Polysulfone-Azo Composite Membrane: New Preparative Approach, Importance in Bactericidal and Biofilm Inhibition Activities

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Received 19 February 2009; accepted 17 June 2009
DOI 10.1002/app.31014
Published online 13 November 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The study in particular relates to a process for modifying surface of polymeric membrane and their biocidal activities. The modification process is based on absorption of 1, 3, phenylene diamine on the asymmetric polysulfone membrane and its diazo reaction. The azo compound (Bismarck brown) is characterized by LC-mass, uv–vis spectra. The incorporation of azo compound into polysulfone asymmetric membrane is proved by ATR-FTIR, SEM studies. The decrease in contact angle for modified surface proves the development of hydrophilic character. The modified membrane shows higher biocidal activity (for marine bacteria *Vibrio* sp.) compared to the virgin polysulfone membrane. The biofilm formation is inhibited for the modified membrane compared to virgin Polysulfone membrane.
 J Appl Polym Sci 115: 3710–3715, 2010

Key words: polysulfone; bismarck brown; *Vibrio* sp.; biocide; biofilm

INTRODUCTION

A major constraint associates with the use of membrane in water treatment is the deterioration in performances during the course of functioning. It may contain many microorganisms as well as humic acid and micropollutants—all of these species being potential contributors of membrane fouling. The consequence of the fouling is the deteriotion in performance as well as life time of the membranes. The attachment of bacteria to any solid surface from any aquatic environment forms the colonies over which the settlement of macro flora and fauna occurs.[1] This gives the habitat for attachment of that fouling and boring organism (e.g., gastropods, tubeworms, and mussel).[2] The bacterial attachment results in shortage of lifetime and membrane performances.[3] Sometimes, the bacterial colonization results the biofilm on the membrane. It is the natural phenomena for bacteria as self-defense against any odds to them. The inhibition of biofilm formation is helpful to expose pathogenic bacteria against any environmental stress (pH, salinity, alkalinity, some heavy metal, nutrient scarcity etc.). The aim of the membrane researchers is to find out the easy solution so that bacterial attachment can be avoided.

To avoid the attachment of undesired organisms, there are many methods. Removal and prevention of biofilms are caused by chemical treatment (chlorine dioxide, sodium hypobromite, mono, di, tri chloro amines, ozone, etc.) as well as UV-disinfection. Apart from these methods, membrane researchers are in a path to find out the possible approaches to inhibit the formation of biofilm. The simplest possible approach is to modify the membranes to inhibit the formation of biofilm i.e., the modification develops the biocidal activity. The art of incorporation of material into the membrane (through blending, grafting, and adsorption techniques) draws attention in the present context. The polymers and materials are mixed and membrane is prepared in the blending method.[4] In the grafting technique, compound is chemically bonded to the polymer through their active sites[5–10] whereas material is adsorbed into the polymeric membrane surface from its solution or vapor state in the adsorption technique. The electrostatic interaction as well as hydrophobicity/hydrophilicity factor is the possible reason for adsorption. In the present technique, the novel approach is adopted through adsorption of the reactants and the

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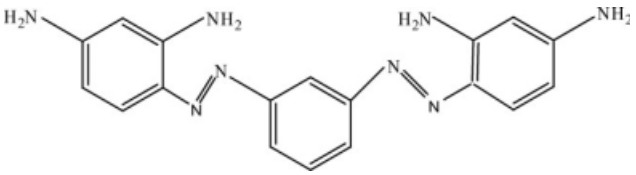


Figure 1 Structure of Bismarck Brown.

formation is within the polymeric matrix through diazo reaction. In this method, the azo compound (Bismarck brown) (Fig. 1) is incorporated in to the Polysulfone asymmetric membrane.

