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Studies on Permeation of Bovine Serum Albumin (BSA) Through Photo-Modified Functionalized Asymmetric Membrane

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UV-initiated photo-modification by hydrophilic functional moieties (viz. acrylic acid, acrylamide) is performed on the photo-responsive Polyether sulfone (PES) membrane. Characterization of the modified membranes has been done by porometry, Fourier Transform-IR (FTIR), contact angle, thermogravimetry, X-ray diffraction and gel permeation chromatography. The grafting of acrylamide is higher compared to acrylic acid, as is evidenced from weight increase, as well as porometry studies. The separation abilities of Bovine Serum Albumin (BSA) have been experimented through all the membranes and results show that modified membranes possess a better separation ability compared to virgin polyether sulfone. The separation order follows the trend: PES-g-(AM) > PES-g-(AA) > PES. pH dependence conformational changes of BSA macromolecule influence the separation. The increase in pH results in the decreasing trend in separation. The water flux recovery ratio (FRR) for the virgin PES membrane is minimum compared to other two grafted membranes.

Keywords: Polyether sulfone, acrylic acid, acrylamide, photoirradiation, grafting, Bovine Serum Albumin, pH

1 Introduction

During the last few decades, ultrafiltration has made a breakthrough in the biotechnological arena. It involves the bioseparation process for purification of different bio-products including enzymes, drugs, hormones, antibodies etc. (1–5). As the process is basically dependent on pores, it is possible to separate macromolecular solutes having comparable molecular weights/molecular dimensions. Today, research focused on the direction of modification of polymeric materials or membranes. The modification can be tailor-made i.e., properties are improved as the requirement. The modifications not only control the permeation through pores, they also have the abilities to influence by their functionalized part. Among the methods of modification of polymers, grafting is one of the promising methods. In principle, graft copolymerization is an attractive method to impart a variety of functional groups to a polymer. The tailor-made properties of the membranes through grafting

make them environmentally sensitive. The membranes can rapidly response to a stimulus from their surrounding environment. Permeability through them and selectivity can be tuned by altering the easily controlled parameters viz. pH, temperature. Therefore, these membranes have found many potential applications ranging from separations to controlled release systems (6–10). The grafted moiety on the membranes could change their conformation by tuning the pH value of the contacting solution. Graft copolymerization is initiated by chemical treatment, photo-irradiation, high-energy radiation technique, etc. (11). Photochemical surface functionalization of polymers through grafting of monomers has several advantages. Apart from its simplicity, mild reaction conditions and low temperature feasibility make it more attractive at this time (12).

In this research work, water soluble monomers (acrylic acid and acrylamide) are grafted on the polyether sulfone (PES) membrane by a photo-irradiation technique. The monomers change their conformation, as the pH of the medium. Apart from the membranes, the feed solute Bovine Serum Albumin (BSA) can also have the ability to change their conformation. In the present context, the permeation of BSA through the modified membranes is studied. BSA is an example of a protein which is well described hydrodynamically as a prolate ellipsoid (13, 14). The dimensions are 140Å for the major axis and 40Å for

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the minor axis (15, 16) BSA has three principal domains with individual pH dependent net charges and an isoelectric point (IEP) around 4.8. The different conformers are in a reversible form in solution at different pH values. The normal form (N-form) of BSA is at pH 5 to 8, whereas the Fast form (F-form) and Expanded form (E-form) prevails at pH 3-4 and pH < 3, respectively.

2 Experimental

2.1 Materials

Polyether sulfone with an inherent viscosity of 1.27 dl/g in N-methyl pyrrolidone was procured from Gardah Chemicals, India. Dimethyl formamide (Qualigen, India) was used for the preparation of the membrane. Acrylic acid (SRL, India), Acrylamide (SRL, India) and Dextran (71KDa) were obtained from Sigma, USA. Folin reagent (SD Fine Chem, India), Di sodium Tartarate (SD Fine Chem, India), BSA fraction V (SRL, India) was also used.

A light induced experiment was done by a UV-lamp (Philips HPR-125 watt). The membrane samples were kept at a 20 cm distance from the light. The lamp generated 300–400 nm. All the experiments were carried out at ambient temperature. The photo-irradiation was carried out at a nitrogen atmosphere. The radiation density flux on all the surface area was assumed to be constant in each run.

