.
- Small and controllable pore size.

Polysaccharides are natural biopolymers with various blessings for medicine applications, equivalent to biocompatibility, biodegradability, non-antigenicity, and nontoxicity. Electrospinning may be a versatile and facile technique that might turn out continuous fibres with nanoscale from a good vary of natural and artificial polymers. The medical textile, regenerative medicine and pharmaceutical industry is a market area that will never lose value and is a constantly growing sector. Functional membranes obtained from polysaccharides are used in their potential biomedical applications such as tissue engineering, wound healing, and drug delivery.

Polymer blending creates a very useful method for improving or changing the physicochemical properties of polymeric materials. Polymer blends provide to improve mechanical properties, morphology, permeability, and degradation. PVA could be a semi-crystalline hydrophilic chemical compound with smart chemical and thermal stability. It is a biocompatible and non-toxic polymer and can be easily processed and has high water

permeability. PVA can be used in a wide variety of applications in the medical, cosmetic, food, pharmaceutical and packaging industries. It has good flexibility and toughness, so it is a typical synthetic polymer used to improve physical properties by mixing it with other materials with poor physical properties.

The greatest advantages of the use of nanofibers from polysaccharides to produce functional membranes:

- a) Pullulan/PVA functional nanofibers membrane is non-toxic.
- b) The nanofibers allow high permeability and selectivity.
- c) Pullulan is non-immunogenic, non-carcinogenic and non-mutagenic.
- d) Pullulan is widely used in various biomedical applications, e.g., drug delivery, tissue engineering, grafting, plasma substitution, medical imaging, insulinotropic activity, pharmaceutical dose formation, coating, etc.
- e) A large variety of polymeric solution can be used for the preparation of nanofibers.
- f) The surface can be modified easily.

Based on the explanations listed higher than, varied nanofiber webs were elect for this thesis. The selection of the various nanofibers was made according to; easy to electro spun, low cost, repeatable and even web surface.

This thesis aimed to produce functional nanomembranes that are biodegradable, do not harm the environment and human beings, have a wide range of use, are easy to produce, have less production cost compared to other chemical polymers and have antibacterial properties.

The specific objectives of the thesis are:

1. To produce biodegradable and harmless nanofibers that allow functional uses by using polysaccharides.
2. Development of biodegradable nanofiber membranes from polysaccharides, a natural polymer with inherently antibacterial properties.
3. Development of non-toxic nanofiber membranes.
4. The development of antibacterial has a wide range of use nanofiber membranes.
5. Possibility of obtaining thin film by the surface modification, non-porous, hydrophobic nanofilm which can be used in sectors such as food package, filtration etc.

4.EXPERIMENTS

4.1Materials and Methods

Materials such as Pullulan, PVA, NaCl, epoxy, hardener (were obtained from Sigma Aldrich, Czech Republic). Nanofibers were prepared using NS-1S500U Nanospider™ (Elmarco, Czech Republic) under the controlled conditions. Preparation conditions are given in Table 4.1.

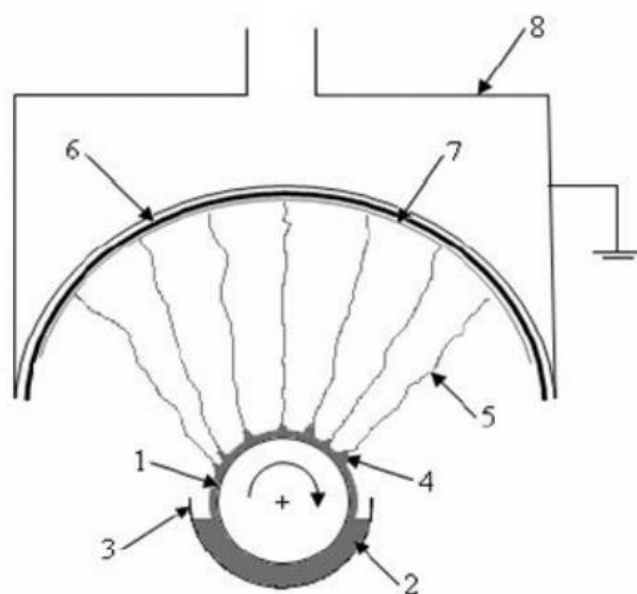


Fig.4.1. The scheme of Nanospider™ system [34].

1. metal roller; 2. polymer solution; 3. reservoir; 4. Taylor cones; 5. fibers; 6. polypropylene non-woven fabric; 7. nanofiber sheet; 8. negative electrode

Nanospider™ has been developed as a new, patented, needle-free, high-voltage, free-liquid surface electrospinning process. The generation is based upon the discovery, that it is possible to create Taylor Cones and the following flow of material not only from the tip of a capillary, however additionally from a thin film of a polymer solution [34].

Table 4.1. Preparation conditions of the nanofiber.

Air Input	Temperature 21,6 °C
Air Humidity %	24,7%
Air Temperature	Temperature 22,3 °C
Air Output	Temperature 22,2 °C
Air Input flow (m³/h)	24,9 m ³ /h
Air Output (m³/h)	102,9 m ³ /h
Electrode Distance (mm)	179 mm

Rewinding Speed (mm/min)	12 min/sec
EMW Speed (mm/sec)	354 mm/sec
High Voltage Supply Current (kV)	49,9 kV

Pullulan: Pullulan is a complex sugar (polysaccharide) made by a yeast-like fungus *Aureobasidium pullulans*. Pullulan biodegradable and water-soluble with low oxygen permeability, and it is used for food coating and drug processing. It is compatible with the FDA (U.S. Food and Drug Administration) and non-toxic. Therefore, for food products, it can be used in repeated contact [13].

Early estimations of the mass of Pullulan showed tidy variability, with values starting from $4,5 \times 10^4$ to around $2,5 \times 10^5$. Pullulan molecular weight (M_w) about 362 kDa. Some of this variability is also because of the various strategies of mass determination used, however, because the mass of Pullulan might decrease with fermentation time. Since Pullulan has a high affinity to liver cells, the use of Pullulan in liver tissue engineering is anticipated. The chemical structure of Pullulan is shown in Figure 4.1 [13].

The manufacturing technique is conducted below conditions of fine producing practices and uses raw materials and method aids that food-grade specifications. Pullulan is created by mesophilic fermentation of starch sweetening by the chosen non-toxigenic strain of *Aureobasidium pullulans*. The strain has been selected by ancient techniques, i.e., the strain isn't the merchandise of genetic modification victimisation recombinant technologies. The assembly strain encompasses a high yield of pullulan, low production of pigment and doesn't manufacture *Aureobasidium pullulan*. After completion of the fermentation, the plant cells are removed by microfiltration [13].

Table 4.2. Pullulan properties 10% wt. [13].

Viscosity (10% wt. 30 °C)	265,2 ± 22 cP
pH (10% wt.)	7,3
Molecular Weight (M_w)	about 362 kDa
Water Solubility	Soluble

Average Pore Radius (Å) ± SD	29,59±0,08
Total Pore Volume (cc/g) ± SD	0,06313±0,01
Surface area (m² /g) ± SD	42,679± 1,75
Conductivity (mS) ± SD	13,66 ± 2,5
Glass Transition Temperature (T_g)	43 C°

Another reason for choosing nanofiber blend in this study is the mechanical properties.

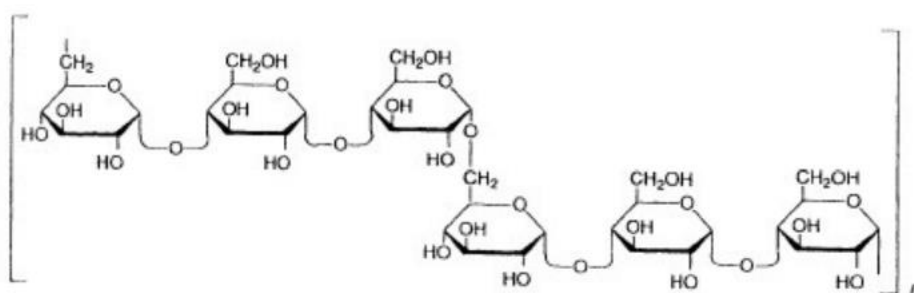


Fig.4.2. Chemical structure of pullulan [13].

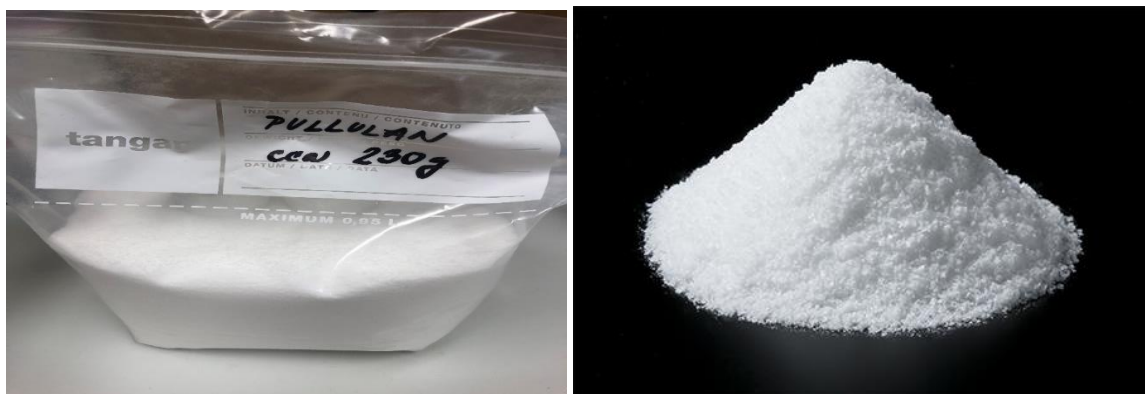


Fig.4.3. Pullulan

PVA: Polyvinyl alcohol (PVA) is $[\text{CH}_2\text{CH}(\text{OH})]_n$ and the most ordinarily used soluble artificial chemical compound for medical speciality applications. PVA is insoluble in organic solvent and solely sparsely soluble in grain alcohol. Owing to its solubility pattern and simple degradability, PVA is additionally called a “green polymer”. PVA shows

compatibility with a variety of polymers, and it is simply entailed with numerous materials that extend the vary of its relevancy [35].

