Chapter 4 Synthesis of PVDF Based Graft Copolymeric Antifouling Membranes Showing Affinity Driven Immobilization of Nucleobases

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4.1. Introduction:

A remarkable development in biotechnology and genetic engineering has accelerated the rate of production of biologics *i.e.*, proteins, enzymes and nucleic acids.^{1,2} A significant attention is therefore focused on the downstream purification and recovery of these biomolecules from crude cell culture media. In the purification of biomolecules, affinity chromatography has emerged as a very important tool due to the basis of separation being highly specific supramolecular interactions.^{2,3} Affinity chromatography generally is done using columns packed with resin beads (*i.e.*, packed bed chromatography) bound with affinity ligands. Replacing resin beads with membranes as the stationary phase offers several advantages that include improved mass transfer efficiency, lower pressure droop, efficient ligand utilization, easier scalability and cost effectiveness.⁴ Membrane chromatography operates by convective flow through process unlike slow diffusion of biomolecules within bead pores.^{3,5} Therefore, in case of the former, separation occurs at a much faster rate than the later. Despite their potential, the major disadvantage of membrane adsorbers is their low binding capacity per unit volume relative to beads. Physical and chemical properties of the membranes have a significant role over their performance. The poor binding capacity, typically observed in commercial membranes, originates primarily from their significantly poor specific surface area (SSA). Furthermore, broad pore size distribution leads to inefficient membrane utilization for differences in solute residence times and capacities of differently sized pores. Modification of membrane surface or pore walls with suitable chemical functional groups, metal affinity ligands *etc.* or polymeric chains can significantly impact over the membrane performance. Husson and coworkers have shown that ion exchange membranes can be prepared with 2-fold higher dynamic capacities than leading commercial resins at >15 fold volumetric throughput by polymer grafting using surface initiated atom transfer radical polymerization (si-ATRP) technique.¹ Therefore, introduction of chemical functionalities (capable of affinity interactions) in the pre or post membrane fabrication stage and control over the pore sizes should be an acceptable strategy for designing efficient membranes for chromatographic separation.

Polyvinylidenefluoride (PVDF) is an excellent membrane forming material due to its chemical inertness and high mechanical/thermal stability.⁶ However, due to the presence of

very much strong C-F bonds (485 kJ/mole),⁷ its chemical functionalization is difficult and significant hydrophobicity triggers biofouling.⁸ These should be the possible reasons behind relatively poor popularity of PVDF based membranes in affinity chromatography applications during bio-processing compared to commercially available nylon,⁹ polyethersulfone,¹⁰ polyvinylalcohol¹¹ or cellulose based membranes.^{12,13} Despite the favorable anti-fouling properties, hydrophilic membrane materials¹⁴ suffer from poor mechanical, thermal stability and susceptibility towards chemical attack.^{15,16} Therefore, integration of superior bulk properties of hydrophobic materials and improved surface properties of hydrophilic materials is desirable for fabrication of advanced membranes.⁸ In this regard, covalent attachment of hydrophilic polymers/functional groups on PVDF chain is a better approach compared to coating or composite formation in view of leaching problem during application.^{8,16} A significant progress in respect of grafting of polymer chains on PVDF backbone, by conventional free radical polymerization or controlled polymerization technique like ATRP, is observed in last two decades.^{18,19} The former technique however requires use of γ -ray irradiation or peroxide treatment, which associates threat of gelation or backbone degradation. Application of ATRP with PVDF macroinitiator eliminates these problems, but poor initiation efficiency of PVDF macroinitiator, owing to the high (C-F) bond strength and lower stability of secondary radical centers restrict its application on a large range of vinyl monomers.^{6,18,20} Thus, reports on grafting of new homo or copolymeric chains directly from the PVDF backbone, particularly polymer chains having reactive functionalities capable of further modifications, should be of significant value.