Ultrafiltration experiments were performed with a Sepa-200 ultrafiltration cell at room temperature (25°C). The complete setup is presented in Figure 1. The transport of

BSA (Mol. wt 67,000, 100 mg/lit, 10 mM phosphate buffer) was taken as feed solution. The filtration was carried out at 0.2 MPa at 200 rpm.

2.2 Methods

A two-step process was used to prepare grafted membranes viz.: (1) Preparation of asymmetric Polyethersulfone membrane and (2) Preparation of acrylic acid/acrylamide grafted polyethersulfone membrane. The processes are described below.

2.2.1. Preparation of asymmetric polyethersulfone membrane

Polyethersulfone solution in dimethyl formamide (15% w/w) was prepared through slow dissolution in heating conditions over a long time. The viscous polymer solution (prepared in dimethyl formamide) was first spread into a thin film on the nonwoven polyester fabric, and immediately immersed in a non-solvent medium (water). The solidified membrane was rinsed with reverse osmosis treated water for 18 h to remove the solvent and then dried for 48 h at room temperature before modification.

2.2.2. Preparation of asymmetric acrylic acid/ acrylamide grafted polyethersulfone membrane

Acrylic acid/acrylamide solutions in water (0.1% in weight) were spread on the polyethersulfone membranes (asymmetric side) fitted on glass tray for 3 mins time duration. The solutions were decanted from the membrane surface and

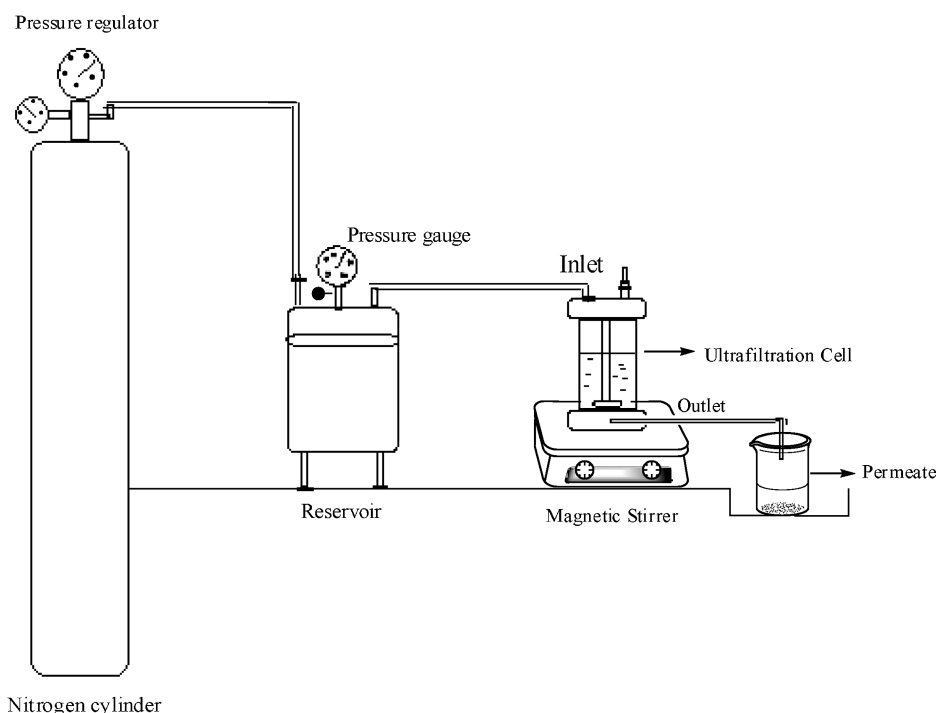


Fig. 1. Set up of dead-end filtration arrangements.

photo-irradiated for 2 mins ambient temperature and nitrogen atmosphere. BSA (100 mg/lit) solution in 10 mM phosphate buffer was prepared. The solution pH was adjusted as the composition of phosphate and citrate buffer changed. Reverse osmosis treated water was used in all the cases during the experiment.

2.2.3. Techniques

The amount of BSA of the feed and the permeate through the membranes were estimated by the standard method of Lowry et al., using Folin Ciocalteu's Phenol reagent (2N), sodium carbonate, copper sulfate, sodium potassium tartarate (17). The absorption was measured at 750 nm by Spectrophotometer (Varian, Carry 500 Scan, USA). The performance of the membranes was checked through a dead end filtration unit, by applying nitrogen gas pressure.