Table 4.3. PVA properties [35].

Molecular Weight (M_w)	About 26,300 and 30,000
Melting Point (T_m)	185 °C
Glass Transition Temperature °C	80 °C
Water Solubility	Soluble
pH	range of 5,0 to 6,5
Density (ρ) g/cm³	1,19-1,31 g/cm ³

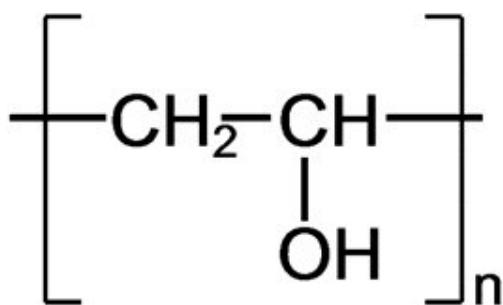


Fig.4.4. Chemical structure of PVA [35].

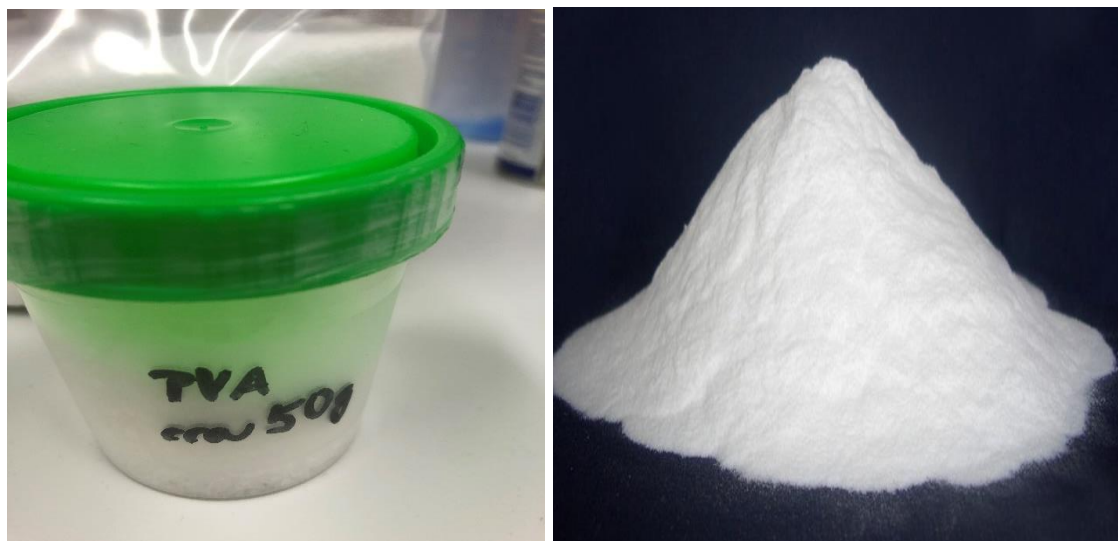


Fig.4.5. PVA

NaCl (Sodium Chloride): Sodium chloride is often referred to as salt (although ocean salt conjointly contains different chemical salts), is an ionic compound with the formula NaCl, representing a 1:1 magnitude relation of metal and chloride ions. Common salt is that the salt most chargeable for the salinity of H₂O and the humour of the many cellular organisms. In its edible variety of flavourer, it is ordinarily used as a seasoning and food preservative. Giant quantities of common salt are utilised in several industrial processes, and it is a severe supply of metal and atomic number 17 compounds used as feedstocks for additional chemical syntheses [36].

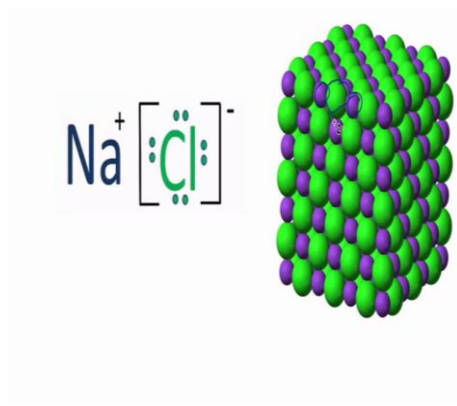


Fig.4.6. Chemical structure of NaCl [36].



Fig.4.7. NaCl

Hardener: Hardener (Polyamines Liquid) may be a part of certain forms of mixtures. Polyamine's liquid uses epoxy. Polyamine hardeners are created of an organic molecule containing two or a lot of amine groups. In some mixtures, a hardener is employed merely to extend the resilience of the mixture once it sets. In different mixtures, a hardener is employed as a solidification part. A hardener is often either a chemical or a catalyst within the chemical action that happens throughout the blending process. A hardener might also be called an associate degree accelerator [37].

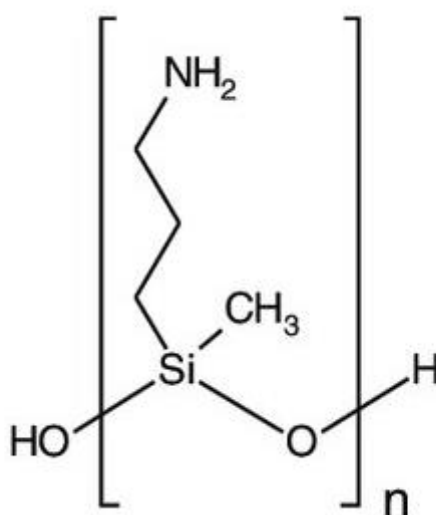


Fig.4.8. Chemical structures of polyamine hardener [37].

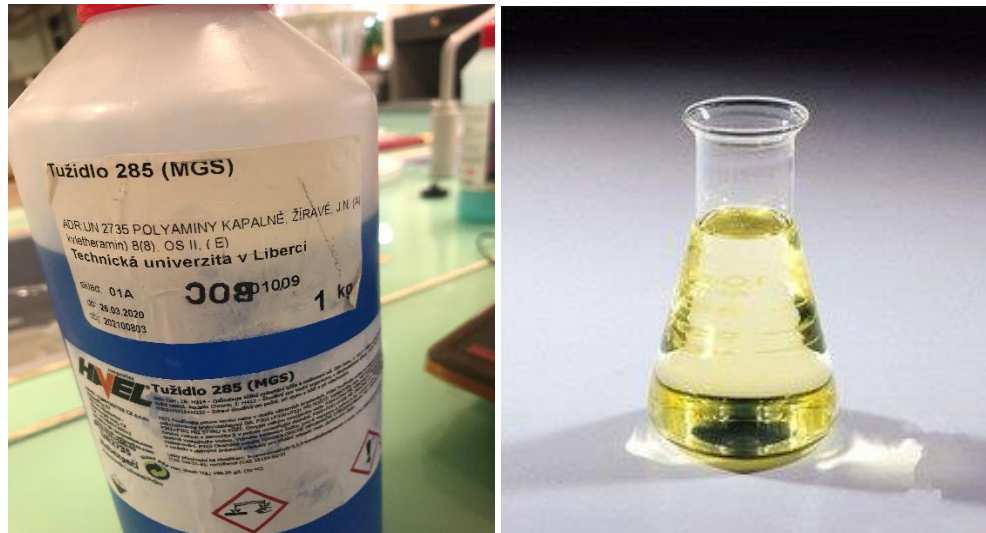


Fig.4.9. Hardener

Epoxy Resin: The resin binds the fibres together, permits loads to be transferred between the fibres and provides a barrier to weather, water, or chemicals. Epoxies are sometimes sold as a two-pack system; half A (resin) and half B (hardener). Half A (resin) is that epoxy, and there are many alternative types available. Epoxy resin and hardener are each equipped as liquids. Once they are mixed, they react, and cross-links are formed between the two chemicals. Once several the cross-links have formed, the system forms a gel and is claimed to be gelled when most of the cross-links have formed. The system forms a solid [38].



Fig.4.10. Epoxy resin

4.2. Production of Nanofiber

In the experimental part of the study, firstly, solutions of different sizes required for electrospinning were prepared according to ČSN P CEN ISO/TS 80004-8. These solutions are described below:

- **PVA solution:**

PVA (Sigma Aldrich, 87%-89% hydrolysed) 10 weight percentage (%wt.) distilled water we dissolve at 80 ° C.

- **Pullulan solutions:**

Pullulan 10 weight percentage (wt. %) and PVA 10 wt. % in distilled water with the assistance of heating and magnetic stirring was used. The solution was prepared separately then mix in a weight ratio (Pullulan/PVA) 0.05 wt.% NaCl (Sodium Chloride).