Reports on affinity chromatography of nucleic acids are limited. DNA affinity chromatography is reported previously where fragmented nuclear DNA is used as ligands for non-specific or sequence specific binding of nucleoproteins or nucleic acids.²¹⁻²³ On the other hand, being of non-biological origin boronates (for RNA immobilization)²⁴ or polycations have received application in immobilization of nucleic acids.²⁵⁻³⁴ However, polycations show cytotoxicity as well as significant non-specificity in respect of nucleic acid immobilization from a bioanalyte.³⁵ Alternative to the polycations, literature also reports application of neutral molecules which rely on complementary H-bond formation with nucleobases for efficient nucleic acid transfection.^{35,36} The present work reports synthesis and application of PVDF based graft copolymeric affinity membranes containing neutral, H-bond capable affinity ligands for immobilization of nucleobases/nucleic acids. Synthesis of PVDF based

different graft copolymers having amphiphilic, stimuli responsive random copolymeric graft chains consisting poly(n-butyl methacrylate) (PBMA), poly(diethyleneglycolmethylether methacrylate) (PDEGMEM)/ poly(oligoethyleneglycolmethylether methacrylate) (POEGMA) and poly(furfuryl methacrylate) (PFMA) are carried out by Cu based ATRP in N-methylpyrrolidone (NMP) solution. Post polymerization modification of the graft copolymers is subsequently carried out to introduce H-bond capable succinimide moieties via Diels-Alder (DA) reaction of the pendant furan rings with maleimide. Membrane fabrication is carried out following breath figure and immersion-precipitation techniques.³⁷⁻⁴⁰ A comparison of their potential in respect of membrane porosity, surface hydrophilic group composition hence, anti-fouling properties and possibilities of stimuli responsive pore generation is presented. The membranes fabricated from the synthesized graft copolymers have shown significant immobilization of nucleobases or nucleic acids from aqueous medium with desired level of selectivity based on nucleobase structures.

4.2. Experimental:

4.2.1. Materials:

PVDF (Aldrich, M_n = 7.1 × 10⁴, polydispersity index 2.57, head to head (H–H) defect = 4.33 mol%) is recrystallized from its 0.2% (w/w) acetophenone solution. The monomers PBMA, PDEGMEM, POEGMA and PFMA (Aldrich) are purified by passing through a basic alumina column. Maleimide is also used as received. CuCl (Aldrich) is taken in a Schlenk tube under a nitrogen atmosphere and washed with 10% aqueous HCl solution followed by methanol and diethyl ether. 4,4[′]-Dimethyl-2,2[′]-dipyridyl (DMDP, Aldrich) is used as received. N-Methyl-2-pyrrolidone (NMP), dimethylformamide (DMF) are purchased from RANKEM, RFCL limited New Delhi, India and are distilled and stored in an argon atmosphere.

4.2.2. Synthesis of PVDF-g-P(BMA-r-DEGMEM-r-FMA):

In a nitrogen purged tube $(8 \times 2.5 \text{ cm})$, PVDF (0.5 g) is dissolved in 3 mL NMP. The monomer (1.5 mL) [BMA (9.43 mmol)], (0.8 mL) [DEGMEM (4.33 mmol)] and (0.5 mL) [FMA (2.79 mmol)], DMDP (0.2926 g, 1.58 mmol), and CuCl (0.0665 g, 0.665 mmol) are added one by one before sealing with a rubber septum. The reaction mixture is stirred in an oil bath at 60°C. After the predetermined time, the reaction vessel is cooled to 30°C and the reaction mixture is diluted with 3 mL of NMP and is poured into hot water. The precipitate is isolated. The process is repeated twice by re-dissolving and reprecipitating it. The isolated

polymer is allowed to stir with hot water at 50°C to obtain a copper free PVDF graft copolymer. The product is dried under vacuum for three days at 30°C, weighed, and the percent conversion is calculated.

4.2.3. Synthesis of PVDF-g-P(OEGMA-r-FMA):

Similar procedure is followed with PVDF (0.5 g), monomer (0.2 mL) [OEGMA (0.44 mmol)], (0.4 mL) [FMA (2.57 mmol)], DMDP (0.07 g, 0.379 mmol), and CuCl (0.02 g, 0.2 mmol).

