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Biocompatibility studies of polyacrylonitrile membranes modified with carboxylated polyetherimide ☆

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ABSTRACT

Poly (ether-imide) (PEI) was carboxylated and used as the hydrophilic modification agent for the preparation of polyacrylonitrile (PAN) membranes. Membranes were prepared with different blend compositions of PAN and CPEI by diffusion induced precipitation. The modified membranes were characterized by thermo gravimetric analysis (TGA), mechanical analysis, scanning electron microscopy (SEM) and contact angle measurement to understand the influence of CPEI on the properties of the membranes. The biocompatibility studies exhibited reduced plasma protein adsorption, platelet adhesion and thrombus formation on the modified membrane surface. The complete blood count (CBC) results of CPEI incorporated membranes showed stable CBC values and significant decrease in the complement activation were also observed. In addition to good cytocompatibility, monocytes cultured on these modified membranes exhibited improved functional profiles in 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Thus it could be concluded that PAN/CPEI membranes with excellent biocompatibility can be useful for hemodialysis.

1. Introduction

Blood and biocompatibility are essential for blood contacting materials used in biomedical applications such as antithrombogenic implants, hemodialysis membranes, and biosensors [5]. However, synthetic materials usually exhibit poor hemocompatibility and thus biomaterial induced complications remain life-threatening in the employment of blood-contacting devices such as hemodialysis membranes [16]. Prolonged contact of blood with polymer surface leads to dialysis-induced oxidative stress (DIOS) and membrane induced inflammation (MII), both of which create cardiovascular problems in hemodialysis patients [23]. It is generally accepted that nonspecific protein adsorption is the first event that occurs when biomaterial contacts human blood and it controls the biomaterial induced thrombus formation. Platelet adhesion to biomaterials is often followed by the activation of the adherent platelets which accelerates thrombosis by promoting thrombin formation and platelet aggregation [40].

The adsorption of proteins on the polymeric membranes can be decreased by changing the hydrophobicity and wettability of the surface [2,12]. Polyacrylonitrile (PAN) and acrylonitrile based copolymers have been successfully employed as membrane materials for ultrafiltration and hemodialysis application because of its good thermal and mechanical stability and excellent membrane forming properties [3]. However, moderate hydrophilicity, brittleness and relatively poor biocompatibility of PAN membranes necessitate its modification prior to their use in biomedical and bioengineering applications [37]. Many investigations have demonstrated that biocompatibility of the conventional materials could be improved by bulk or surface modification [4]. Among the different modification methods, blending is considered to be simple, highly attractive and inexpensive way of obtaining new materials with improved properties [1].

On the other hand, Poly (ether-imide) (PEI) is a high performance amorphous thermoplastic with considerable chemical resistance, thermal stability, mechanical strength, and good membrane forming properties. Recent research and development in the biomedical field and preliminary study from our group revealed that, PEI has excellent biocompatibility and show low immune response in contact with blood [19,30]. However, PEI has certain drawbacks such as high hydrophobicity, requires strict membrane casting conditions and thus it is necessary to make PEI more hydrophilic before membrane preparation [21,22]. One of the strategies to improve the surface wettability, and surface charge of the PEI is surface modification by incorporating different functional groups. In the literature it was pointed out that polymer carrying functional groups show remarkable blood-contacting properties, since these materials may act like anticoagulant heparin [34]. Thus, in the present investigation PEI has been functionalized by carboxylation and blended with PAN matrix

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to improve the hemocompatibility and separation efficiency of PAN membranes for hemodialysis applications.

Present study deals with the functionalization of PEI by carboxylation (CPEI) and was used as the hydrophilic agent for modification of PAN hemodialysis membranes. The CPEI was blended with PAN in different composition and membranes were prepared by diffusion induced precipitation technique in the absence and presence of pore former polyethylene glycol-200 (PEG-200). The effect of blend ratio on the membrane morphology, hydrophilicity, pure water flux, thermal, mechanical and antifouling properties of the membranes was studied. Biocompatibility of the PAN/CPEI blended membranes was also investigated by estimating the amount of protein adsorbed, platelet adhesion and blood count on the membrane surface. Cytocompatibility of the prepared membranes were studied by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)] cell proliferation assay. Attempts have been made to correlate the changes in membrane hydrophilicity with biocompatibility and the results are discussed.

2. Experimental section

2.1. Chemicals and reagents

Commercial grade Polyacrylonitrile (M_w = 115 kDa) provided as a gift sample by Technorbital, (Pvt) Ltd., India was used after drying in a vaccum oven at 100 °C for 6 h. Poly (ether-imide) (M_w = 30 kDa), Ultem®1000 procured from Sigma Aldrich, India was dried at 100 °C for 8 h prior to use. N-methyl-2-pyrrolidone (NMP) was purchased from SRL Chemicals Ltd., India and sieved through molecular sieves (Type-5 A°) to remove moisture and stored in dry condition prior to use. n-Butyllithium (n-BuLi) solution, in n-hexane (1.6 M) was purchased from Sigma Aldrich., India. Sodium lauryl sulfate (SLS) was obtained from Glaxo India Ltd., and used as surfactant in the coagulation bath. Polyethylene glycol (PEG-200) from Merck Co., India was used as the non-solvent additive in the dope solution and distilled water was used as non-solvent in the coagulation bath for membrane preparation. Bovine serum albumin (BSA) was purchased from Hi-media Laboratories India (Pvt) Ltd. All other chemicals used were of analytical grade.