Some of the marine bacterial groups like *Pseudomonas, Bacillus, and Vibrio* are generally responsible for biofouling in the marine environment.[11] A presumptive *Vibrio* sp. isolated from sea water is used as experimental strain in the study. The natural abundance in sea water, biofilm forming capability, can grow in nonselective media are the characteristics of the bacteria. This biofouling bacteria generally provides space and facility for other fouling organism like barnacles and many molluscan larvae to settle down in the surface. As bacteria can be diluted to a single cell and studied in liquid culture, this mode of operation has been exploited and used to study the biofilm formation on the abiotic (membrane) surfaces. The bacterial study in liquid culture is advantageous to study by dipping the membrane in to it. Membrane modification is an art to inhibit the biofouling.[7,12,13] Literature report is also available that polymeric colored substrate results from functionlization develop biocidal activity.[14] Kobrakov et al.[15] showed attaching heteryl containing azo compounds the fungicidal activity was developed on to textile fibers. Thus, in the present study, we have tried to test the bactericidal activity as well as biofilm inhibition on the modified membrane and biofilm formation for the unmodified membrane.

MATERIALS AND METHODS

Materials used

Polysulfone (Udel, P-3500, Solvay Advanced Polymers), *N,N* dimethyl formamide (Qualigen, India) were used to prepare the asymmetric membranes. *m*-phenylene diamine (Loba, India) and Sodium nitrite (SD fine Chemicals, India) were used for the diazo reaction. All the other reagents used were of laboratory grade for the experiment. m-phenylene diamine was distilled before use. 30% w/v H_2O_2 (SD fine, India) was used for peroxide loading. In all the experiment, reverse osmosis treated water was used.

For biofilm formation study, Thiosulphate citrate bile salt sucrose agar (TCBS), Zobell marine broth, NaCl all were procured from Hi media, India. Glycerol (Hi-media, Molecular Biology grade) was used for the preparation of the membrane samples to take scanning electron microscopic study.

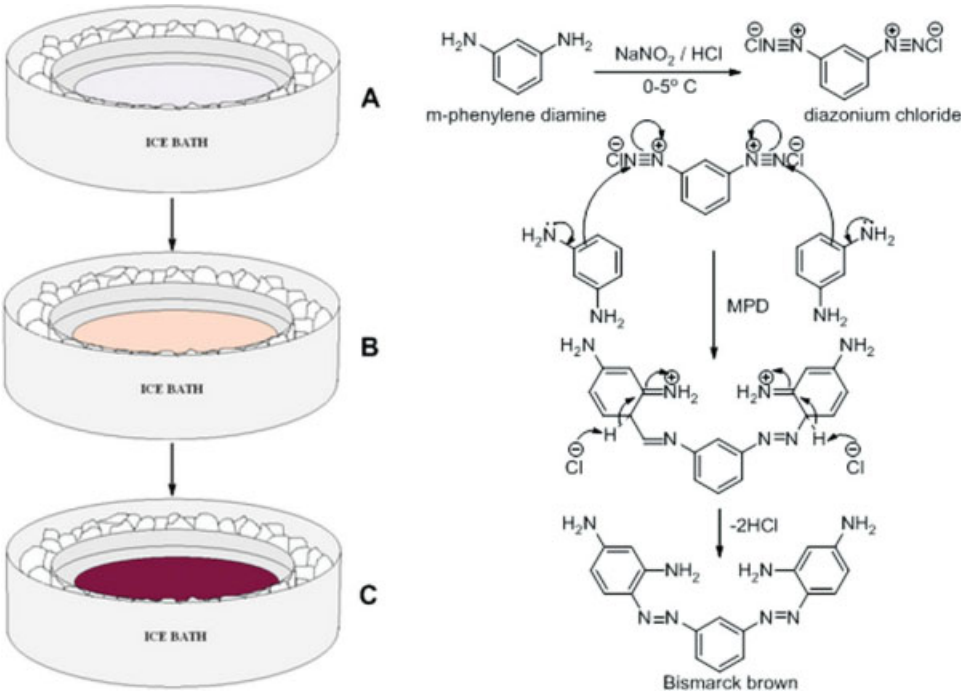
Methods

Polysulfone solutions in *N,N* dimethyl formamide (15% w/w) were prepared through slow dissolution in heating condition over long duration. The viscous polymer solution (in *N,N* dimethyl formamide) was spread into a thin film on the nonwoven polyester fabric (1 m) and immediately immersed in nonsolvent medium (water), mixed with sodium lauryl sulfate. The membrane casting steps were performed in the prototype casting machine, in our laboratory. The membranes were kept in the gelation bath for at least 3 h to complete the wet phase inversion. Then they were washed with water and dried at room temperature.