ATR-FTIR (with a Perkin-Elmer Spectrum GX with a resolution $\pm 4 \text{ cm}^{-1}$, incident angle 45°) and TGA (Mettler) studies in nitrogen environment ($10^\circ\text{C}/\text{min}$) were carried out to obtain evidence of the newer functional groups and thermal stabilities of the grafted membranes compared to virgin polyether sulfone membrane, respectively. Capillary Flow Porometer (Porous Materials Inc, USA, Model 1500 AEX), was used to characterize the pores of the grafted and virgin membranes. The porometry is based (18) on the following equation:

$$D = \frac{4\gamma \cos \theta}{P}$$

D is the diameter of a pore of circular cross-section, here, θ is the contact angle, γ surface tension, P is the differential pressure. The detail of the method was described in our earlier reports (19, 20).

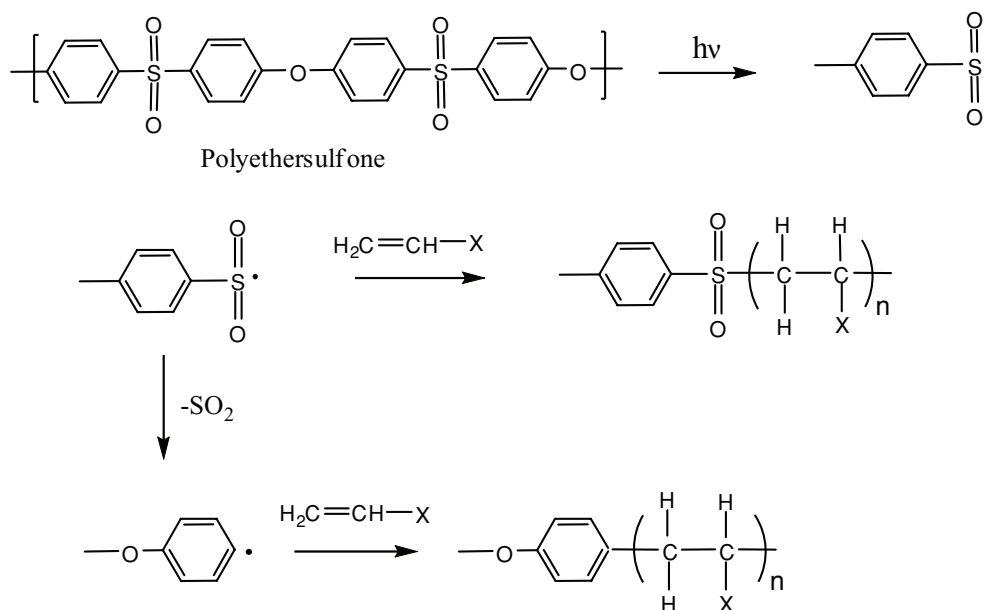
The Dextran (M_w 71,400, 0.5 mg/lit) concentrations were analyzed with gel permeation chromatography (HPLC-GPC Waters Alliance), using an auto injection mode under the following conditions: Column, ultrahydrogel 500, mobile phase 0.1% NaNO_3 in water, flow 0.5 ml/min, and 2414 RI detector, temperature 30°C .

X-ray diffractometer (X'PERT, Philips), $\text{Cu K}\alpha$ radiation as monochromator was used to prove the changes in the amorphous character due to grafting on the asymmetric polyether sulfone membrane. The contact angles of the virgin, as well as grafted membranes in water, were measured by Tensiometer (DCAT 21 from Dataphysics, Germany) by adjusting motor speed 0.2 mm/sec, dipping length 5 mm.

3 Results and Discussion

Polyethersulfone membranes are prepared through a phase separation process by a wet phase inversion technique. Due to the strong interaction between dimethyl formamide and water, dimethyl formamide in the casting solution mixes rapidly into the water gelation bath at the moment the casting solution, solidification of polyether sulfone resulted. As in this case, $\gamma_P < \gamma_S$ (P : Polymer, S : solvent and γ : surface tension), the asymmetric layer tend to form on the gelation bath side (21, 22).

