PREPARATION AND CHARACTERISATION OF FLAT SHEET MICRO/NANOPOROUS MEMBRANES USING POLYSULFONE BLEND WITH PVP/PEG AND CHITOSAN/CHITOSAN NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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In this research, novel asymmetric polysulfone (PSF) membranes were prepared from chitosan/chitosan nanoparticles and PSF/Polyethylene glycol (PEG)/Polyvinyl pyrrolidone (PVP) system via immersion precipitation. The variation in pore size and surface morphology of the prepared membranes were studied by Field Emissive Scanning electron microscope (FESEM) and contact angle measuring instrument. The contact angle measurements demonstrated that hydrophilicity of the PSF membranes was significantly enhanced by addition of chitosan nanoparticles than PVP/PEG, in the casting solution and the improved pore size were confirmed through FESEM studies. These surface modified membranes with better pore morphologies can be used for biomedical applications like hemodialysis.

(Received February 15, 2016; Accepted June 3, 2016)

Keywords: Chitosan nanoparticles, Hydrophilicity, Pore size, contact angle, FESEM

1. Introduction

Micro/Nanoporous membranes are widely used for biomedical applications, for sorting, sensing, isolating and releasing biological molecules [1]. Recent advances in nanoscience have made it possible to precisely control the morphology as well as physical and chemical properties of the pores in nanoporous membranes that make them increasingly attractive for use in smart implantable drug delivery systems, bio-artificial organs, and other novel nano-enabled medical devices. Polymeric materials are vividly used in biomedical applications for preparing membranes [2]. Polysulfone is the most widely used synthetic polymeric material for preparing hemodialyser membranes, due to its properties such as high mechanical strength, chemical inertness and thermalstability [3]. It can withstand sterilization techniques such as steam, ethylene oxide and gamma radiation. Conventional immersion precipitation is used for casting polysulfone into hollow fiber or flat sheet membranes that has high permeability to low molecular weight proteins and high endotoxin retention, when used for hemodialysis[4] . The disadvantage of polysulfone is its hydrophobicity that prevents it from blood contacting applications. In hemodialysis operation, the adsorption of proteins onto the membrane results in complication due to the activation of complement alternative pathway. These problems can be overcome by increasing the hydrophilicity of the polysulfone membrane, thereby reducing the membrane fouling.

The surface morphology of membranes can be modified by the various surface modification techniques [5,6]. PVP and PEG are commonly used additives for obtaining controlled pore size and stability in membranes [7-8]. Akon Higuchi et al prepared chemically modified PSF hollow fiber membranes with improved hydrophilicity, by covalently conjugating with PVP/PEG[9,10].Surface modification of the membranes using additives, improves the porous structure, pore size, hydrophobicity/hydrophilicity and imparts antibacterial activity to the surface, preventing protein adsorption and biofouling[10-12]. Nurul Nabilah Aminudin et al compared the effect of adding PVP and PEG as additives for modifying the surface of PSF membranes [13].

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Chitosan (1-4)-2-amino-2-deoxy-D-glucan, is the N-deacetylated derivative of chitin, is a naturally occurring biopolymer and is less cytotoxic, hydrophilic, elastic, biocompatible, and biodegradable material. These unique properties make it an attractive material for biomedical applications. Chitosan is also commonly used for preparing nanoporous membranes for various biomedical applications [14]. Polysulfone can blend with various nanoparticles to obtain desired pore and surface characteristics [15]. Chitosan can blend with PSF to prepare membranes with improved hydrophilicity[16]. Addition of chitosan imparts antibacterial activity to the membrane and prevents fouling. Rajesha Kumar et al prepared ultrafiltration membranes by blending chitosan with PSF [17]. The prepared membranes had improved flux, hydrophilicity and antibacterial activity. Chitosan and its nanoparticles are gaining importance because of their interesting properties and are widely used in preparing composite hollow fiber PS-chitosan membranes for applications like artificial liver [18-20].Yun-Ju Chuang et al fabricated microdialysis probe using chitosan nanoporous membrane for separation of biomolecules[21].

In this work, flat sheet membranes (micro/nano scale) were prepared using PSF blended with PVP/PEG as well as chitosan/chitosan nanoparticles as additives. In the process of preparing chitosan nanoparticles, particle size analyser was used for characterizing the size of nanoparticles formed. The prepared membranes were characterized using FESEM and contact angle measurements to study the changes in the surface morphology. From the analysis, we report the successful formation of flat sheet membranes using immersion precipitation method. The membranes prepared using chitosan/chitosan nanoparticles revealed better pore size and hydrophilicity. Chitosan is of low cost and is natural in origin, it's a potential candidate for preparing hollow fiber hemodialysis membrane by blending with PSF to prevent protein adsorption and biofouling.