The asymmetric Polysulfone membrane was dipped in m-phenylene diamine (2%) for 30 min. The thin polymer rich phase adsorbs *m*-phenylene diamine on to it. It was dried by evaporation. The membrane was taken in petridish and placed in ice. Sodium nitrite solution mixed with hydrochloric acid was cooled in ice-water bath and poured on to it. The solution was decanted after 10 min from the surface of the membrane and 2% *m*-phenylene diamine solution was added over the membrane. Sodium Hydroxide (0.1%) solution was used to cease the reaction. The brown color appearance on the membrane indicated the preparation of modified Polysulfone-Bismarck Brown (PS-BB) membrane. The membrane was washed with water (reverse osmosis treated). The aforementioned reaction was also followed without membrane to get it in powder form. The peroxide loading was done by dipping the membranes (6×2.5 cm) in to 1 : 1 diluted H_2O_2 (30% w/v) for 18 h. The biofilm forming activities was tested after autoclave the membranes.

First, the *Vibrio* sp. was isolated from coastal water with a salinity of 30 ppt in thiosulphate citrate bile salt sucrose agar (TCBS) and pure colony was stored in -20°C with 25% glycerol before use. One single colony of experimental strain was inoculated in Zobell broth supplemented with 3% Sodium chloride and allowed to grow for 12 h. Ten microliters of bacterial inoculums was added to 20 mL of Zobell broth in 50 mL test tube. The membranes (virgin Polysulfone and modified Polysulfone) with size of 4×4 cm^2 were dipped single piece in each tube in sterile condition. The systems were allowed in shaking condition (80 rpm) in water bath at 30°C for 48 h and visible growth of bacterial biofilm over the surface of membranes were checked.

Further, to determine the exact concentration of bacteria attached to the membrane surfaces, they are



Scheme 1 Reaction Scheme of formation of Bismarck Brown and its pictorial presentation (a) Polysulfone adsorbed *m*-phenylene diamine, (b) Polysulfone-diazonium chloride, and (c) Polysulfone- azo compound (Bismarck Brown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

gently washed with sterile distilled water and 1 cm^2 area at the center of each dipped membrane were swept with sterile cotton swab and mixed with 1 mL of sterile (0.9 %) NaCl. Then suitable dilution mixtures were plated in TCBS agar to estimate the bacterial concentration from each membrane.

Techniques used

Mass spectrometry was performed using ESI-MS in positive ionization mode. Absorption spectra was studied from 200–750 nm with the UV–vis spectrometry. ATR-FTIR (Perkin Elmer Spectrum GX with a resolution of ± 4 cm^{-1}, incident angle 45°) studies were carried out to get the evidence of incorporation of Bismarck brown in to the membrane. The characterization of the membranes was carried out in terms of contact angle studies (by DCAT21 (Germany) (motor speed 0.09 mm s^{-1}) dipping width 0.5 mm). For water permeability measurements, a laboratory made cross-flow filtration was used. The experimental set up was sketched elsewhere.[16]

The membranes were treated separately for the scanning electron micrograph. It is required to dehydrate the attached bio adhesion without changing the shape. The membranes were dipped in 2% glutaraldehyde for 30 min. They were washed with phosphate buffer pH 7.2 (two times 5 min each). The membranes were transferred to gradually six ascending concentration of ethanol from 10 to 90% and rectified spirit. All the dipping duration was of 30 min and it was of twice in rectified spirit. Finally, the membranes were dried and ready for micrograph.