According to the recipe mentioned above, prepared the solutions separately and then mix in two different mass ratios were prepared 60/40 and 80/20 with 10 wt.%. The prepared solutions were mixed at 280 rpm at 39 ° C. 0.05 g NaCl was added in this study. Conductivity problem occurred in the mixture with NaCl added, and some gaps were observed on the nanofiber surface. It is shown in Figure 4.12, so the solution was prepared without adding NaCl for the second time.

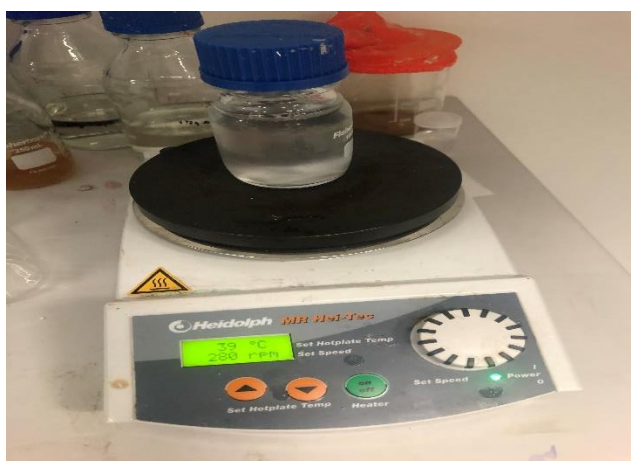


Fig.4.11. PVA / Pullulan solution 60/40 obtained by mixing at 280 rpm, 39 ° C.

A polymer solution is injected through a nozzle at a constant feed rate or high connected needle voltage 49,9 kV.

In this thesis, electrospinning method was produced by using Nanospider™ (Elmarco, Czech Republic) electrospinning machine.

The applied voltage causes a load on the surface of the liquid droplet, and when this is high enough, the surface of the hemisphere extends, and a Taylor cone is formed. Upon further increasing the applied voltage, a charged liquid fountain is ejected. The Taylor cone is pulled into the ground and the collector placed in a fixed place distance from the needle. Meanwhile, the process polymer solution in which the solvent evaporates, leaving the dry polymer fibres in the collector.



Fig.4.12. Produced under the conditions of electrode distance 179 mm, air humidity 24,7%, temperature 21,6 °C nanofibers. 60/40 % Pullulan/PVA

a-)0.05 g NaCl added. b-) Without NaCl on standard spun bond nonwoven base layer.

It was difficult to separate the fibres from the spun bond base layer. Therefore, it was decided to replace the layer with a self-oiled paper base layer, facilitating the separation of nanofibers from the base layer and minimising fibre damage. The new layer used is shown in Figure 4.13. The Pullulan / PVA nanofiber obtained is in water-soluble form. No oil is used or oiled

in the production of the base layer. The oily texture on the substrate is wax, and we obtained the paper from the producer.



Figure 4.13. A self-oiled paper base layer

4.3. Surface Modification

The purpose of the surface modification is to obtain a non-porous nanofilm from porous and water-soluble nanofiber. The aim of this thesis is to enable Pullulan / PVA nanofiber to be used functionally in areas such as the energy sector, sensor and filtration, food package industry, pharmaceutical industry, etc.

The aim of this study makes the production of Pullulan/PVA films to produce a thin film by the surface modification. A new approach in this work, epoxy resin modified to nanofiber surface.

Epoxy organic compound represents one of the foremost necessary engineering polymers and has found wide applications reminiscent of adhesives and electronic encapsulation materials. Epoxy resins have replaced steels as structural elements in craft and satellites thanks to their high mechanical strength. Epoxy resin and hardener are each equipped as

liquids. When the epoxy resin and hardeners are mixed at area temperature, they react, and cross-links are shaped between the two chemicals. When several cross-links have formed, the system forms a gel and is claimed to be gelled when most of the cross-links have formed, the system forms a solid and is said to be cured.

Nano-miniaturization of epoxy resins will more improve their mechanical performance and expand their application and is usually wont to meet high strength and/or high hardness necessities. Epoxies are compatible with most composite producing processes, significantly dust bag moulding, autoclave moulding, pressure bag moulding, compression moulding, filament winding and hand lay-up.

In this work, epoxy resin was obtained together with a hardener. The prepared film obtained more homogenous. Beside membrane is thinner because some amount of the solvent evaporated. The processes of epoxy resin application on Pullulan/PVA nanofiber shown in Figure 4.15.

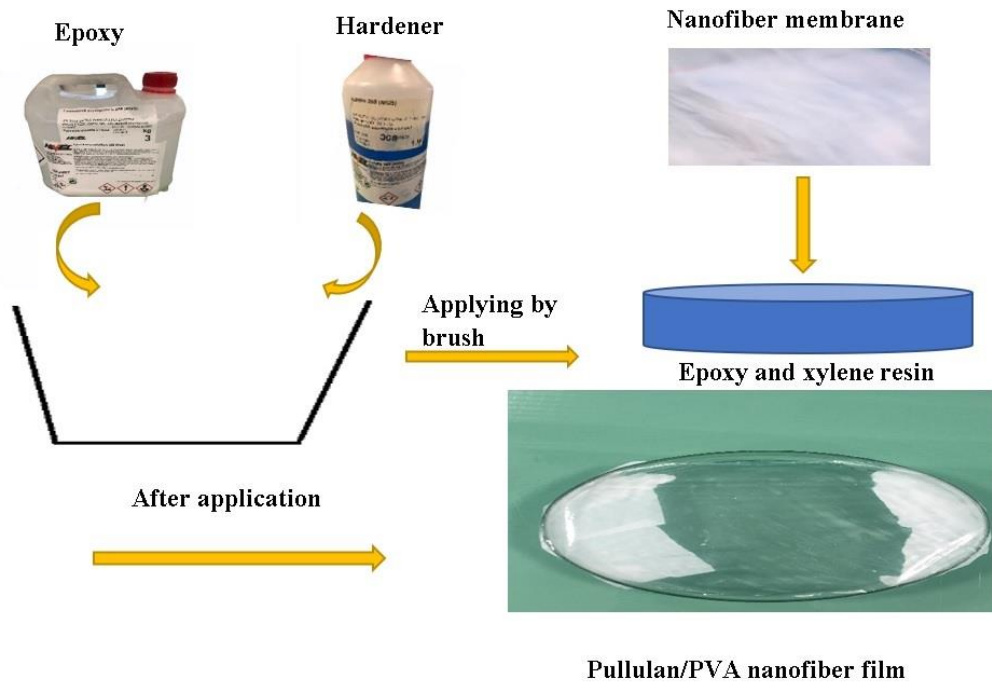


Fig.4.14. Process of epoxy/ hardener resin application on nanofiber.

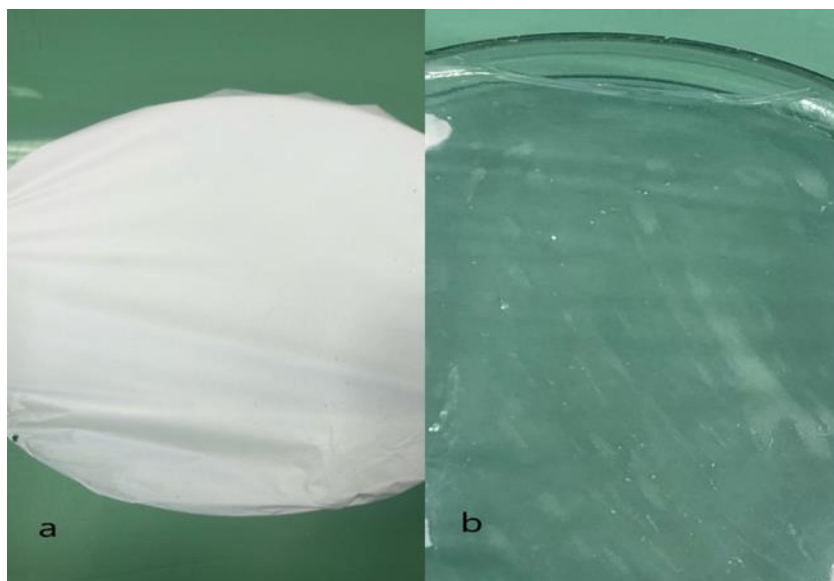


Fig.4.15. Epoxy resin-modified Pullulan /PVA nanofiber transparent film.

a-) Before the resin application. b-) After the resin application.

Amounts and application conditions used for five different samples are shown in table 4.4.

Table 4.4. Receipt of resin application

Sample	Amount of Resin	Drying Conditions
1	Epoxy 3 g - Hardener 1 g.	20 °C± 2 °C 65% air humidity 24 hours
2	Epoxy 3 g - Hardener 1 g.	80°C 2 hours
3	Epoxy 5 g - Hardener 1 g.	20 °C± 2 °C 65% air humidity 24 hours
4	Epoxy 5 g -Hardener 1 g.	80°C 2 hours
5	Epoxy 6 g - Hardener 1 g.	80°C 2 hours

6	Epoxy 6,024 g - Hardener 2,115 g.	20 °C $\pm$ 2 °C 65% air humidity 24 hours
7	Epoxy 6,024 g - Hardener 2,115 g.	80°C in oven 2 hours

The total amount of solvent prepared is 11,229 g. The amount used for the samples is 5,634 g.

Deformation was observed in the sample kept in the oven at 90 °C for more than 10 minutes. For this reason, the optimum temperature was determined as 80 °C for 2 hours for the oven. The samples kept at 90 °C degrees for 2 hours at 80 °C are indicated in Figure 4.16. The Pullulan / PVA nanofilm obtained is in water-insoluble form. The surface had an adhesive form after the resin was applied.

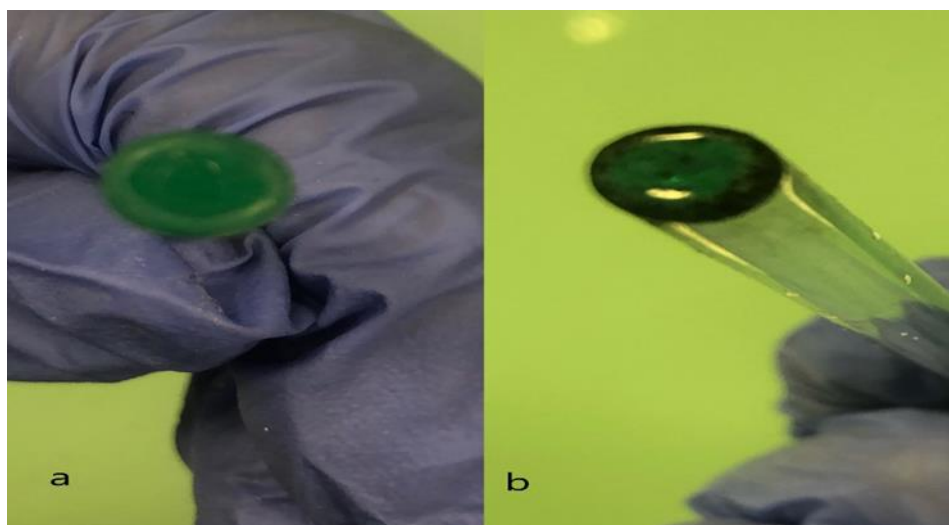


Fig. 4.16. Carbon residue visible on the glass rod shows adhesivity.

a-) Glass rod is contacted with the membrane surface-) The glass rod, which was brought into contact with the surface, was then transferred to the carbon to detect adhesivity.

5.RESULTS and DISCUSSION

5.1. Surface Morphology (SEM)

A scanning microscope (SEM) is a kind of microscope that uses a centred ray of electrons to scan a sample's surface to form a high-resolution image. SEM produces pictures that will show data on a material's surface composition and topography. Membrane morphology was tested using SEM images.

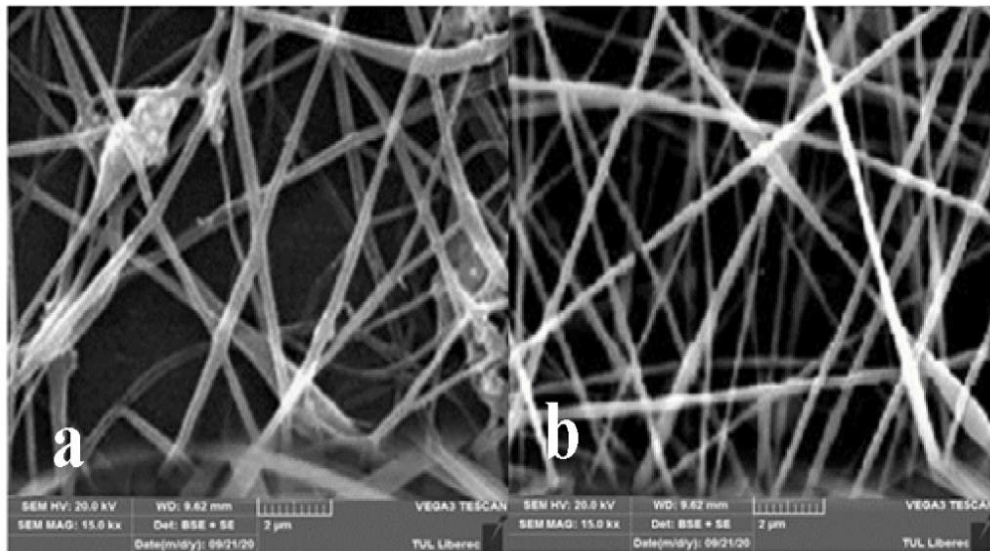


Fig.5.1. SEM images of Pullulan (a), PVA (b). Nanofiber production of applied voltage = 49,9 kV and scale bars in images represent 2 μm .

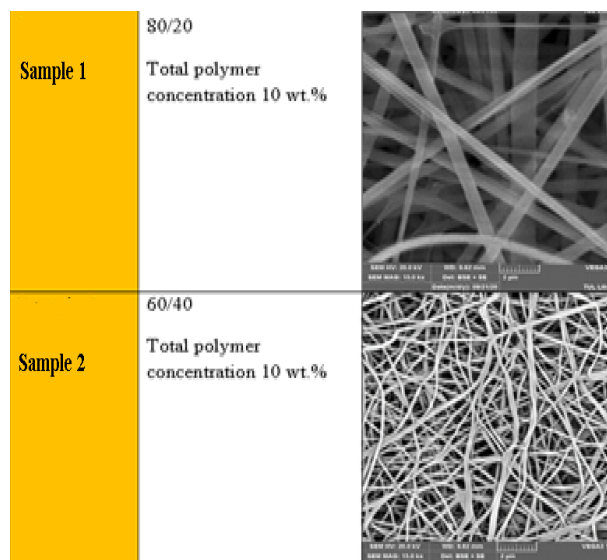


Fig.5.2. SEM images of electro spun Pullulan/PVA blend nanofibers with various mass ratios. Scale bars in images represent 2 μm .

Sample 1: 80/20, sample 2: 60/40.

The acidic solution tends to give higher electrospinning because of the presence of H^+ ion concentration, aiding in the attraction of chemical compound solution droplet from the tip of the collector towards the negative electrode once field may be applied. So, the Pullulan with neutral to alkaline pH scale was not obtaining electro spun with any carrier polymer. Higher zeta potential and conductivity led to increasing the charge carrying ability of the polymer jet that subjected to higher tension underneath the electrical field, resulted in poor fibre formation. The viscosity range of various polymer solutions at which electrospinning is completed is a major variable. The higher viscosity of extracted pullulans aided them to give continuous smooth fibres when electro spun, whereas pullulan having lower consistency would give beaded fibres. PVA was used as a carrier polymer because it is comparatively inexpensive, with chemicals and thermally stable, and not degradable under most physiological conditions. Pullulan / PVA blend fibre is shown in Figure 5.3.

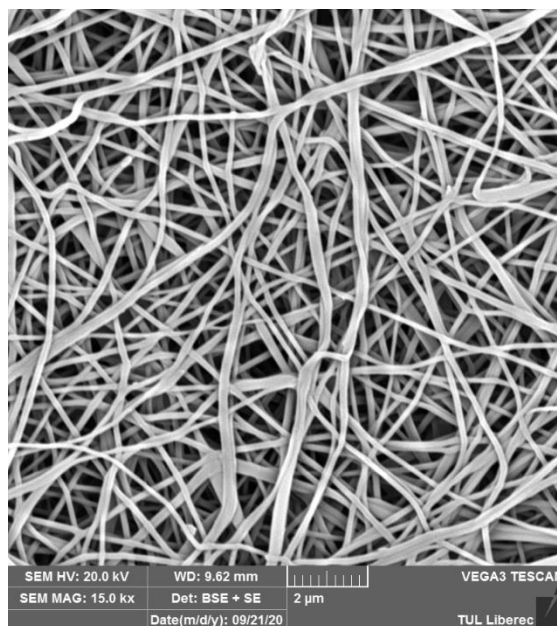


Fig. 5.3. SEM images of Pullulan/PVA blend nanofibers that electro spun with various total polymer concentrations of Applied voltage = 49,9 kV. Blend ratio (Pullulan/PVA) = 60/40, and Total polymer concentration = 10 wt.%) with 2 μm zoom.

The SEM images analysed in Image J software; the average diameter range of nanofibers was found to be 220 nm for pullulan blended with PVA.

Table 5.1. Average diameter range of nanofibers with different ratio.

Electro spun nanofiber	Diameter in nm (Average $\pm$ SD)
Pullulan	830 $\pm$ 50,498087
PVA	490 $\pm$ 61,567565
Pullulan 60% + 40 % PVA	213 $\pm$ 21,068474
Pullulan 80%+ 20% PVA	512 $\pm$ 36,096819

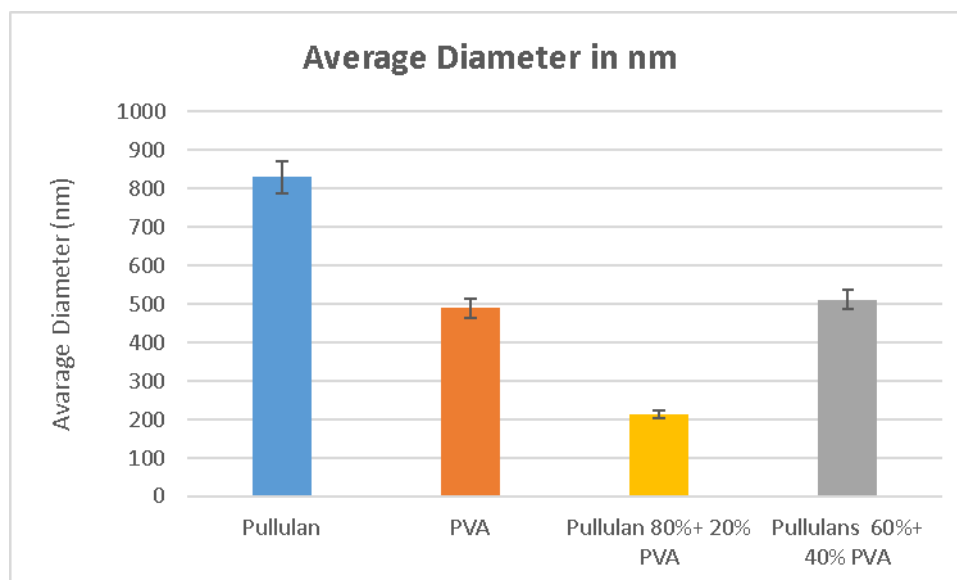


Fig.5.4. The average diameter of Pullulan/PVA nanofiber

The fibres obtained by electrospinning pullulan and PVA were larger in diameter compared to their blended fibres. It is seen in table 5.1. and Figure 5.4. that when the pullulan ratio decreases, the diameters also decrease.