The photo-responsive nature of polyether sulfone makes the feasibility of photo-modification by suitable monomers. The probable mechanism for the modification is shown in Scheme 1. The mechanism involves the sequence of steps (23, 24). viz. absorption of light by the phenoxyphenyl sulfone chromophores in the polymeric chain, the photoexcitation of the homolytic cleavage of C-S bond at the



Sch. 1. Reaction mechanistic pathways of vinyl monomers to Polyethersulfone.

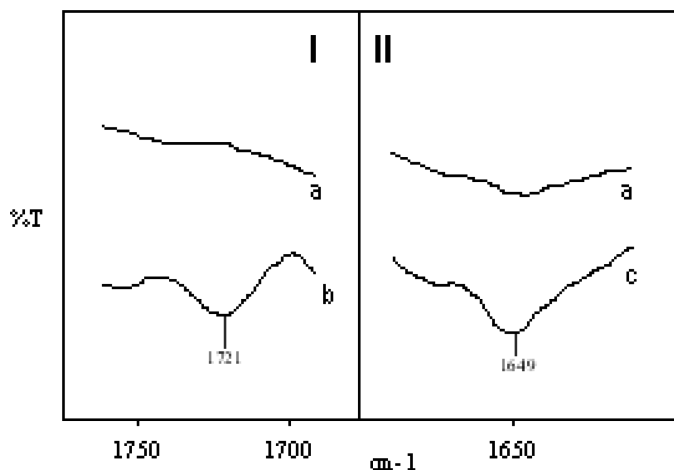


Fig. 2. ATR-FTIR spectra of grafted and virgin polyether sulfone; (a) virgin PES and (b) PES-g(AA) and (c) PES-g(AM) membranes.

sulfone linkage and the reaction of monomers with the aryl and sulfonyl radical. There may be alternative features that the sulfonyl radical may lose sulfur dioxide to generate an additional aryl radical which also may initiate polymerization.

The evidence of grafting of acrylamide and acrylic acid onto PES membranes are evaluated by FT-IR, TGA, XRD, porometry studies. The presence of the grafted monomers is confirmed by using FT-IR spectroscopy. In the spectrum (Figure 2) (I), of PES-g(AAc) shows the characteristic band of PAAc $\sim 1721\text{ cm}^{-1}$, which is ascribed to the C=O stretching of AAc. In the spectra of PES-g-AAm (shown in Figure 2) (II), the characteristic peak at 1649 cm^{-1} appears due to the carbonyl absorption of amide group, respectively.

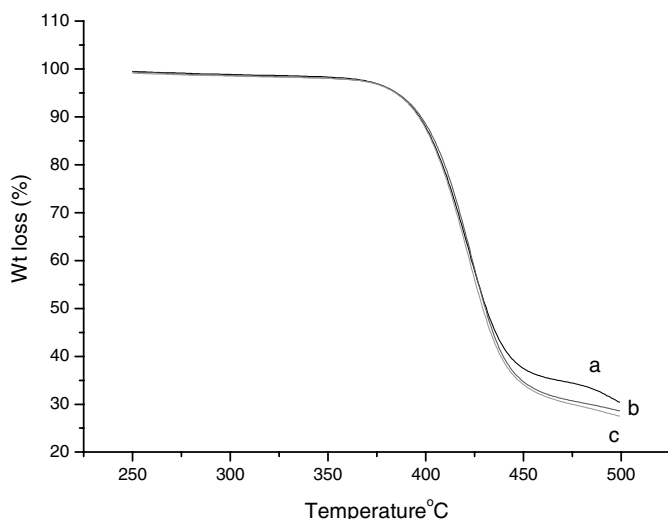


Fig. 3. Thermogravimetric pattern of (a) virgin PES, (b) PES-g(AA) and (c) PES-g(AM) membranes.

Table 1. Some characteristic data of membranes

Membrane	Weight increase [$\text{gm}/\text{cm}^2 (\times 10^4)$]	FWHM(cm)	Mean contact angle ($^\circ$)
PES	—	4.0	74.73
PES-g(AA)	2.38	6.0	72.88
PES-g(AM)	7.95	4.5	72.52

Primary thermograms of virgin PES and PES-g(AAc) and PES-g(AAm) membranes are presented in Figure 3. The thermograms show the differences at the 250 to 500°C range. The degradation of grafted membranes in this range is slightly more, compared to the virgin membranes.