RESULTS AND DISCUSSION

The polysulfone solution (in *N,N* dimethyl formamide) is cast it into thin film on the nonwoven polyester fabric and quench it in to the *N,N* dimethyl formamide (solvent)/nonsolvent (water) gelation bath. The entire phase separation process was the result of solvent and nonsolvent exchange during the quench step.[17–19] The process is termed as "phase separation process." Sodium lauryl sulfate was added to the gelation bath to achieve the uniform pores through the membrane.

The diazo reaction was carried out as described in the experimental section. The reaction scheme as well as mechanistic steps is presented in Scheme 1. The *m*-phenylene diamine in presence of $NaNO_2$/HCl (0–5°C) forms Diazonium chloride. The diazonium ions coupled with active substrate (*m*-phenylene diamine) and nitrogen retaining reaction occurred.[20] In similar condition, the azo compound was prepared in powder form to characterize the compound.

The m/z 347.69 $[M + H]^+$ is observed for the particular powder from the mass spectrometry results. The mass spectrum is presented in Figure 2. The

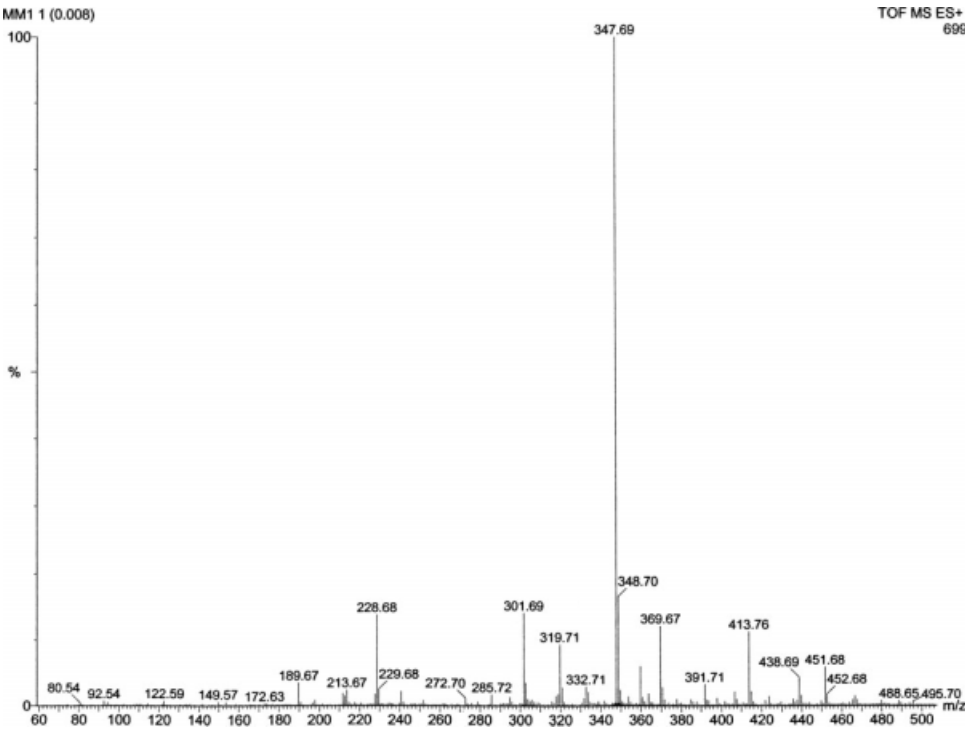


Figure 2 ESI-MS spectra of azo compound in powder form.

calculated molecular mass of Bismarck Brown is 346.17. An absorption maxima (λ_{max}) is observed at 460 nm.

Figure 3 shows the FTIR-ATR spectra of modified and virgin membranes. The development of peak at $\sim$ 3400 cm^{-1} for modified membranes features the aromatic amine stretching vibrations. 1625 cm^{-1} is due to N—H bending vibration studies. These peaks are the characteristic of amine group and prove the presence of amine compound in to the membranes.

The scanning electron micrograph (Fig. 4) of the membrane samples shows that presence of azo compound onto the membrane surface. There is distinct difference in morphological point of view between modified membranes (b) and virgin Polysulfone membranes (a).