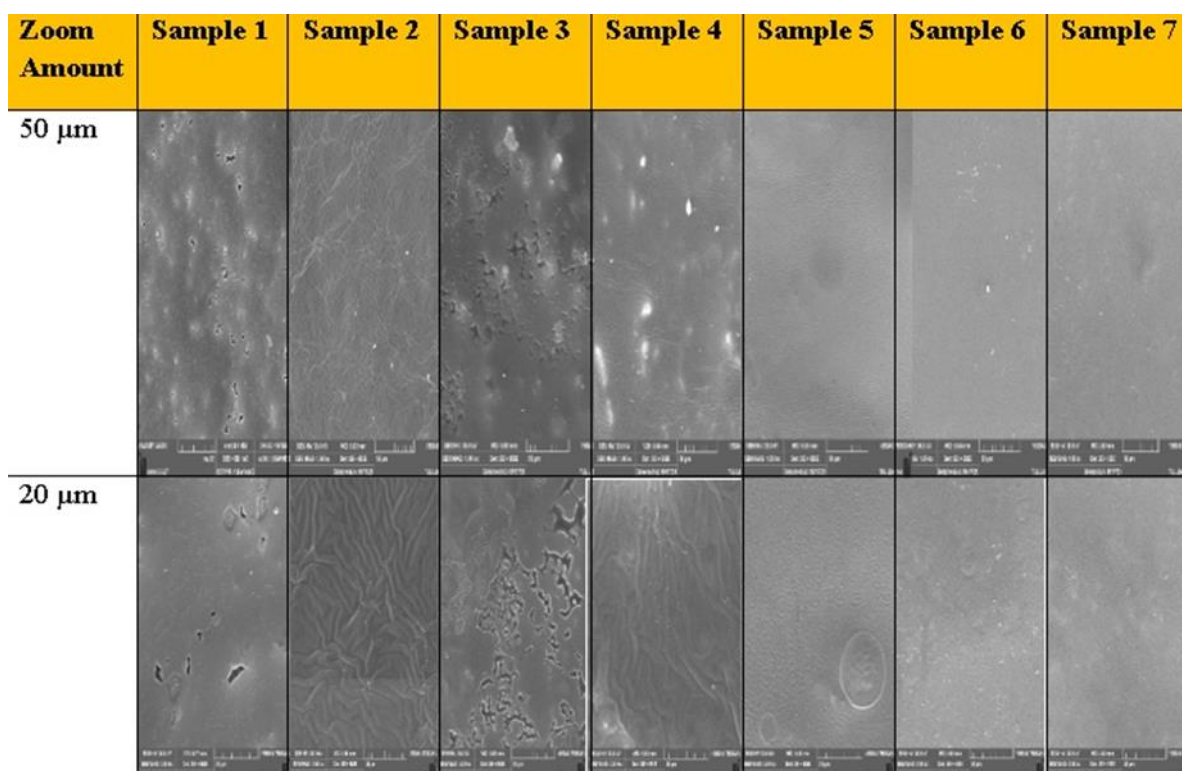


Fig. 5.5. Surface morphology of modified samples by epoxy resin with 30 kV. Working distance is 9,82 mm.

The properties of the samples are given in Table 4.4. Figure 5.5. shows at 50 μm and 20 μm magnification nanofiber films obtained using epoxy and hardener are seen. The reason for the differences in the SEM appearance of the nanomembranes produced by the recipe given in Table 4.4 is due to the different amounts of resin used and the way they are dried. Samples 1,2,3, and 4 are much thinner than samples 5,6,7. A significant loss of strength and surface deformation was observed in samples 1, 2, 3, 4 and 5. However, Samples 6 and 7 have a smooth surface and a thicker structure. The reason is the amount of resin used is less than the others, and some of the solvents evaporate from the samples during drying, causing the membrane to be thinner. Increasing the amount of resin used enabled to obtain of more durable and smoother nanofilms.

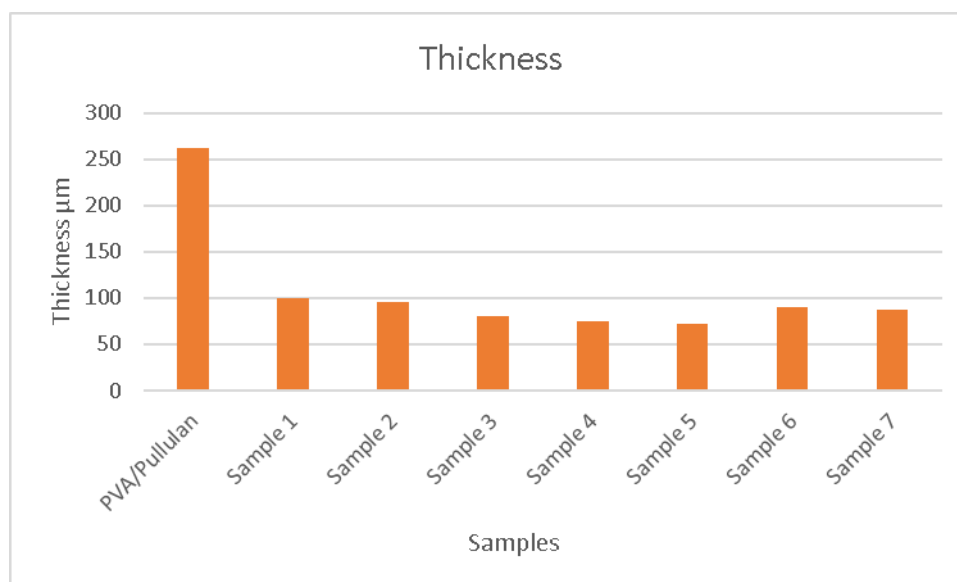


Fig.5.6. Thickness values for nanofiber and nanofilms

Table 5.2. Thickness values for samples with different amount of solvent

Sample	Thickness μm
PVA/Pullulan	261,6
Sample 1 (Epoxy 3 g - Hardener 1 g. 20 °C± 2 °C 65% air humidity 24 hours dried)	99,6

Sample 2 (Epoxy 3 g - Hardener 1 g. 20 °C± 2 °C 65% air humidity 24 hours dried)	95,4
Sample 3 (Epoxy 5 g - Hardener 1 g. 20 °C± 2 °C 65% air humidity 24 hours dried)	80,0
Sample 4 (Epoxy 5 g - Hardener 1 g. 80°C 2 hours dried)	75,2
Sample 5 (Epoxy 6 g - Hardener 1 g. 80°C 2 hours)	72,0
Sample 6 (Epoxy 6,024 g - Hardener 2,115 g. 20 °C ± 2 °C 65% air humidity 24 hours dried)	90,5
Sample 7 (Epoxy 6,024 g - Hardener 2,115 g. 80°C in oven 2 hours)	97,1

The thickness determination methodology is often quicker by a software system that is appropriate for constructing laboratory primarily based programs for knowledge grouping and processing during a graphical interface.

For verification of the film thicknesses, samples are analyzed by a scanning electron microscope. Specimens are ready cross-sectional, and skinny film thicknesses are measured by microscope's software ImageJ[®]. The thickness values have changed according to the solvent ratio shown in Table 4.4. The reason why the thickness values of Pullulan / PVA films added with less ratio is low is that the epoxy is high viscosity without polyamine. The epoxy cannot penetrate the fiber system, and the viscosity of the epoxy decreases with the

added polyamine. The prepared film is more homogeneous, as shown in Figure 5.4. However, the membrane was thinner because some of the throttling evaporated. The amount of solvent used to eliminate this effect was increased. The longer the drying time provided more penetration.

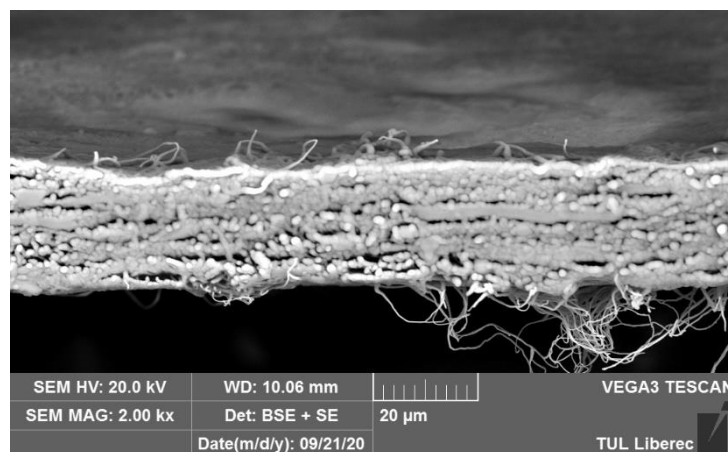


Fig. 5.7. Cross-sectional SEM images PVA/Pullulan 60/40 % nanofiber membrane produced at 49,9 kV. Working distance is 10,02.