4.2.4. Modification of PVDF-g-P(BMA-r-DEGMEM-r-FMA) with Maleimide via Diels-Alder reaction:

In a nitrogen purged tube (8×2.5 cm), PVDF-g-P(BMA-r-DEGMEM-r-FMA) (1 g) is dissolved in 15 mL DMF. Then maleimide (0.22 g, 2.26 mmol) is added before sealing with a condenser. The reaction mixture is stirred in an oil bath at 60°C for 7 days. After the predetermined time, the reaction vessel is cooled to 30°C and evaporated the solvent up to 5 mL and then is poured into hot water. The precipitate is isolated. The process is repeated twice by re-dissolving and reprecipitating it. The isolated polymer is allowed to stir with hot water at 50°C to obtain a copper free PVDF graft copolymer. The product is dried under vacuum for three days at 30°C, weighed, and the percent conversion is calculated.

4.2.5. Modification of PVDF-g-P(OEGMA-r-FMA) with Maleimide via Diels-Alder reaction:

Similar procedure is followed with PVDF-g-P(OEGMA-r-FMA) (0.5 g) and maleimide (0.11 g, 1.13 mmol) in DMF (15 mL).

4.3. Characterization:

Size Exclusion Chromatography: The molecular weight and molecular weight distribution is measured by gel permeation chromatography (GPC) performed at room-temperature using Waters model 510 HPLC pump, 2414 Refractive Index Detector with Styragel[®] HR2 (THF) and HR4 (THF) combination. HPLC grade DMF (Spectrochem, India) is used as the eluent at a flow rate of 0.5 ml/min. Before injection into the GPC system the polymer solutions are treated with cation exchange resin Dowex 50 W (Fluka) to free them from cupric salts. They are then filtered through a prefilter-filter combination system compatible with organic solvents. The M_n of PVDF, PVBDF and PVOF graft copolymers are determined by conventional calibration by PMMA standards.

¹**H NMR Spectroscopy:** The ¹H-NMR spectra of PVDF, PVOF, PVBDF, PVBDFM, and PVOFM graft copolymers are recorded in d⁶-DMSO; for PVBDF and PVBDFM it is also recorded in CDCl₃ in a 500 MHz Bruker instrument.

FESEM Analysis: Analysis of surface morphology of the samples are done in FESEM (JEOL, JSM 6700F) instrument operating at 5 kV after coating with platinum for 90 s. Samples are prepared for FESEM analysis either through casting from dilute DMF solution or casted films by *breath figure/immersion-precipitation* techniques.

EDS Analysis: EDS analysis of samples are also performed by FESEM (JEOL, JSM 6700F) instrument.

AFM Analysis: Surface topography of the samples after casting from dilute solution of DMF on freshly cleaved mica surface is observed through atomic force microscopy (AFM) (Veeco, model AP0100) in noncontact mode at a tip resonance frequency of 300 kHz.

FTIR Analysis: The FTIR spectroscopy is carried out in FTIR-8400S instrument (Shimadzu) using KBr pellets.

ATR-IR Analysis: ATR-IR spectra of the membrane films are recorded by Bruker ATR-IR Spectrometer.

Photoluminescence Analysis: Photoluminescence (PL) studies are conducted in Horiva-Jovin Yvon Fluoromax-3 instrument.

WAXS Analysis: The WAXS analysis of the samples are performed by using a Bruker AXS diffractometer (D8 advance) using CuK α radiation (λ =1.54 Å), a generator voltage of 40 kV and a current of 40 mA.

XPS Analysis: XPS analysis of the samples is performed by using a focused monochromatized A1 K α X-ray source (1486.8 eV) in Omicron Nano-Technology 0571 XPS instrument.

DSC Analysis: Thermal studies are performed using a Mettler Toledo DSC1 STARe differential scanning calorimeter (DSC) at various heating rates in a N_2 atmosphere with sample weights of ~4–5 mg.

TGA Analysis: Thermogravimetric analysis (TGA) is done at 10°C/min heating rate with a sample weight of \sim 5–7 mg in a N₂ atmosphere using a Mettler Toledo TGA/SDTA 851e instrument.