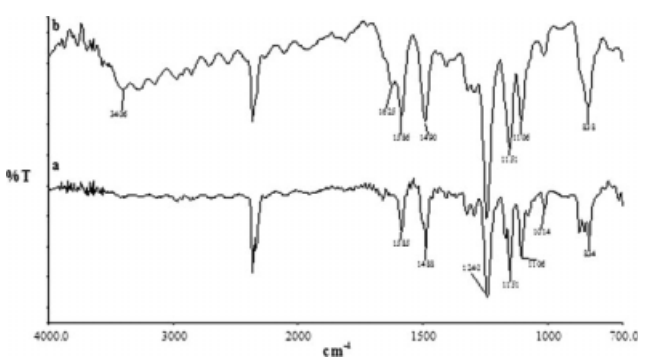


Figure 3 FTIR-ATR spectra of azo-modified (b) and virgin polysulfone(a) membranes.

The hydrophobic interaction between the bacterial cell and membrane overcome the repulsive forces active with in a certain distance from the polymeric surface and irreversibly attached.[21] Contact angle studies show that with the modification of Bismarck Brown, the mean contact angle of the modified membrane decreased $\sim$ 3° (67.26° from 70.21°) with respect to virgin PS membrane. The decrease in contact angle shows the development of hydrophilic character on the membrane. The hydrophilicity of

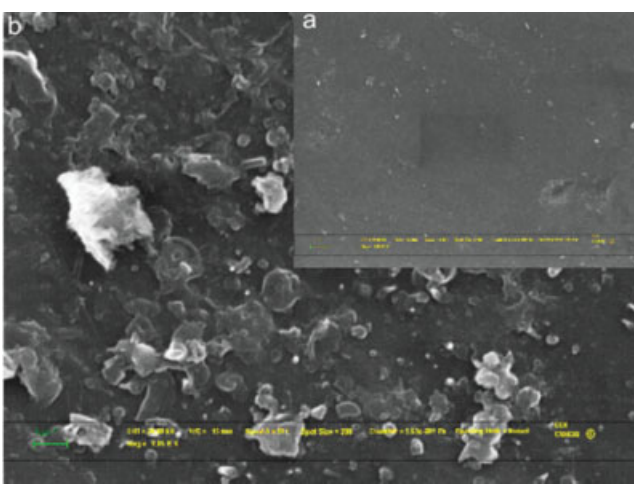


Figure 4 Scanning electron micrograph of azo-modified (b) and virgin polysulfone(a) membranes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the PS-BB membranes show low adhesion of bacterial cell compared to virgin PS membrane. Similar results are also there for different substrates in the literature.[22,23] The Pure water permeability results show that the Polysulfone- Bismarck Brown (PS-BB) membranes are of low water permeability with respect to Polysulfone virgin membrane (236.8 to 152.6 lm^{-2} h^{-1} at 0.69 MPa). This is due to the pore blocking of the membranes.

The biofilm formation was tested for virgin PS and PS-BB membranes. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. The bacterial attachment is counted for different membranes and the results are presented in Table I in two sets. From the results, it shows PS-BB membrane has better biofilm inhibition behavior compared to PS membrane. Of course, it needs peroxide loading. The bacterial attachment of virgin membrane is $\sim 10^4$ times more (per sq. cm area) with respect to modified PS-BB membrane. This may be due to the development of hydrophilic character of the modified membrane as it is evidenced from the contact angle studies. The biofilm is visually observed from the photograph (Fig. 5).

A clear picture of the attachment is proved by scanning electron microscopy studies. It shows that the attachment depends upon of the nature of the polymeric substrate. Figure 5(a,c) show that multilayer growths of bacterial attachment on the polysulfone virgin membrane where as for azo-modified membrane [Fig. 4(b)] the bacterial count are much less. The interbacterial attachment in the colonial growth is also seen in Figure 5(c).