The XRD patterns of the virgin and grafted membranes prove the incorporation of grafted moieties. The width of the half height of the peak found in the range $15\text{--}35^\circ$ is changed for the grafted membranes. It shows a broadening of peak width for the grafted membrane, and suggests the amorphous character is relatively more with respect to the unmodified membrane. Table 1 features the full width at half maxima (FWHM) for the three membranes. FWHM value suggests that a more broadening nature of the peak is due to acrylic acid grafting rather than grafting of acrylamide.

The porometry data of the unmodified and modified membranes shows that with the incorporation of the grafted moiety, the pore diameter decreases (Table 2). The mean flow pore diameters are reduced $>30\%$ from the virgin polyether sulfone membrane and the mean flow pore pressure increases 1.5 times. The bubble point pore diameter and the characteristic of the asymmetric membrane are also decreased on grafting, whereas the bubble point pore pressures obviously increased. Thus, the mean flow/bubble point pore diameter for PES-g (AM) is smaller compared to the acrylic acid grafted membrane (PES-g(AA)) as well as the unmodified one.

The incorporation of acrylic acid/acrylamide is also evidenced by contact angle studies. The mean contact angle of the grafted membrane is decreased $\sim 2^\circ$ from the value of a virgin PES membrane (Table 1).

The separation abilities of the membranes are also checked by passing the macromolecule marker, Dextran. The grafted membranes are more capable of separating Dextran, with respect to unmodified membranes (Figure 4). Thus, it can correlate that grafted membranes are more capable of separating BSA in general conditions, as Dextran. Moreover, BSA conformations are dependent on the variation of pH.

The grafting of acrylamide is higher than acrylic acid, as is shown from the weight measurements (Table 1) and the porometry, as well as MWCO of the membranes. This can be explained from the reactivity ratio of the monomers. Moreover, it is also seen that as pH increases, the acrylic acid reactivity decreases, whereas for acrylamide, the reactivity increases (25). The change in reactivity ratios may

Table 2. Porometry results of membranes

Membrane	Bubble pt. pore diameter (μm)	Bubble pt. pore pressure (psi)	Mean flow pore diameter (μm)	Mean flow pore pressure (psi)
PES	0.0848	78.279	0.0834	79.623
PES-g(AA)	0.0531	125.116	0.0530	125.234
PES-g(AA)	0.0581	114.237	0.0553	120.17

be attributed to the dissociation of acrylic acid and protonation of acrylamide. At pH (=3.9 (for 0.1% w/v), the reactivity of acrylic acid is low compared to acrylamide at pH (=6.1 (for 0.1% w/v)). (26). The separation performance of the membranes depends on mainly two controlling factors, viz. the pore size of the membranes and the nature, as well as the conformation of the feed solutes. The separation ability of the membranes increases as the conformation (in size) of the feed solute increases. As described earlier, BSA can exist in three different conformations depending upon pH of the medium. The size increasing trend of BSA molecule follows the order: N-form (at pH 5 to 8) < F-form (at pH 3-4) < E-form at pH < 3.

The rejection performances of the membranes are a decreasing trend with the increase in pH (Fig. 5) as the BSA molecules are in N-form, compared to the expanded F-form. The decreasing trend is somewhat higher for the grafted membranes compared to the ungrafted one at higher pH. At pH (<isoelectric point (pH 4.8)), both size exclusion, as well as the electrical repulsion factor, prevails at pH 3.1, as the membrane (for pKa (PAA) 4.8) and BSA both are positively charged (27, 28). PES-g-(AM) shows better rejection compared to PES-g(AA) and the ungrafted one, as the blocking effect is greater, due to higher grafting, already discussed in porometry and weight increase studies. Moreover, in acidic conditions, protonation develops a positive charge on the membrane. (26). The repulsive force