Fabrication of Membrane:

'Breath figure' technique: In this technique, membranes are casted from THF (7% w/v) solution (of PVBDFM) in a glass slide under a condition of steady air flow at 25°C and 85% humidity. The membranes are finally dried under vacuum for 24 h after complete evaporation of THF. In order to study the effect of casting temperature on the membrane morphology, similarly casted three membranes as stated above are immersed in water baths having temperatures 10°C, 30°C and 60°C before complete evaporation of THF. This is followed by complete drying of the films by keeping at the same temperature out of the water baths for 24 h.

Immersion-precipitation technique: In this technique, membranes are casted from NMP (15% w/v) solution (of PVOFM) on a glass slide using a manual film applicator (Doctor Blade) at 25°C. Then they are immersed and kept in a non-solvent (water) coagulation bath for 24h for phase inversion. The membranes are finally dried under vacuum for 24 h after complete evaporation of water.

Analysis of Antifouling Property of Membrane Receptor: Membranes (PVDF, PVBDFM and PVOFM) having normalized PVDF content are dipped separately into aqueous 5 μ M solution (10 mL) of lysozyme and kept for 24 h. The protein adsorption analysis from each solution is monitored after withdrawing aliquot time to time by Fluorescence spectroscopy (PL).

Analysis of Nucleobase/RNA Immobilization: Membranes (a set of four membranes, total weight of 100 mg) are dipped separately into aqueous solutions of RNA [5 mL, 5 (μ M)] and aqueous solution (4 mL) having different concentrations (1 mM, 2 mM, 3 mM and 4 mM) for each of adenine, melamine and uracil at 25°C for 15 minutes and then the study of immobilization of RNA, nucleobases and melamine are carried out by measuring the concentration (after withdrawing aliquots) before and after incubation by CAPCELL PAK C18 column (4.6 mm I.D x 250 mm) in HPLC chromatogram analysis. The adsorption data obtained for nucleobases/melamine are used for construction of Langmuir type plot (as below). In a similar experiment 6 mL aqueous solution of mixture of adenine, melamine and uracil (1 mM for each) is used for assessing competitive adsorption of these bases.

Construction of Langmuir-type Plot: The Binding Constants (K) corresponding to the adduct formation between the membrane receptors (PVBDFM) and nucleobases/melamine determined by the Langmuir adsorption isotherm³⁶ as follows-

$$c/(\theta A) = c/A + 1/(KA)$$

(4.1)

Here, 'c' is the equilibrium concentration of free guest in the liquid phase, whereas 'A' and ' θ ' are the maximal amount of the adsorbed guest and the surface area coverage respectively.

4.4. Results and Discussions:

4.4.1. Synthesis of PVDF graft di/ter-copolymers:

Synthesis of the desired membrane materials are carried out following a two-step methodology. In the first step random ATRP of BMA, DEGMEM, FMA or OEGMA, FMA, is carried out for the synthesis of PVBDF or PVOF graft copolymers respectively, after initiated directly from PVDF macroinitiator, following "*grafting from*" strategy. The polymerization reactions are carried out in NMP solution using CuCl/DMDP catalyst at 60^oC (experimental section) (**Scheme 4.1**). The polymerization kinetics for PVBDF system (**Figure 4.1a**) shows a linear monomer disappearance with time for BMA and DEGMEM monomers, indicating controlled growth of the corresponding polymer chains. However, for FMA the plot shows somewhat non linearity indicating occurrence of termination reactions. The monomer disappearance with time for individual monomer is determined from the ¹H-NMR analysis of the graft copolymers precipitated in various time intervals.



Figure 4.1: (a) First order monomer disappearance plot for the monomers during graft copolymerization, (b) plot for molecular weight evolution during grafting.