CONCLUSIONS

The Bismarck Brown is incorporated into the asymmetric Polysulfone membrane based on diazo reaction. The formation of Bismarck brown is evidenced from LC-Mass, UV–vis absorption maxima. The incorporation of azo compound is evidenced from FTIR-ATR, SEM. Contact angle decrease ($\sim 3^\circ$) show better hydrophilicity of the modified surface with respect to virgin polysulfone membrane. The hindrance of the biofilm formation by *Vibrio* sp. for the modified azo-polysulfone membrane is due to better hydrophilic character. The bacterial count is less than 10^{-4} for the modified membranes.

TABLE I
Bacterial Count on Membranes

Membrane details	Set 1 (CFU mL^{-1})	Set 2 (CFU mL^{-1})
Azo modified- H_2O_2	1.1×10^2	7.3×10^2
Azo modified	2.4×10^5	3.9×10^5
Virgin polysulfone- H_2O_2	1.6×10^6	2.4×10^6
Virgin polysulfone	3.7×10^6	2.9×10^6

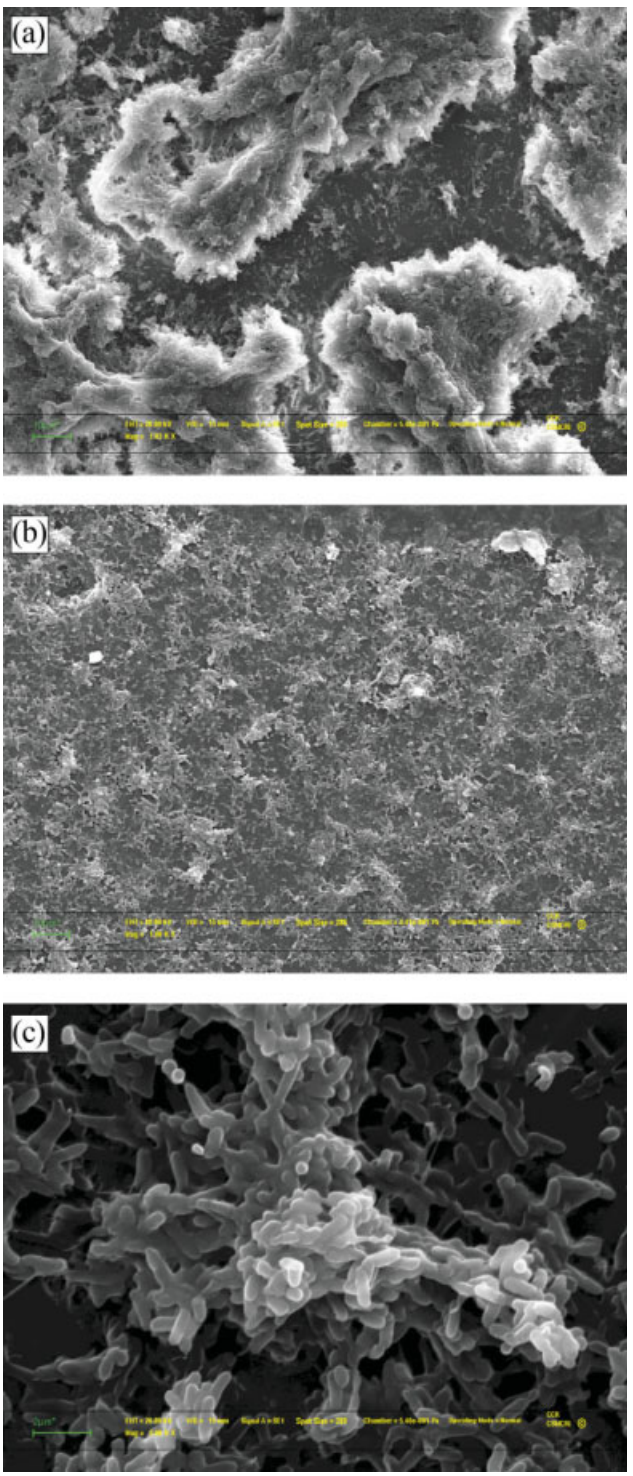


Figure 5 Scanning electron micrograph showing bacterial attachment of virgin polysulfone (a), (c), and azo-modified (b) membranes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The authors thank BRNS, DAE (India), and CSIR (India).

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