2. Experimental Section

2.1 Materials

The raw materials required for membrane casting are the commercially available polysulfone (22,000 mw), N-dimethyl formamide (99%) is the solvent , pore forming agents or additives are polyethylene glycol (400) and polyvinyl pyrrolidone, N-methyl pyrollidine(NMP),chitosan(medium molecular weight) and acetic acid. Chitosan is employed for the synthesis of nanoparticles and for blending with polysulfone. All the above chemicals were purchased from sigma-aldrich. Distilled water was used as the non-solvent in the coagulation bath.

2.2. Preparation of PSF Membrane

One gram of Polysulfone (10wt%) was weighed and dissolved in 9.5mL(90wt%) of N-dimethyl formamide under magnetic stirring at a temperature of 60°C and 300 rpm. Stirring was performed for four hours and the polymer solution was obtained. The polymer solution was cast on the petri plates and after one minute, the petri plate containing polymer solution was immersed in the non-solvent (water) for the occurrence of phase inversion process. Then the set up was undisturbed for 18 hours of gelation period. After the gelation process, the membrane was taken and rinsed with distilled water and then dried in oven at 50°C for 72 hours. The prepared membranes were then stored safely for characterization.

2.3. Preparation of PSF/PVP/PEG Membrane

10% Polysulfone solution was prepared using N-dimethyl formamide and additives Polyethylene glycol/Polyvinyl pyrrolidone. 1gram of polysulfone was dissolved in 8.95mL of N-methyl formamide under magnetic stirring at 300 rpm and the temperature was maintained at 60°c. After one hour of magnetic stirring, polyethyleneglycol (5%) was added and again stirred for 3 hours.Further Polyvinylpyrrolidone (5%) was added and again stirred for 8 hours. Then the prepared solution was cast on a clean glass plate using thin film applicator maintained at a thickness of 200µm. After the evaporation of solvent for 30 seconds, the glass plate containing the casted film was gently immersed in the coagulation bath containing distilled water.After 18 h of gelation, the membrane was removed from the coagulation bath and washed thoroughly with

distilled water to remove residual N-dimethyl formamide present in the membrane. Then the membrane was dried at 45-50°c in the hot air oven for 72 hours and then stored for characterization.Similarly, membranes were casted for 10%, 15%, 20% PEG/PVP formulations. They were also dried at 45-50°cin the hot airoven for 72 hours and then stored for characterization.

2.4. Preparation of PSF /Chitosan Membrane

2% chitosan solution was prepared using 1% acetic acid solution and the chitosan solution was magnetically stirred for 4 hours at 500 rpm. Meanwhile, 10%polysulfone solution was prepared using 8.7 mL of N-methylpyrrolidone (NMP) as solvent and was magnetically stirred for four hours at a temperature of 60°C and at 300rpm. Then 5 mL of 2% chitosan solution was added to the polysulfone solution. Then extra 5 mL of N-methylpyrrolidone was added to the above polysulfone solution and stirred vigourously for 24 hours at 60°C.Then casting of the polymer solution was performed. After 3 hours of evaporation time, the cast membrane was immersed in the coagulation bath. After 18 hours of gelation period, the membrane was taken and washed with distilled water and was kept for drying at room temperature. Then the dried membrane was stored and characterized using FESEM and contact angle measurement.

2.5 Synthesis of Chitosan Nanoparticles Using Ionic Gelation Method

Stock solutions of 1% acetic acid solution (1 mL of acetic acid dissolved in 100 mL distilled water) and sodium tri-polyphosphate solution of concentration 0.7 mg/mL were prepared. Chitosan powder (medium molecular weight) was added to 1% acetic acid solution to obtain concentrations of 0.05%, 0.1%, 0.15%, 0.2% chitosan solutions. The chitosan solutions were magnetically stirred at 500rpm for 2 hours. Then 1:3 ratio of sodium tri-polyphosphate solution was added drop by drop to the chitosan solutions under stirring (17 ml of sodium tri-polyphosphate solution to 50 ml of chitosan solution).Then the chitosan solutions of different concentrations were magnetically stirred for 2 hours at 500 rpm. Then the chitosan solutions were stored and characterized using particle size analyzer.

2.6 Preparation of PSF/ChitosanNanoparticles blend membrane

0.05% chitosan solution was prepared using 1% acetic acid solution and the chitosan solution was magnetically stirred for 2 hours at 500 rpm. Then 1:3 ratio of sodium tri-polyphosphate solution was added drop by drop to the above chitosan solution under stirring (17 ml of sodium tri-polyphosphate solution to 50 ml of chitosan solution). Then the chitosan solution was magnetically stirred for 2 hours at 500 rpm for obtaining chitosan nanoparticles.

Meanwhile, 10%polysulfone solution was prepared using 8.7 mL of N-methylpyrrolidone (NMP) as solvent and was magnetically stirred for four hours at a temperature of 60°C and at 300rpm. Then 5 mL of 0.05% chitosan solution was added to the polysulfone solution. Then extra 5 mL of N-methylpyrrolidone was added to the above polysulfone solution and stirred vigorously for 24 hours at 60°C.Then casting of the polymer solution was performed. After 3 hours of evaporation time, the cast membrane was immersed in the coagulation bath. After 18 hours of gelation period, the membrane was taken and washed with distilled water and was kept for drying at room temperature. Then the dried membrane was stored and characterized using FESEM and contact angle measurement.

3. Characterisation

The flat sheet membranes prepared using PSF with additives PVP/PEG and chitosan/chitosan nanoparticles were characterized using FESEM and contact angle measuring instrument. The chitosan nanoparticles were characterized using particle size analyser.

4. Results and Discussion

The sample of membrane cast on petri plate is shown below. Pure PSF membranes prepared was white in color.

Fig.1. PSF membrane cast on petriplate.

4.1. Particle Size Analysis of Chitosan Nanoparticles

Particle size analysis revealed that the nanoparticle formation was best in case of 0.05% chitosan solution and this concentration of chitosan solution can be used for blending with polysulfone. The 0.05% chitosan solution produced nanoparticles in the range of 30-200nm, 0.1% chitosan solution produced nanoparticles in the range of 100-400nm, 0.15% chitosan solution produced nanoparticles in the range of 300-1000 nm and 0.2% chitosan solution produced nanoparticles in the range of 300-1500 nm.0.15% chitosan solution was added with PSF for preparing the membrane.

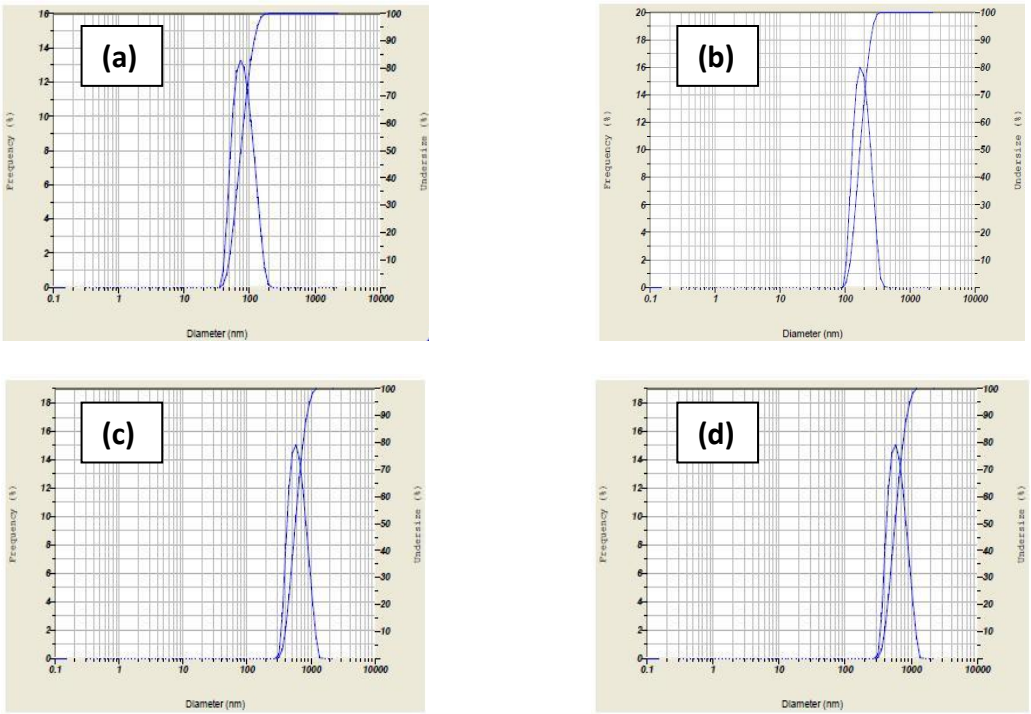

Fig.2. Particle size analysis of (a) 0.05% chitosan solution (b) 0.1% chitosan solution (c) 0.15% chitosan solution and (d) 0.2% chitosan solution.

4.2 Characterization of Membranes using Contact Angle Tester

The contact angle tester, Kruss GMBH, Germany was used to measure the contact angle for the membranes. Water was used as the liquid to measure the contact angle. The contact angle

vs position and force position plots were obtained for the membranes. The contact angle for hydrophilic samples would be < 90° and for hydrophobic samples, the contact angle would be >90°.

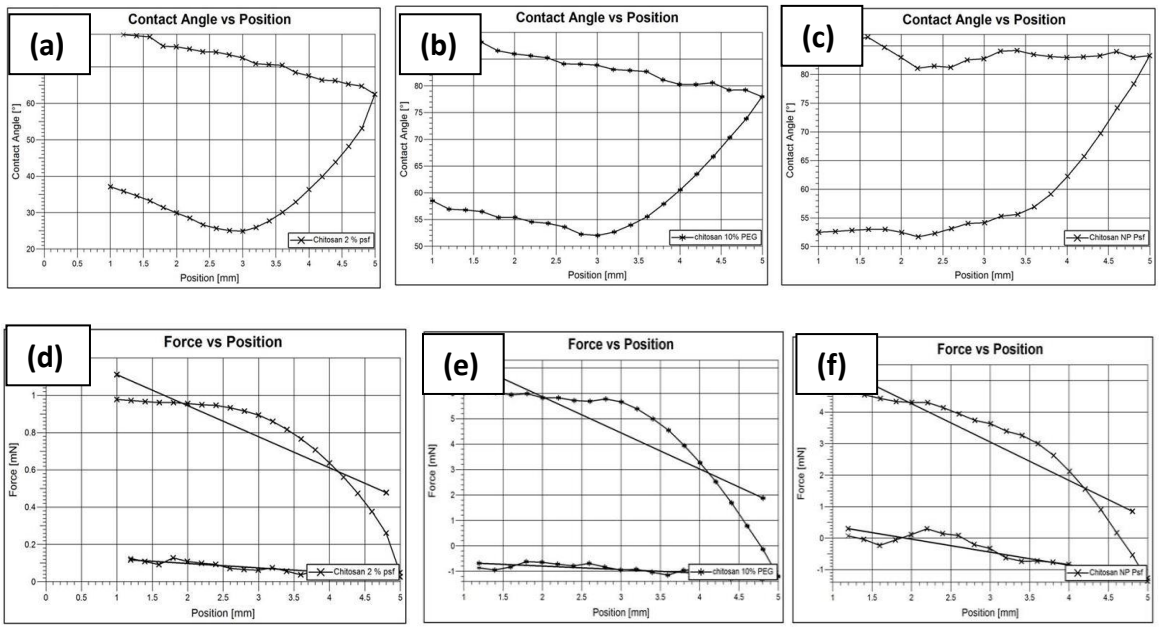

Fig.3.Contact angle vs Position of (a) Chitosan 2% - PSF membrane (b) 10% PEG-PSF membrane and (c) Chitosan NP - PSF membrane and Force vs Position graph of (d) Chitosan 2% - PSF membrane (e) 10% PEG-PSF membrane and (f) Chitosan NP - PSF membrane.The contact angle for the membrane samples are listed in table1.

Table 1. Contact angle measurement

S. No	Sample	Contact angle (°)
1.	10% PEG-PSF	92.29
2.	Chitosan 2%-PSF	83.92
3.	Chitosan NP-PSF	84.71

The 10% PEG - PSF sample was slightly hydrophobic since the contact angle was slightly greater than 90°. The contact angle obtained for Chitosan NP-PSF membrane (chitosan nanoparticle blended polysulfone) was almost closer to the the contact angle obtained for Chitosan 2% PSF membrane and the chitosan and chitosan nanoparticles blended polysulfone membranes were hydrophilic since the contact angle obtained for the membranes was less than 90°.

4.3 Field Emission Scanning Electron Microscope – Analysis

The pore diameter was measured using Image J and tabulated (Table.2), for the images shown in fig.4.

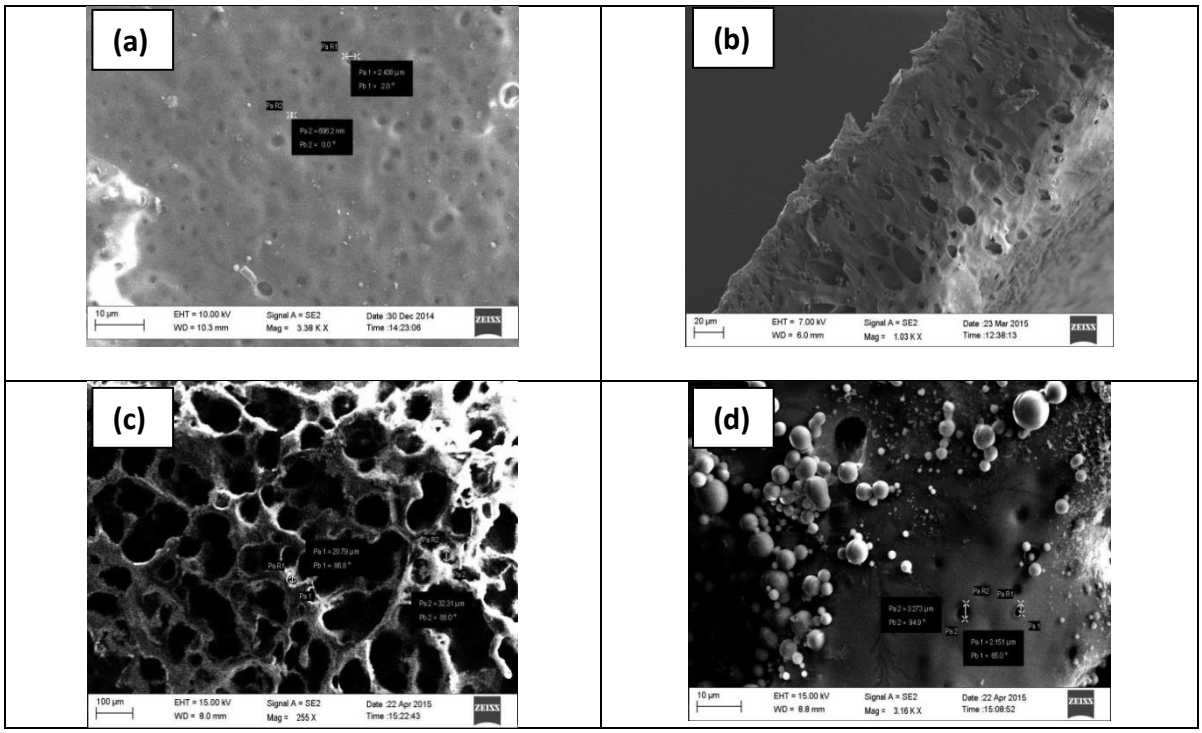

Fig.4. FESEM images of flat sheet membrane prepared using (a) PSF (b) PSF/PEG/PVP blend (c)PSF/chitosan and (d)PSF/chitosan nanoparticles.

Table 2. Pore diameter of membranes from Image J analysis.

S.No	PSF (microns)	PSF/PEG/PVP (microns)	PSF/Chitosan (microns)	PSF/chitosan nanoparticles (microns)
1	2.564	12.008	44.801	2.749
2	1.026	4.163	46.364	2.499
3	1.538	5.065	22.067	2.263
4	2.364	12.008	66.2	3.534
5	1.642	9.159	42.067	1
6	2.293	5.266	55.14	1.819
7	0.769	10.163	64.749	2.609
8	0.573	6.713	85.255	2.703
9	0.811	6.713	127.557	2.249
10	1.538	3.33	80.453	1.999

From Table.2, it is evident that the pore size obtained by blending PSF and chitosan nanoparticles varies between 1 to 4 microns. The pore size obtained for PSF membrane without adding additives ranges between 0.5 microns to 3 microns, whereas the pore size of the PSF membranes prepared using PVP and PEG varies between 3 to 12 microns. The pore size of PSF/chitosan blend membranes is larger in diameter when compared to membranes prepared using PSF/chitosan nanoparticles. In order to obtain membranes with controlled pore size, PSF can be mixed with chitosan nanoparticles with more rigorous stirring. The blending of chitosan nanoparticles should be optimized to obtain better membranes otherwise there are chances for nanoparticles to aggregate on the membrane surface.

5. Conclusion

The flat sheet membranes were prepared using polysulfone as the base material. Additives like PVP/PEG and chitosan/chitosan nanoparticles were added to the base material to impart

hydrophilicity to the membrane. The membranes prepared using chitosan nanoparticles have controlled pore size in the range of 1 to 4 microns. The water contact angle measurement of the membranes revealed that chitosan nanoparticles blended PSF membranes were more hydrophilic in nature compared to others. The particle size analyzer helped in optimizing the concentration of chitosan solution, suitable for the synthesis of nanoparticles in the expected dimensions. The results obtained from the FESEM micrographs revealed that the pores were obtained in micro and nanoscale dimensions. The PSF/chitosan nanoparticles blend membranes have controlled pore dimensions and are more hydrophilic in nature, which tends to reduce biofouling and hence, potential candidate for preparing hemodialyser membranes.

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