The evolution of molecular weight with conversion for the graft copolymers and for each of the constituting graft chain polymers, as determined from their ¹H-NMR analysis (*vide infra*) is presented in **Figure 4.1b**. The plot shows a linear increase in molecular weight for the three constituent polymers as well as random copolymeric graft chains indicating their controlled growth. Finally the SEC analysis of the PVDF macroinitiator and PVDF-g-

P(BMA-r-DEGMEM-r-FMA) shows a clean sweep for the latter to lower elution volume without leaving any peak residue in the PVDF macroinitiator region (**Figure 4.2a**). A similar shift in SEC trace of PVDF-g-P(OEGMA-r-FMA) copolymer with respect to PVDF macroinitiator is also observed in **Figure 4.2b**. The column calibration during SEC analysis is done with commercially available linear polystyrene due to the non-availability of graft/branched polymer as calibration standard. Therefore molecular weight characterization cannot be performed from SEC analysis.



Figure 4.2: Overlay of the SEC traces (a) PVDF and PVBDF and (b) PVDF and PVOF. **4.4.2. Characterization by NMR spectra:**

The ¹H-NMR spectra (in d⁶-DMSO solvent) of various synthesized graft copolymers along with assignments of the characteristic signals for the backbone and graft chain protons are presented in **Figure4.3**. The signals observed at 2.65 ppm and 2.23 ppm represents backbone PVDF chain protons corresponding to (H-T, $-CH_2$ -CF₂-) and (T-T, $-CH_2-CH_2$ -) linkages respectively.²⁰ The signals for ester methylene protons (-O- CH_2 -) for all of FMA (proton 'G'), DEGMEM (proton 'f') and BMA (proton 'e') repeating units are observed at 4.72, 3.78 and 3.66 ppm respectively [**Figure 4.3A(i**)]. Apart from these, characteristic absorptions for the side chain protons ('c' and 'd') of DEGMEM repeating units are observed in the range of (2.98-3.37) ppm; signals for the aromatic furan ring protons 'H+I' and 'J' of PFMA block are observed at 6.22 ppm and 7.41 ppm respectively.⁴¹⁻⁴³ However, due to very high molecular weight of the PVBDF graft copolymer (**Table 4.1**), limited solubility of PBMA block and appreciably high viscosity of d⁶-DMSO, the signals for the ester methylene protons (-O-<u>*CH*</u>₂-) of the respective methacrylate repeating units are not well resolved. A more distinct appearance of the said signals is observed in CDCl₃ as solvent (**Figure4.4**), but due to the non-solvation of PVDF chains in this medium, signals for PVDF backbone protons are

absent. Now signal area ratios of proton 'G' and PVDF backbone protons (at 2.65 ppm and 2.23 ppm) can be precisely determined from **Figure 4.3A(i)**, which gives the mole ratio of VDF and FMA repeating units in the graft copolymer.



Figure 4.3: ¹H-NMR spectrum with signal assignments for PVBDF and PVBDFM in A[(i) and (ii)] PVOF and PVOFM in B[(i) and (ii)], recorded in d⁶-DMSO.

On the other hand, signal area ratio of 'G', 'f' and 'e' protons at 4.95 ppm, 4.07 ppm and 3.92 ppm respectively, representing the relative mole ratio of FMA, DEGMEM and BMA repeating units may be precisely determined from **Figure 4.4(i)**.

$$M_{n,graft} = M_{n,PVDF} \left\{ 1 + x_1 \left(\frac{M_0^{BMA}}{M_0^{VDF}} \right) + x_2 \left(\frac{M_0^{DEGMEM}}{M_0^{VDF}} \right) + x_3 \left(\frac{M_0^{FMA}}{M_0^{VDF}} \right) \right\}$$

Therefore, these area ratios are utilized for the determination of the graft chain composition, monomer conversion and molecular weights of the graft copolymers during polymerization. The calculation of the average molecular weight (M_n) for the entire graft copolymer (PVBDF) or various grafted chains are carried out using the following equation²⁰ and presented in **Table 4.1**.