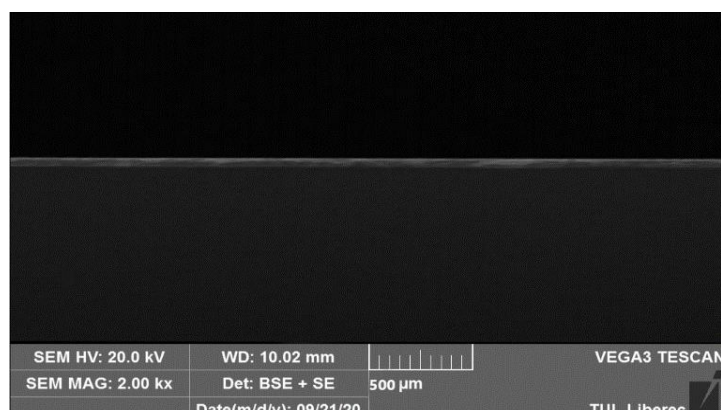


Fig. 5.8. Cross-sectional SEM images PVA/Pullulan film membrane with Epoxy resins with 49,9 kV. Working distance is 10,02.

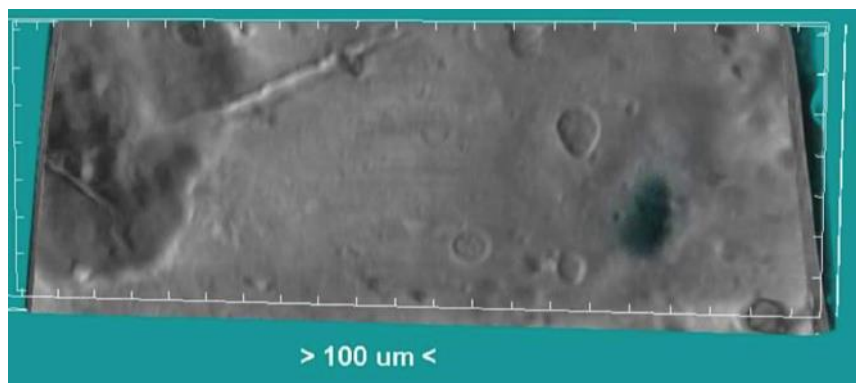


Fig.5.9. Pore observe after surface modification.

A non-porous surface was obtained because of PVA / Pullulan nanofiber epoxy surface modification. At the voltage of 12 kV.

5.2.BET Analysis

Determination of the specific surface area of solids by gas adsorption is the BET method. The area is quantified by using the method developed by Brunauer, Emmett and Teller (BET method) that is an extension of Irving Langmuir theory of monolayer surface assimilation to multilayer adsorption. This is the maximum widely to be had and applied approach for determining aerogel porosity. In this approach, a gas, commonly nitrogen, at its boiling point is adsorbed at the sample. The quantity of gas adsorbed depends on the dimensions of the pores inside the sample and at the partial pressure of the gas relative to its saturation pressure. By measuring the quantity of gas adsorbed at a selected partial pressure, the Brunauer, Emmet and Teller (BET) offer the specific surface area of the material.

This analysis was analysed according to ČSN P CEN ISO/TS 80004-6 standard. Samples were degassed at 160 °C for 2 hours under vacuum before. All the parameters were analysed in triplicates and were expressed as average $\pm$ standard S.D.

Table 5.3. BET analysis of nanofibers with different ratio.

Electro spun pullulan nanofiber	Average Pore Radius (Å) ± SD	Average Total Pore Volume (cc/g) ± SD	Average Surface area (m² /g) ± SD
Pullulan	15.80 ± 0,855497	0.1390 ± 0.002	182.60 ± 5.5
PVA	17.40 ± 3,021175	1.1322 ± 0.009	1302 ± 32
Pullulan 80%+ 20% PVA	17.87± 4,487585	0.1194 ± 0.0012	146.30 ± 5
Pullulans 60%+ 40% PVA	15.61 ± 1,846691	0.1176 ± 0.0024	150.68 ± 4.5

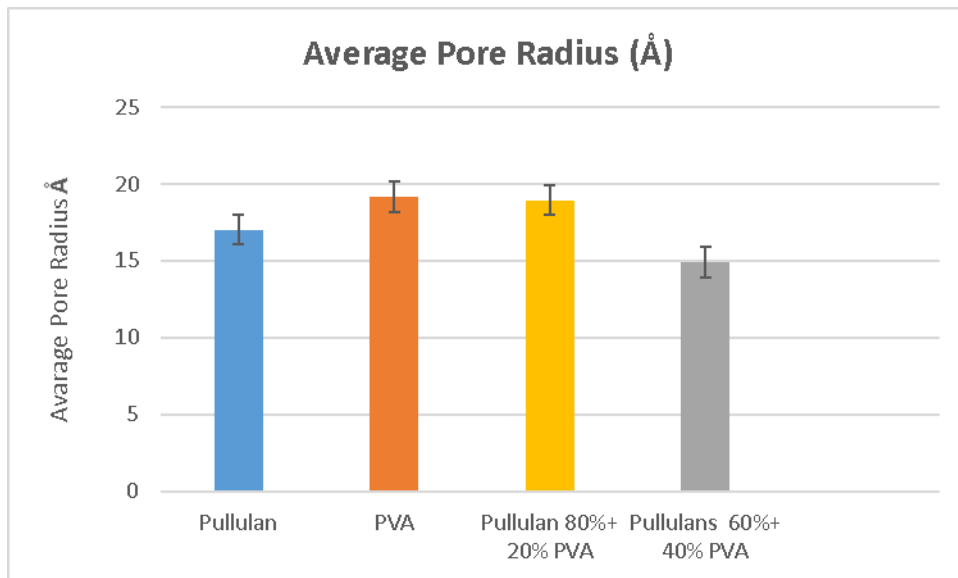


Fig.5.10. Average pore radius for nanofibers

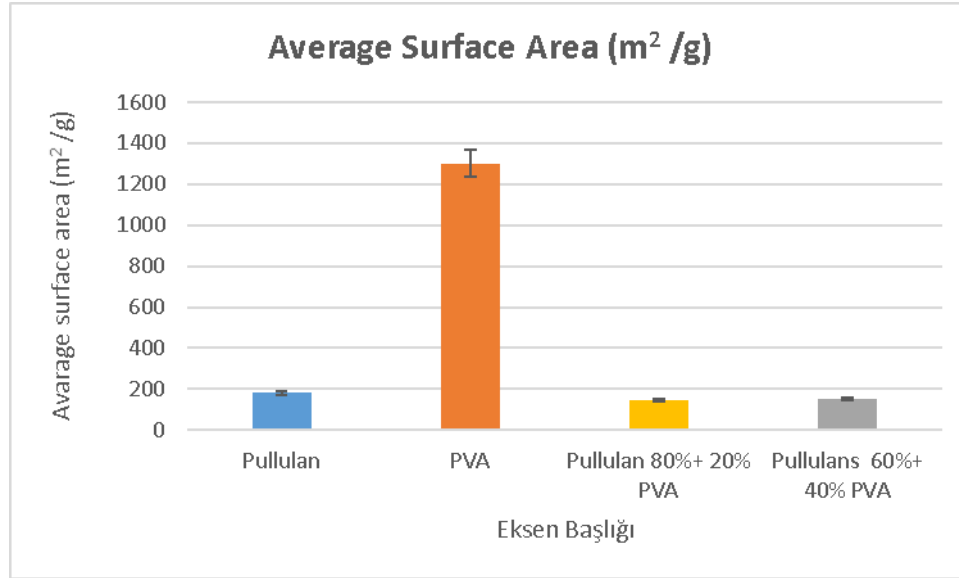


Fig.5.11. Average surface area for nanofibers

The smaller pore size of the 60/40 ratio Pullulan / PVA has a higher diameter and higher tensile strength than others and a smoother nanofiber form. Therefore, surface treatment was carried out with this nanofiber.

Nanofilms are non-porous even in the sample. Therefore, these film materials are not breathable.

5.3. Air Permeability

The breathability was determined by a Text-FX 3300 air permeability tester purchased from Textest A.G. (Switzerland). The breathability of the nanomembranes was measured several times by the ČSN EN ISO 9237 standard. ISO 9237 is the method and standards for testing the air permeability of the fabric. It can be applied in various kinds of materials like nonwovens, protective, technical, industrial textiles, etc. The measure of airflow that passes sheer from the given material space therein provided time and pressure difference. The experiment was performed with breathability. The test was on 200 Pa, the pressure at

20 cm² (l/m²/s). The larger the pore radius, the greater the apparent permeability. Air permeability is shown in Figure 5.13.



Fig.5.12. Air permeability test device

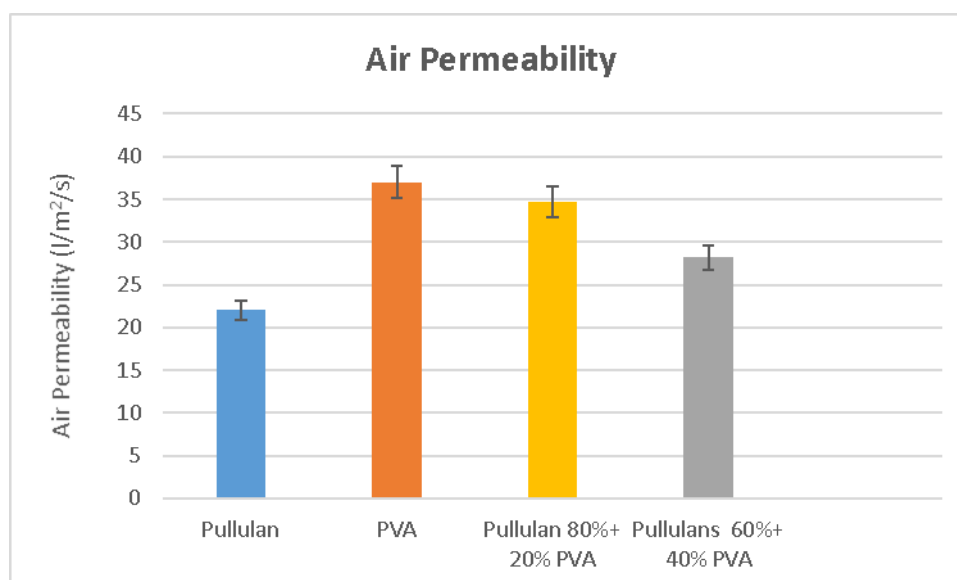


Fig.5.13. Comparison of air permeability for nanofibers with different blend ratio 10% wt.

Pullulan had a lower air permeability than others. The pore size increased with the addition of PVA. The average pore diameter is shown in Table 5.2.

The pore observation after surface modification is shown in Figure 5.9.

5.4. Water Solubility

Samples (2 cm x 2 cm), solubility of nanofibers in water. The samples were thrown into a beaker containing 100 ml of distilled liquid water (25°C) mixed with a magnetic stirrer. Nanofibers dissolved completely in water in 10 seconds. However, the nanofilm did not dissolve in water. It means nanofilms were hydrophobic. Pullulan / PVA nanofiber is hydrophilic. The higher hydrophilicity is due to the hydroxyl in the numerous pullulan molecular chains.

5.5. Differential Scanning Calorimetry Analysis

Differential scanning calorimetry (DSC) is used for thermal analysis. The distinction within the quantity of heat required to extend a sample's temperature and reference is measured as a temperature performance. Each illustration and respect are maintained at nearly identical temperature throughout the experiment. Generally, the temperature program for a DSC analysis is supposed such the sample holder temperature can increase linearly as a function of time. The test was conducted consistent with the standard ČSN EN ISO 11357-1.



Fig.5.14. DSC test device

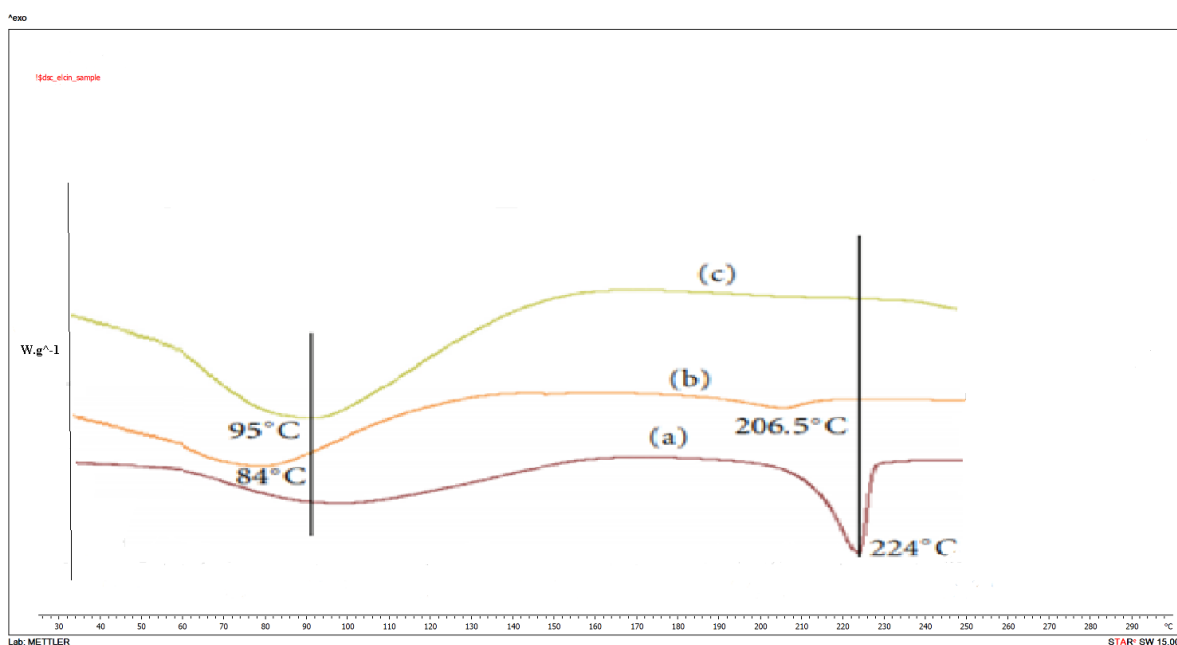


Fig. 5.15.DSC curves for polymers blend membrane using electrospinning method (Applied voltage = 49,9 kV. Blend ratio (Pullulan/PVA) = 60/40, and Total polymer concentration = 10 wt.%)

a-) PVA b-) Pullulan/PVA c-) Pullulan

----- PVA -----Pullulan/PVA -----Pullulan

In Figure 5.15, DSC curves for polymers blend membrane using electrospinning method (Applied voltage = 49,9 kV. Blend ratio (Pullulan/PVA) = 60/40, and Total polymer

concentration = 10 wt.% A comparatively large and sharp endothermic peak is determined at concerning 224 °C in Figure 5.15 (a) temperature of pure PVA. This peak is shifted to 206,50 °C with the addition of 10 wt. Pullulan in Figure 5.15 (b), and this shifts the melting temperature to a lower value; this occurred because of the addition of pullulan, whose melting temperature changes from 95 °C to 84°C in Figure 5.15 (b). This is because most of the chains are in a non-crystalline state due to the rapid solidification process of stretched chains during electrospinning. Pure pullulan shows a large thermogram peak of melting transition (T_m) at 95°C in Figure 5.16 (c).

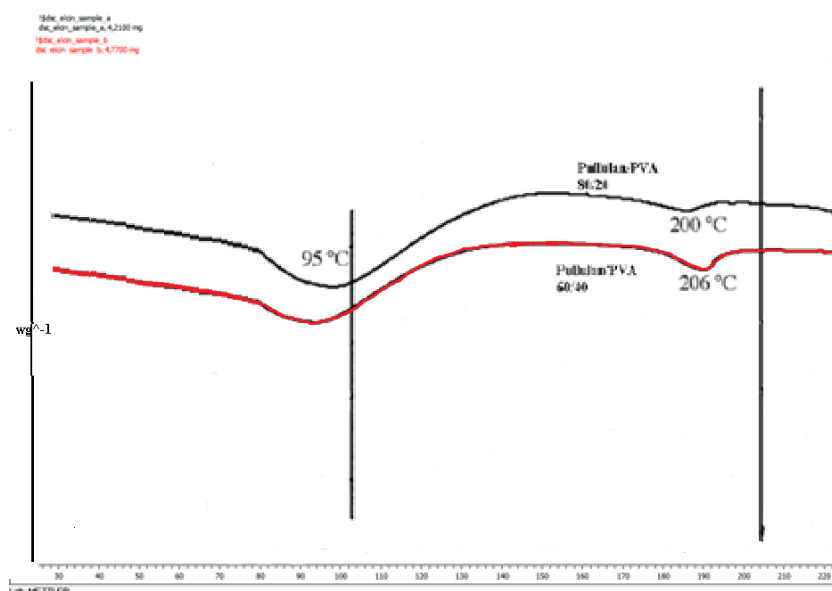


Fig. 5.16. DSC curves for polymers blend membrane using electrospinning method (Applied voltage = 49,9 kV. Blend ratio Pullulan/PVA 80/20 and 60/40.

----Pullulan/PVA 80/20 blend ratio

----Pullulan/PVA 60/40 blend ratio

DSC thermograms electro spun PVA / Pullulan mixture nanofibers with different mixing ratios. A relatively large and sharp endothermic peak blend ratio of 80/20 was observed at

200 C° 80 C° for Pullulan / PVA nanofiber, and when the pullulan ratio decreased, this peak was shifted to 206 C° for Pullulan / PVA nanofiber from 60/40. Mountains towards temperature, indicating that melting decreases with increasing pullulan content temperature of the mixture nanofibers. This decrease in melting temperature probably occurred because most of the chains were not crystalline due to the rapid solidification of the stressed chains during the electrospinning process.

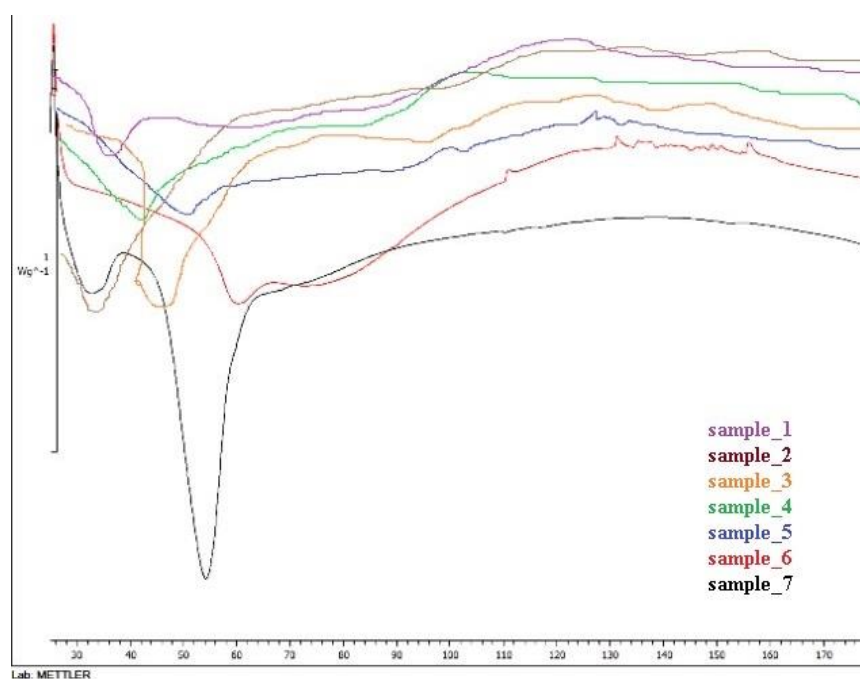


Fig. 5.17. DSC curves comparison of samples method (Applied voltage = 49,9 kV. Blend ratio (Pullulan/PVA) = 60/40, and Total polymer concentration = 10 wt.%)

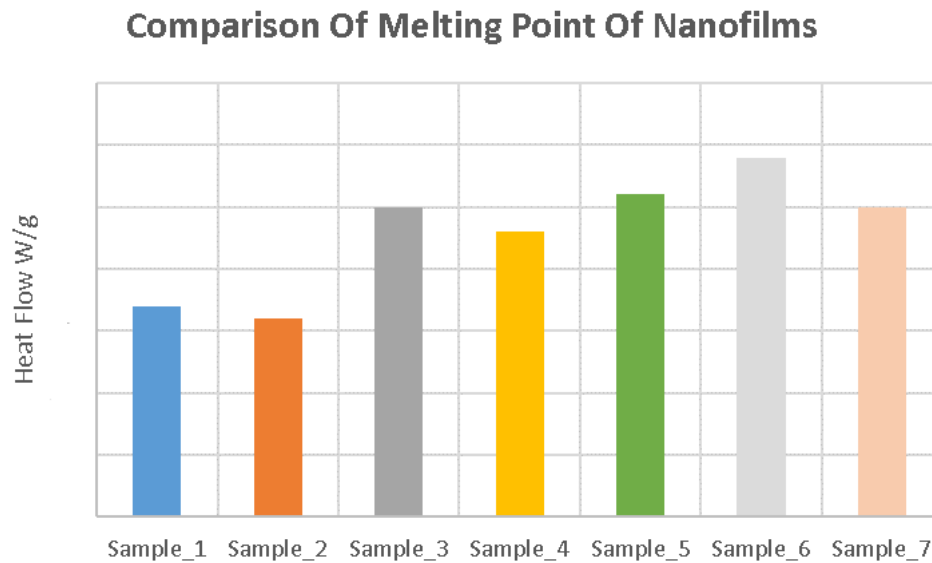


Fig.5.18. Comparison of melting point for nanofilms

DSC analysis of the nano film membranes is shown in Figure 5.18 comparatively. The physical properties of the samples were measured as a function of temperature when applied to nanofilm controlled heat. These curves show the temperature program consists of a series of alternating isothermal and heating segments. The recipe for nanofiber surface treatment is given in Table 4.4. Sample_1 has 34 °C, Sample_2 32 °C, Sample_4 46 °C, Sample_3 50 °C, Sample_5 52 °C, Sample_6 58°C, Sample_7 50 °C melting point. When cross-linking to a polymer is increased, you also limit the melting ability of the polymer by means of epoxy resin.

In Figure 5.17 and Fig.5.18, where the resin is dried under laboratory conditions and penetrated the permeate nanomembrane, it is seen that the thermal resistance of the resin evaporated in laboratory conditions is better than the sample removed by waiting in the oven.

6.CONCLUSION

Use of natural polymers in biomedical applications, the fact that they are readily available, non-toxic, and biocompatible. In addition, low cost, and functionalisation of properties by chemical modification of functional groups, their desired property. One such biocompatible polymer is the pullulans that find use in a special variety of biomedical.

In this thesis, the properties of Pullulan / PVA nanofiber membranes, pore size, selectivity, diameter, thickness, air-permeability, surface morphology have been examined. The hydrophilic and porous properties of nanofilm have been acquired by surface modification with a hydrophobic, non-porous nanofilm structure, which enables it to be used functionally in other sectors such as food packaging.

When mixed with Pullulan / PVA, PVA was used as a carrier polymer because it is relatively inexpensive, chemically, and thermally stable, and is not degradable under most physiological conditions. Moreover, pullulan has a beaded structure in the nano-surface obtained by the electrospinning method alone, while the nanofiber structure obtained by mixing with PVA is more uniform happened and the fibres obtained with electrospinning pullulan and PVA were larger in diameter compared to their blended fibres.

The SEM results and measurement of the mean diameter range of the nanofibers showed that the addition of PVA helped reduce the diameter of the nanofibers from pullulan. Pullulan nanofiber average diameter was only 830 nm, PVA nanofiber fibre average diameter was 490 nm, Pullulan 80/20% 512, Pullulan / PVA 60/40% 213 was determined by SEM images using ImageJ[®] software.

The average pore radius provided by the BET result was measured as Pullulan nanofiber 15.80 Å, PVA nanofiber 17.40 Å, Pullulan / PVA nanofiber 80/20% 17.87 Å, Pullulan 60/40% 15.61 Å. On the other hand, the average total pore volume was measured as 0.1390 cc/g for Pullulan nanofiber, 1.1322 cc/g for PVA nanofiber, Pullulan / PVA 80/ 20%, 0.1194

cc/g, Pullulan / PVA 60/40% nanofiber was measured as 0.1176 cc/g. Average surface area for Pullulan nanofibers $182.60 \text{ m}^2 / \text{g}$, PVA nanofibers $1302 \text{ m}^2 / \text{g}$, Pullulan/PVA 80/20% nanofibers $146.30 \text{ m}^2 / \text{g}$, Pullulan/PVA 60 %/40 nanofibers $150.68 \text{ m}^2 / \text{g}$. Pullulan / PVA, 60/40% nanofiber, showed smaller pore size, diameter and total pore volume than other results. This proves that the finest nanofibers were produced from Pullulan /PVA 60/40%.

Permeability provides the larger the pore radius, the greater the apparent permeability. VA nanofiber had higher air permeability, whereas pullulan had the least air permeability. Pullulan/PVA 80/20%, more air-permeable than Pullulan/PVA 60 /40%.

DSC analysis is provided, DSC curves for polymers blend membrane using electrospinning method (Applied voltage is 49,9 kV), blend ratio Pullulan/PVA 60/40, and total polymer concentration is 10 wt.% a comparatively large and sharp endothermic peak is determined at concerning 224°C temperature of pure PVA. This peak is shifted to $206,50^\circ\text{C}$ with the addition of 10 wt. Pullulan and this shifts the melting temperature to a lower value; this occurred because of the addition of pullulan, whose melting temperature changes from 95°C to 84°C for Pullulan/PVA nanofiber. This is because most of the chains are in a non-crystalline state due to the rapid solidification process of stretched chains during electrospinning. Pure pullulan shows a large thermogram peak of melting transition (T_m) at 95°C in Figure 5.14 (c). DSC thermograms electro spun PVA / Pullulan mixture nanofibers with different mixing ratios. A relatively large and sharp endothermic peak blend ratio of 80/20 was observed at 200°C , 80°C for Pullulan / PVA nanofiber, and when the pullulan ratio decreased, this peak was shifted to 206°C for Pullulan / PVA nanofiber from 60/40. Mountains towards temperature, indicating that melting decreases with increasing pullulan content temperature of the mixture nanofibers. This decrease in melting temperature probably occurred because most of the chains were not crystalline due to the rapid solidification of the stressed chains during the electrospinning process. The significant change in melting temperature of the blended electro spun mats as observed in DSC thermograms could be attributed to the addition of PVA.

After surface treatment, when epoxy resins and hardeners are combined below laboratory conditions, they react, and cross-links are shaped among the two chemicals. In this work, epoxy resin changed into obtained with the hardener. The organised film has become extra homogeneous. Also, the membrane is thinner as several the hardener evaporates. A non-porous structure was obtained from a porous structure with epoxy resin. As a result of surface treatment, it became insoluble in water. In the DSC analysis of the membranes, the enthalpy value was reduced. It is seen that the thermal resistance of the resin evaporated under laboratory conditions by drying under laboratory conditions and penetrating the nanofiber is better than the sample taken by waiting in the oven. When cross-linking to a polymer increases, you also limit the solubility of the polymer through the epoxy resin.

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