2.2. Preparation of carboxylated polyetherimide

Polyetherimide was carboxylated by a two-step reaction as reported earlier [6]. Briefly, a solution of polyetherimide (PEI) was prepared by dissolving 15 g of PEI in 500 mL dimethylformamide (DMF) by stirring at 50 °C for 5 h in nitrogen atmosphere. After complete dissolution, the polymer solution containing flask was cooled to −40 °C by immersing it in a dry ice/acetone bath and 5 ml of 1.6 M n-Butyllithium in hexane was added drop wise under constant stirring. Then the solution was vigorously stirred for another 15 min and then dry ice was added to the lithiated PEI. After the addition of dry ice the solution was kept as such for 24 h to complete the carboxylation reaction. Carboxylated PEI (CPEI) in the lithium salt form (COO-Li) was recovered by agitating the precipitate with isopropanol and dried in a vacuum oven. The COO-Li form of PEI was converted in to the acid form (PEI-COOH) through acidification of the lithiated form by stirring with 6N diluted HCl for 24 h followed by two consecutive washings with hot distilled water. Scheme 1 shows the reaction steps involved in the carboxylation of PEI. Subsequently, carboxylated polyetherimide (CPEI) was characterized for functional groups determination by FTIR (Perkin-Elmer, model-Spectrum RX1) and ^{1}H-NMR (Bruker AM-400 spectrometer, 400 MHz) techniques.

2.3. Preparation of PAN/CPEI blend membranes

The dope solutions for the membrane preparation were prepared by dissolving PAN and CPEI in different compositions in the absence and presence of additive PEG-200 using NMP as solvent under constant mechanical stirring in a round-bottom flask for 6–8 h. A series of such solutions were prepared by varying the composition of PAN and CPEI as shown in the Table 1. The dope solutions was then cast onto a clean glass plate and spread with a doctor's blade maintained at a thickness of 0.15 ± 0.02 mm. Prior to casting, a 2 L gelation bath, consisting of 2% (v/v) NMP (solvent) and 0.2 wt.% surfactant, SLS in distilled water (non solvent), was prepared and kept at 10 ± 1 °C. After casting, the solvent present in the cast film was allowed to evaporate for 30 s and the cast film along with glass plate was gently immersed in the gelation bath. After 2 h of gelation, the membrane was removed from the gelation bath and washed thoroughly with distilled water to remove residual NMP and surfactant present in the membranes. The membrane sheets were subsequently stored in distilled water, containing 0.1% formalin solution to prevent microbial contamination. The preparation method involved is the same as that of the "phase inversion" method employed in our earlier works as reported by other researchers from our lab [20,25].

2.4. Membrane characterization

2.4.1. Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) analysis

The ATR-FTIR spectra of pure PAN and PAN/CPEI blend membranes were recorded using a Spectrometer (Thermo Nicolet, Avatar 370) at room temperature. The membranes were dried before FTIR measurement and spectrum was recorded in the region of 1000 cm^{-1} and 4000 cm^{-1}.

2.4.2. Scanning electron microscopy analysis

Structure of the prepared PAN and PAN/CPEI blend membranes was studied by a scanning electron microscope (SEM, Cam Scan MV2300). The membranes of appropriate size (2 cm × 2 cm) were dried using a blotting paper and fractured in liquid nitrogen for 60–90 s. These dried membranes were gold sputtered to produce electric conductivity and images were obtained at high vaccum of 10 kV.

2.4.3. Contact angle measurements

The contact angle values of the PAN and PAN/CPEI blend membranes were measured by sessile drop method using a goniometer (GBX Instruments, Germany). The sessile drop was formed by depositing 5 μL of Milli-Q water slowly and steadily onto the membrane surface using a microsyringe. The contact angle was measured at room temperature within 10 s of the addition of water drop and reported values are the average of five measurements.

2.4.4. Thermo gravimetric analysis (TGA)

The thermal degradation studies of the PAN/CPEI blend membranes were carried out in a Universal V4.5A TA DTG analyzer in nitrogen atmosphere. The samples were scanned from room temperature to 800 °C at a heating rate of 20 °C/min. From the thermo grams, the thermal degradation characteristics such as onset of degradation (T_{on}), temperature of maximum rate of degradation (T_{max}), and percentage weight losses at different temperatures (300, 400 and 800 ° C) have been calculated.

2.4.5. Mechanical properties

The tensile properties of the PAN/CPEI blend membranes was investigated using computerized universal testing machine (Tinius Oslen H10KS, UK) at a cross head speed of 10mm/min. Tensile testing was carried out at room temperature according to ASTM D 638 using rectangular shaped samples size of 2.5 cm × 17.5 cm. At least three specimens were tested for each sample and the mean values were reported.

2.4.6. Protein adsorption test

Membrane samples of 1 × 1 cm^2 were taken and immersed in phosphate buffer saline PBS at pH 7.4 for 2 h to equilibrate the