| Sample | Feed Ratio | Mole ratio | PVDF (wt%) | T _m (| °C) | Enthal py of fusion (-ΔH J/g) | Degree of crystalli zation (%) | PFMA (wt%) | Convers ion (%) in D.A. reaction | PFMA M (wt%) |
|--|----------------|---------------|---------------|------------------|-------|---|--|---------------|---|--------------------|
| PVDF | - | - | 100 | 166.8 | 170.3 | 53.80 | 52 | - | - | - |
| PVBDF [PVDF _{71k} -g- P(BMA _{169k} -r- DEGMEM _{107k} -r- FMA _{54k})] | 2.9:1. 35:1 | 3.6:1. 7:1 | 18 | 165.8 | 173.3 | 6.28 | 31 | 13 | - | - |
| PVBDFM | - | - | 18 | 163.2 | 168.7 | 5.84 | 28 | 7 | 46 | 6 |
| PVOF [PVDF _{71k} -g- P(FMA _{19k} -r- OEGMA _{20k})] | 6:1 | 3:1 | 64 | 164.6 | 168.9 | 23.78 | 33 | 17 | - | - |
| PVOFM | - | - | 64 | 164.6 | 168.4 | 32.83 | 49 | 11 | 35 | 6 |

Here x_1 , x_2 and x_3 represent signal area ratio for BMA, DEGMEM and FMA repeating units respectively with VDF repeating units as obtained from the ¹H-NMR analysis and M₀'s are the molar masses of the respective monomer units (a similar equation is also used in case of PVOF graft copolymers taking FMA and OEGMA units in consideration). In case of PVOF [**Figure 4.3B(i**)], the ester methylene protons (-O-<u>*CH*</u>₂-)for POEGMA and PFMA blocks are well resolved and this graft copolymer is insoluble in CDCl₃, therefore spectrum obtained from d⁶-DMSO solvent is used for the above analyses. In order to ensure the solubility of PVBDF in volatile solvent like THF, a significantly higher feed load of the methacrylate monomers is used, which results in formation of appreciably high molecular weight ($M_{n,NMR}$) for this graft copolymer. On the other hand, during synthesis of PVOF, a much lower feed load of the methacrylate monomers are used, which is consistent with relatively lower molecular weight ($M_{n,NMR}$) observed for this graft copolymer (**Table 4.1**).



Figure 4.4: ¹H-NMR spectra for i) PVBDF and ii) PVBDFM samples along with signal assignments, determined in CDCl₃ solvent.

The table further shows that, agreement between the calculated mole ratios of different methacrylate monomers present in the graft copolymer and their feed ratios is acceptable in case of PVBDF. This indicates appreciable degree of control is expressed during synthesis of the PVBDF graft copolymer. However, in case of PVOF, the said agreement is relatively poor. A possible reason behind this may be the poor degree of control over the polymerization of OEGMA under the viscous polymerization conditions owing to its higher k_p (propagation rate constant) and poor k_t (termination rate constant) values.⁴⁴

In the next step, Diels-Alder reaction between the pendant furan rings of PFMA block in the graft chains of PVBDF or PVOF and extraneously added maleimide is carried out at 60⁰C in DMF medium to prepare PVBDFM or PVOFM. The ¹H-NMR spectra presented in **Figure 4.3**[**A**(**ii**) **and B**(**ii**)] show development of new signals 'g', 'j', 'i', 'h' for the Diels-Alder adduct along with 'G', 'H', 'I', 'J' signals for yet unconverted furan ring protons. The analysis of the signal ratio for 'j' and 'J' protons indicates about 46% and 35% conversion of the pendant furan rings of PFMA chains to the adduct (named PFMAM) has occurred in cases of PVBDFM and PVOFM systems respectively. Nevertheless, in both of the cases of PVBDFM and PVOFM, a comparable weight percentage of the PFMAM residues containing *imidodicarbonyl(-CO-NH-CO-)(IDC)* moieties are present. This may be further mentioned here that, these moieties are capable of interacting via H-bonding with nucleobases. The entire reaction is presented in **Scheme 4.1**.



Scheme 4.1: Synthesis of PVBDFM and interactions with nucleobases/melamine. 4.4.3. Characterization by FTIR spectra:

The FTIR spectrum of PVBDF in **Figure 4.5b** shows signal at 3125 cm⁻¹ corresponding to aromatic (C-H) stretching vibration of furan rings of PFMA. