POLYETHERSULFONE/MULTI-WALLED CARBON NANOTUBES MIXED MATRIX MEMBRANES FOR BOVINE SERUM ALBUMIN REMOVAL AND BIOCOMPATIBILITY STUDIES

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Dedicated to my beloved parents,

(*Zainol Abidin Bin Anas, Azizah Binti Senawi and Sharuwiah Binti Jaafar*)

my lovely wife,

(*Noresah Binti Said*)

family and friends who gave me inspiration, encouragement and endless support throughout the success of my study.

May this thesis be an inspiration and guidance in the future.

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ABSTRACT

Hemodialysis is a process of purifying the blood of a person whose kidneys are not working normally. The design of a sustainable and high performance hemodialysis membrane is of great demand to solve the existing issues and heighten the hemodialysis performance. Hence, the objective of this study is to fabricate polyethersulfone/multi-walled carbon nanotubes (PES/MWCNTs) mixed matrix membrane (MMM) and evaluate its potential as a hemodialysis membrane. Prior to MMM fabrication, MWCNTs were purified by acids mixture (H_2SO_4/HNO_3; 3:1 v/v) through chemical oxidation to remove carbonaceous and metallic impurities. Subsequently, the oxidized MWCNTs were functionalized with citric acid monohydrate via polycondensation process to form poly (citric acid)-grafted-MWCNTs (PCA-g-MWCNTs). The MMMs comprised of 17.6 wt% PES, 4.8 wt% polyvinylpyrrolidone and 0-0.2 wt% MWCNTs were fabricated via dry-wet spinning technique. The MMMs were characterized using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), atomic force microscopy (AFM) and contact angle goniometer. The MMMs biocompatibility were studied in terms of compliment activation, protein adsorption and blood coagulation time. For separation and antifouling studies, the MMMs were subjected to permeation test at the pressure of 0.7 bar using pure water and 500 ppm bovine serum albumin (BSA) solution as the feed solution to obtain pure water flux (PWF), BSA rejection and PWF recovery rate. The matched FTIR spectra obtained showed that the MWCNTs have been successfully incorporated in the MMM. Based on the microscopic analyses using SEM and AFM, MMM incorporated with PCA-g-MWCNTs possessed larger pores and smoother surface. Besides, the decrease in the MMM contact angle value showed that the surface hydrophilicity of the MMM has been improved. The biocompatibility test results showed that the MMM incorporated with PCA-g-MWCNTs displayed the least complement activation and protein adsorption while keeping a normal blood coagulation time, hence demonstrating modest interaction with blood. The permeation test results showed that MMM incorporated with PCA-g-MWCNTs has better PWF and BSA rejection (J= 95.36 $Lm^{-2}h^{-1}$; R= 95.2 %) as compared to the MMM added with oxidized MWCNTs (J= 56.15 $Lm^{-2}h^{-1}$; R= 93.7 %) where the optimum PCA-g-MWCNTs loading was 0.1 wt%. The MMM incorporated with 0.1 wt% PCA-g-MWCNTs also achieved the highest PWF recovery rate (81 %) and showed less fouling effect. The PES/MWCNTs MMM was successfully fabricated and showed good biocompatibility and enhanced separation performance hence secures the essential properties to serve as hemodialysis membrane.

ABSTRAK

Hemodialisis ialah proses membersihkan darah seseorang yang buah pinggangnya tidak berfungsi seperti biasa. Reka bentuk sebuah membran hemodialisis yang mampan dan berprestasi tinggi mendapat permintaan yang tinggi bagi menyelesaikan isu-isu semasa dan meningkatkan prestasi hemodialisis. Justeru, objektif kajian ini adalah untuk menghasilkan membran bermatrik campuran (MMM) polietersulfon/tiub nano karbon berbilang dinding (PES/MWCNTs) dan menilai potensinya sebagai sebuah membran hemodialisis. Sebelum penghasilan MMM, MWCNTs telah ditulenkan oleh campuran asid (H_2SO_4/HNO_3; 3:1 v/v) melalui pengoksidaan kimia untuk menyingkirkan bendasing berkarbon dan berlogam. Selepas itu, MWCNTs yang dioksida telah difungsikan dengan asid sitrik monohidrat melalui proses polikondensasi untuk membentuk MWCNTs-dicantumkan-poli (asid sitrik) (PCA-g-MWCNTs). MMMs yang terdiri daripada 17.6 wt% PES, 4.8 wt% polivinilpirrolidon dan 0-0.2 wt% MWCNTs telah dihasilkan melalui teknik putaran kering-basah. MMMs dicirikan menggunakan spektroskopi infra merah jelmaan Fourier (FTIR), mikroskopi imbasan elektron (SEM), mikroskopi daya atom (AFM) dan goniometer sudut sentuh. Biokeserasian MMMs dikaji dari segi pengaktifan pelengkap, penjerapan protein dan masa pembekuan darah. Bagi kajian pemisahan dan anti cemar, MMMs telah menjalani ujian penyerapan pada tekanan 0.7 bar menggunakan air tulen dan 500 ppm larutan serum albumin bovin (BSA) sebagai larutan suapan untuk mendapatkan fluks air tulen, penyingkiran BSA dan kadar pemulihan PWF. Spektra FTIR sepadan yang diperoleh menunjukkan bahawa MWCNTs telah berjaya dimasukkan ke dalam MMM. Berdasarkan analisis mikroskopik menggunakan SEM dan AFM, MMM yang dimasukkan dengan PCA-g-MWCNTs mempunyai liang yang lebih besar dan permukaan yang lebih rata. Selain itu, penurunan nilai sudut sentuh MMM menunjukkan bahawa sifat hidrofilik permukaan MMM telah dipertingkatkan. Hasil ujian biokeserasian menunjukkan bahawa MMM yang dimasukkan dengan PCA-g-MWCNTs mempamerkan pengaktifan pelengkap dan penjerapan protein paling sedikit sementara mengekalkan masa pembekuan darah yang normal, justeru membuktikan interaksi yang memuaskan dengan darah. Hasil ujian penyerapan menunjukkan bahawa MMM yang dimasukkan dengan PCA-g-MWCNTs mempunyai fluks air tulen dan penyingkiran BSA yang lebih baik (J= 95.36 $Lm^{-2}h^{-1}$; R= 95.2 %) berbanding dengan MMM yang dicampurkan dengan MWCNTs yang dioksida (J= 56.15 $Lm^{-2}h^{-1}$; R= 93.7 %) di mana kandungan PCA-g-MWCNTs yang optimum adalah 0.1 wt%. MMM yang dimasukkan dengan 0.1 wt% PCA-g-MWCNTs juga mencapai kadar pemulihan PWF tertinggi (81 %) dan menunjukkan kesan cemar yang kurang. PES/MWCNTs MMM telah berjaya dihasilkan dan menunjukkan biokeserasian yang baik dan prestasi pemisahan yang dipertingkatkan justeru menjamin ciri-ciri penting untuk berkhidmat sebagai membran hemodialisis.

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LIST OF ABBREVIATIONS

AFM	-	Atomic force microscopy
APTT	-	Activated partial thromboplastin time
AR	-	Analytical reagent
BSA	-	Bovine serum albumin
CA	-	Citric acid
CCVD	-	Catalytic chemical vapor deposition
CVD	-	Chemical vapor deposition
Da	-	Dalton
DER	-	Dope extrusion rate
EDX	-	Energy-dispersive X-ray spectrometry
ESRF	-	End-stage renal failure
FESEM	-	Field emission scanning electron microscopy
FTIR	-	Fourier transform infrared spectrometry
HRP	-	Horseradish peroxidase
ID	-	Inner diameter
IUPAC	-	International union of pure and applied chemistry
LER	-	Linear extrusion rate
LOD	-	Limit of detection
LOQ	-	Limit of quantification
MMMs	-	Mixed matrix membranes
MW	-	Molecular weight
MWCO	-	Molecular weight cut off
MWCNTs	-	Multi-walled carbon nanotubes
NF	-	Nanofiltration
NKF	-	National Kidney Foundation
NMP	-	N-methyl-2-pyrrolidone
OD	-	Outer diameter

O-MWCNTs	-	Oxidized multi-walled carbon nanotubes
PAN	-	Polyacrylonitrile
PBS	-	Phosphate buffer saline
PCA	-	Poly(citric acid)
PCA-g-MWCNTs	-	Poly(citric acid)-grafted-multi-walled carbon nanotubes
PES	-	Polyethersulfone
PEG	-	Polyethylene glycol
PFSA	-	Perfluorosulfonic acid
PMMA	-	Polymethylmethacrylate
PPP	-	Platelet poor plasma
PRP	-	Platelet rich plasma
PSf	-	Polysulfone
PT	-	Prothrombin time
PVDF	-	Polyvinylidenefluoride
PVP	-	Polyvinylpyrrolidone
PWF	-	Pure water flux
RO	-	Reverse osmosis
SDS	-	Sodium dodecyl sulfate
SEM	-	Scanning electron microscopy
STEM	-	Scanning transmission electron microscopy
SWCNTs	-	Single-walled carbon nanotubes
TEM	-	Transmission electron microscopy
THF	-	Tetrahydrofuran
TGA	-	Thermogravimetric analysis
TMP	-	Trans-membrane pressure
UF	-	Ultrafiltration

LIST OF SYMBOLS

A	-	Membrane surface area
Ad	-	Adsorption amount
C_f	-	Concentration of solute in feed
C_p	-	Concentration of solute in permeate
d_i	-	Inner diameter of hollow fiber membrane
d_o	-	Outer diameter of hollow fiber membrane
J	-	Flux
l	-	Membrane effective length
M_W	-	Molecular weight
n	-	Number of trials
ρ	-	Density of liquid
ΔP	-	Trans-membrane pressure
P_f	-	Feed pressure
Q_d	-	Dialysate flow rate
Q_f	-	Feed flow rate
r_m	-	Mean pore radius
R	-	Solute rejection
R_a	-	Average roughness
T	-	Temperature
t	-	Time
V	-	Permeate volume
w_1	-	Weight of wet membrane
w_2	-	Weight of dry membrane
ε	-	Porosity
$\%G$	-	Percent graft yield

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Over the past fifteen years, the number of chronic kidney diseases patients has increased terrifically where these patients suffer from the incapability of filtering and removing body waste. According to Malaysia's National Renal Registry, it has been reported that the total amount of people undergoes hemodialysis had risen from 6,689 to 21,159 people in 2009 (Cheng, 2011). The number did not stop there. The latter report in May 2013 indicated the increase of dialysis patients to 26,159 people (Cheng, 2013). The latest statistics issued by National Kidney Foundation (NKF) in 2014 revealed the total number of 30,000 Malaysians on dialysis (Cruez, 2014). This shows the growth of about 4,000 newly registered patients each year. In human blood circulatory system, the blood carries soluble wastes such as the end-products of metabolism reactions occurred in body, together with the accumulated sodium, potassium, and chloride ions in the body. The accumulated wastes cause toxins to build up in the body and may render further complications. Thus, blood must be cleaned by removing those substances as waste. The most widely applied extracorporeal treatment to filter and purify blood is hemodialysis.

Hemodialysis is considered as a highly successful therapy that provides the second chance to live. Since the commencing of the first semipermeable membranes for hemodialysis, the membrane technology keeps developing until it has been successfully used for hemodialysis treatment for patients who suffer from acute renal disease and end-stage renal failure (ESRF). In general, the main component of hemodialysis machine is dialyzer, where semipermeable membrane is situated. The membrane is arranged in the middle, serves as membrane contactor to form separate adjacent paths for blood and dialysis fluid (dialysate). It filters waste products (i.e. urea, creatinine, β_2-microglobulin), removes excess water and balances electrolytes such as sodium, potassium, and bicarbonate. Hemodialysis treatment utilizes 4 principles of movement across semipermeable membrane, namely diffusion, convection, ultrafiltration (UF) and osmosis. Diffusion is the movement of solutes across concentration gradient while convection is the movement of solvent and dissolved solutes across hydrostatic pressure gradient. UF is a convective movement of water following pressure gradient and osmosis is a movement of water across water concentration gradient, separated by membrane.

Among hydrophobic polymers, polyethersulfone (PES) is usually employed for blood purification (Zhao *et al*., 2013) due to its hydrophilic-hydrophobic characteristic that can be easily tailored to ensure higher biocompatibility. Currently, materials used for commercialized hemodialysis membranes are polysulfone (PSf) and PES. PES shares the same properties as PSf but offers outstanding oxidative stability, greater mechanical, chemical and heat resistance. Thus, PES could endure many kinds of sterilization method which is crucial for clinical purpose. PES is more hydrophobic compared to PSf, which is favorable in terms of mechanical strength of membrane. However, some studies concluded that membrane fouling is directly proportional to hydrophobicity. Thus, modification of PES membrane is performed to improve the hydrophilicity. Polar additive such as polyvinylpyrrolidone (PVP) is usually added for this purpose.

Recently, the advancement of synthetic membranes for hemodialysis is not centering on synthetic polymers alone. The expansion of nanotechnology has exposed people around the world on the exceptional properties of nanomaterials. In this context, nanomaterials have been promisingly used as nanofiller of polymer matrix (Favvas *et al*., 2014; Ng *et al*., 2010; Japip *et al*., 2014), forming a new class of membrane known as mixed matrix membranes (MMMs). The incorporation of nanoparticles in membrane matrix for surface modification and performance enhancement of membranes is an emerging trend in membrane technology. There are two types of commonly used nanoparticles, i.e. (i) carbon nanoparticles such as carbon nanotubes (CNTs) and graphene and (ii) metal oxide nanoparticles like titanium dioxide and iron oxide nanoparticles. Their major roles include enhancing the durability of polymeric membranes towards chemical degradation, fouling and thermal instability as well as heightening the performance of the resultant MMMs through their unique properties (Souza and Quadri, 2013; Cao *et al*., 2006).

In some cases, hydrophilic nanomaterials are placed specifically in the membrane pores, where they have a promising effect on the flux improvement and fouling mitigation. For example, the incorporation of CNTs into membrane mainly aims at providing numerous additional transport channels to improve the membrane mass-transfer properties. The study by Irfan *et al*. (2014) comprehensively highlights the advantages offered by functionalized multi-walled carbon nanotubes (MWCNTs) towards PES membrane. The improved characteristics like porosity and hydrophilicity subsequently results in the enhancement of the membrane pure water permeation rates, antifouling capabilities (Sianipar *et al*., 2015) and separation performance (Nie *et al*., 2015).

In this study, MWCNTs were incorporated in PES membranes for hemodialysis application. To further enhance the hydrophilicity and water transport properties, the MWCNTs were functionalized with poly (citric acid) (PCA), forming PCA-grafted (g)-MWCNTs. The effects of MWCNTs modification and loading on the MMMs were evaluated in terms of morphology, separation features and antifouling performance. In brief, this study would be beneficial to those interested in the design of carbon nanocomposites and the development of a sustainable and high performance membrane for efficient liquid separation especially in UF and hemodialysis treatment. Besides, the employment of unique nanoparticles in hemodialysis membrane would broaden people's horizons and provide the insight towards its potential commercialization for hemodialysis application.

1.2 Problem Statement

One of the major problems arising in hemodialysis is the membrane fouling which caused by adsorption of nonpolar solutes, hydrophobic protein or bacteria onto the membrane surface (Van der Bruggen, 2009; Koh *et al*., 2005). Membrane fouling caused a reduction in water permeability and separation performance of membrane. As a result, the performance of the membrane deteriorates with time. There are a number of factors contributing to membrane fouling. The first one is the hydrophobic property of polymer matrix. Naturally existing hydrophobic proteins in blood tend to deposit on membrane surface. The second one is due to the bio-incompatibility of membrane which induced inflammatory responses such as complement activation. Immunological cells will be triggered and block the opening of pore, minimizing pore size. Thirdly is related to inner surface roughness of membrane. The possibility of proteins to get stuck on a rough surface is higher. Besides that, morphology of membrane can also be associated with membrane fouling (Yuan and Zydney, 1999). In fact, current commercial membranes could not remove 'middle' size molecules such as β_2-microglobulin efficiently due to inadequate membrane pore size.

Realizing the importance of maintaining the consistency of the membrane separation features, a novel approach in the design of a safe, high performance hemodialysis membrane is of great demand. To tackle the stated problems, surface modification is usually done to hydrophobic polymer like PES. The simplest way to modify PES is by blending with hydrophilic polymers like PVP (Barzin *et al*., 2004). Other than becoming pore former, PVP also increases the hydrophilicity of membrane, thus increasing antifouling properties and biocompatibility of PES membrane (Wang *et al*., 2006). Nevertheless, the tendency of PVP to swell in water and elude during dialysis (Irfan *et al*., 2014) makes it less convincing. The efforts then have been shifted to the development of MMMs by incorporating nanoparticles to overcome the limitation of polymeric membranes.

Among other nanoparticles, MWCNTs received the most attention due to its nanoscale dimension, chemically inertness, remarkable total surface area, high modulus and strength. MWCNTs are used as membrane fillers which can pave massive mass transport channels for solutes, improving separation process (Zhang *et al*., 2014). However, pristine MWCNTs suffer from strong intermolecular forces due to their hydrophobic nature and thus cannot disperse well in organic solvents. Besides, carbon and metal impurities, which could pose threat on patient must be removed from MWCNTs walls. Thus, chemical oxidation of pristine MWCNTs must be performed. Chemical oxidation of MWCNTs also introduces polar (i.e. carboxyl) groups onto the surface, which makes it become dual nature. It has been proven that a small amount of oxidized MWCNTs could enhance hydrophilicity, water permeability, and the antifouling property of polymeric membranes (Gallagher *et al*., 2013; Majeed *et al*., 2012; Ajmani *et al*., 2012), other than increasing mechanical stability and transport property of membranes.

In addition, further functionalization of MWCNTs with dendritic polymers is highly needed to reduce their toxicity effects and lead to safe interaction with cell membranes. Dendritic polymers have been previously demonstrated as suitable nano-carriers for use in biomedical applications because of their large number of functional groups, small sizes and polyvalency. One of dendritic polymers is PCA, which is a highly water soluble polymer and its biocompatibility cannot be denied (Naeini *et al*., 2010; Qian *et al*., 2008). It was anticipated that the addition of PCA-g-MWCNTs might improve the MMM separation performance and antifouling properties, hence pave a way to its potential application in hemodialysis. Therefore, in this study, attempts were made to fabricate PES/MWCNTs MMMs to investigate the synergism between MWCNTs nanofillers and PES matrix in demonstrating far better characteristics, separation performance, and antifouling properties compared to that of neat membrane.

1.3 Objectives of the Study

The main objective of this project is to fabricate PES hollow fiber membrane embedded with MWCNTs and evaluate its potential as hemodialysis membrane. Based on the aforementioned research background and problem statement, the specific objectives of this study are listed below:

1. To functionalize and characterize MWCNTs.

2. To study the effects of purification and functionalization of MWCNTs on the MMMs surface characteristics and biocompatibility.

3. To evaluate the separation performance of the MMMs in terms of pure water flux and BSA removal.

1.4 Scopes of the Study

In order to fulfil the above objectives, the following scopes of work are outlined:

1. Purifying the MWCNTs through chemical liquid phase oxidation process using acids mixture (H_2SO_4/HNO_3; 3:1 v/v) and functionalizing with PCA.

2. Confirming the purity of oxidized and functionalized MWCNTs that were formed using field emission scanning electron microscopy (FESEM), scanning transmission electron microscopy (STEM), energy-dispersive X-ray spectrometry (EDX), thermogravimetric analysis (TGA), and Fourier transform infrared spectrometry (FTIR).

3. Investigating the dispersion stability of oxidized and functionalized MWCNTs in ethanol by observing the suspensions after 24 hours.

4. Preparing dope solutions comprised of 17.6 weight percent (wt%) PES, 4.8 wt% PVP, and 0-0.2 wt% MWCNTs in NMP and water.

5. Fabricating PES/MWCNTs mixed matrix hollow fiber membranes via dry-wet spinning technique at 50 cm air gap.

6. Casting PES/MWCNTs flat sheets via dry-wet phase inversion process with evaporation time of 6 seconds using water as the coagulation bath.

7. Examining the morphology of the fabricated membranes using SEM and atomic force microscope (AFM), thermal stability using TGA, hydrophilicity using contact angle measurement, biocompatibility of the MMMs, and confirming the molecular structure by FTIR.

8. Evaluating the separation features of MMMs in terms of pure water flux (PWF) and protein rejection using 500 ppm bovine serum albumin (BSA) at 0.7 bar.

9. Investigating the antifouling performance of the MMMs based on flux decline behavior and flux recovery rate.

10. Studying the leaching phenomenon of MWCNTs from MMMs during water permeation by direct filtration and using a conductivity meter.

1.5 Significance of Study

This study would have brought upon a huge importance towards the development of science and technology for the sake of mankind. The primary outcome of the research would benefit scientific community in the sense of filling in the knowledge gap in multiple fields which encompass nanotechnology and membrane technology. In addition, the research on hemodialysis membranes in Malaysia is still at early stages. The employment of MWCNTs in hemodialysis membrane for instance could progressively diversify their potential in this biomedical-device application. The ingenious approach which combined both unique properties of MWCNTs and versatility of polymer as a host showed great potential to combat the fouling issues commonly faced by polymeric membranes. This novel invention is believed to become a stepping stone which could provide a valuable information for membranologists and lead the way to further study. The aftermath of the research will also benefit the ESRF patients by providing a sustainable and biocompatible hemodialysis membrane that is capable and reliable to perform exceptional blood purification with minimal adverse effect. Triggered by the general necessities of serving the social community, the study would attract companies that manufacture or supply medical equipment as a platform to patent and market the product. Last but not least, the outcomes of this research would also help to compensate government's burden to accommodate the subsidy cost of performing hemodialysis with the rising population of ESRF patients.

1.6 Limitation of Study

Throughout the study, there were a number of elements that have not received close attention and have not been taken into account. Hence, the corresponding results were generated based on coherent assumptions. The following limitations are disclosed below:

1. All biocompatibility tests were not performed on the identical hollow fiber membrane used for other assessments. Instead, flat sheet membranes from each particular membrane composition were utilized. The size of membrane surface area in contact with blood might influence the results. It has been assumed that the results obtained using flat sheet membrane would reflect the membrane-blood interactions of different membrane's chemical modifications, at least for the comparison purpose. Hollow fiber membrane is hard to handle, since the active surface is at the inner side. On the other hand, the very small lumen made it even difficult to ensure homogeneous interaction with the inner surface.

2. Blood coagulation and complement activation test results might not symbolize the membrane compatibility towards human blood of specific types and conditions. This is due to some issues and constraints regarding the ethics and method of

procuring the supply of ESRF patient’s blood. Instead, the blood samples used for biocompatibility tests were collected from 3 healthy volunteers of random blood groups. The presented results of each membrane were based on the average value obtained from the 3 blood samples. Hence, the outcomes generated from this part of studies represent more on the membrane-blood interactions as a whole.

3. The performance data acquired from the separation and antifouling performance evaluation was collected using outside-to-inside UF system, while the real hemodialysis set up consists of a membrane contactor which utilize both UF and diffusion mechanisms for molecules separation. The feed inlet usually channels the fluid from inside to outside of the module. Still, the used UF system would be sufficient in serving the research purposes at this level. The water permeation, which is the interpretation of blood plasma flow was experimented mainly to compare the PWF achieved by different formulated membranes, regardless of the water inlet direction. As for the protein rejection, the results were highly influenced by the membrane hydrophilicity and PWF.

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