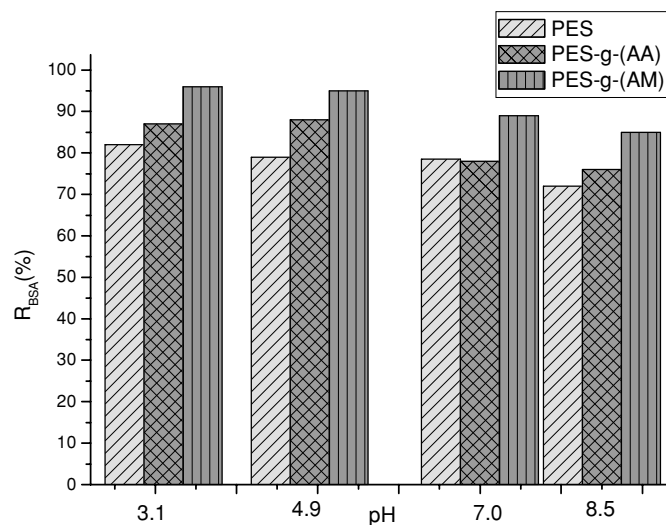


Fig. 5. Variation of rejection of BSA with pH for the three membranes.

due to the same charge between PES-g-(AM) and BSA also has a synergistic effect on it.

The BSA permeate flux is also influenced by pH of the feed solution. At pH 4.9, the permeability shows a minimum value whereas at higher pH it shows an increasing trend. The normalization in flux (with respect to flux at

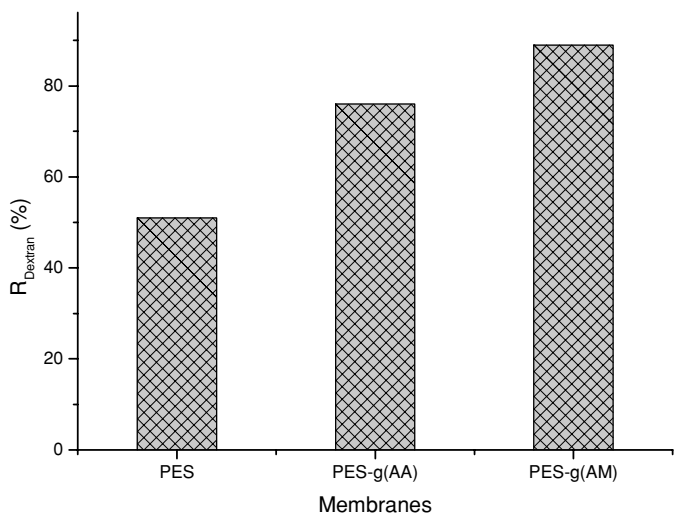


Fig. 4. Characteristic rejection data in terms of Dextran macromolecule marker.

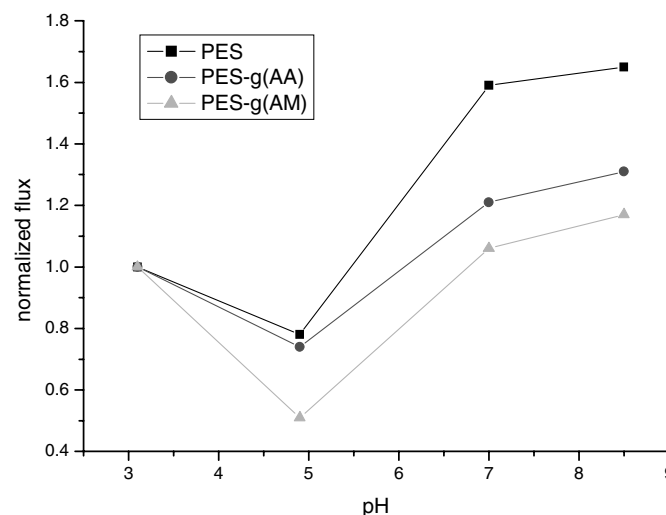


Fig. 6. Variation of normalized BSA flux (with respect to flux at pH 3.1) with pH of the feed solution.

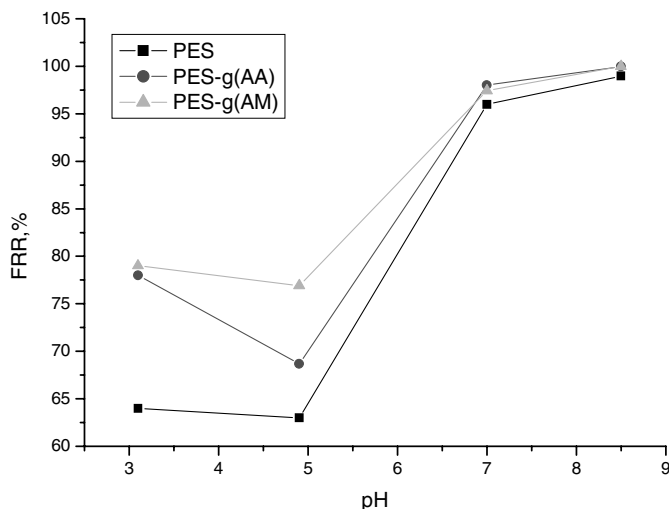


Fig. 7. Variation of water flux recovery ratio (FRR%) after passing BSA solutions having different pH.

pH 3.1) shows the trend in Figure 6. At the iso-electric pH (4.9), there is the possibility of blocking by BSA on the membranes compared to other pH (28). Thus, the flux reduction results at pH 4.9. Moreover, the figure shows that there exists a trend in terms of flux reduction depending upon pH viz. PES-g-AM > PES-g-AA > PES. This observation is supported by the reduction in pure water flux recovery ratio. The flux recovery ratio (FRR) is calculated using the following expression (29):

$$\text{FRR}(\%) = \left(\frac{\text{PWF}_{\text{uf}}}{\text{PWF}_0} \right) \times 100$$

Where PWF_0 and PWF_{uf} are pure water fluxes of the membrane before and after filtration, respectively.

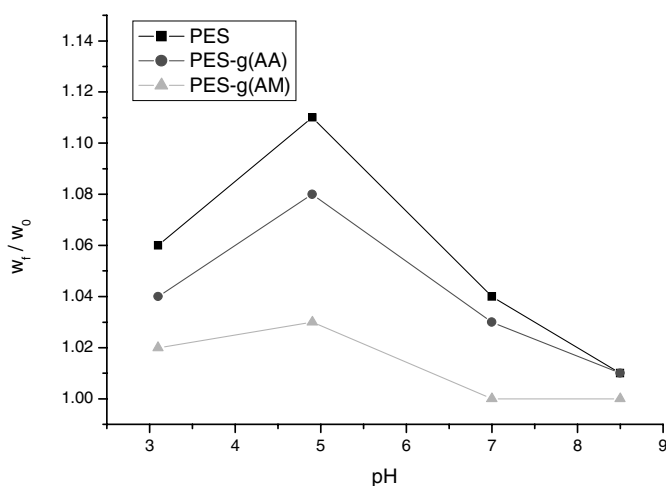


Fig. 8. Variation of weight ratio (weight of membranes after filtration of BSA with respect to the initial weight of the membrane) with BSA solutions having different pH.

The flux recovery ratio (FRR) for the ungrafted PES membrane is minimum compared to other two grafted membranes. The water flux recovery ratio is calculated after passing the BSA solution of different pH. It is presented in Figure 7. It is shown that being grafted, membranes are hydrophilic (contact angle studies), the BSA blocking effect is rather low compared to the ungrafted membrane. The order follows the trend: PES-g-AM > PES-g-AA > PES. This order is further supported by the weight of the membrane after passing the BSA solution at a different pH. Figure 8 shows the gain in weight for a different pH. The blocking order follows the reverse order.

4 Conclusions

The present studies regarding BSA permeation through photo-modified polyether sulfone membranes lead to the following conclusions:

The photo-modification of the polyether sulfone membrane by monomers (acrylic acid and acryl amide) is done without photo-sensitizer, as the photo-responsive part is within the membrane intrinsic. It also shows that the acrylamide grafting is higher than acrylic acid on the membranes.

The photo-modified membranes show better BSA separation ability compared to the unmodified polyether sulfone membrane. The separation abilities show the order PES-g(AM) > PES-g(AA) > PES. This is also supported by porometry, as well as dextran permeability studies.

pH Dependent conformational changes of BSA molecule also influence the performance of the membranes. Low pH favors separation for all the membranes. The permeate flux is also influenced by the pH of the feed solution.

The water flux recovery ratio (FRR) for the ungrafted PES membrane is minimum compared to other two grafted membranes. The order follows the trend PES-g-AM > PES-g-AA > PES. This order is further support by the weight of the membrane after passing BSA solution at different pH.

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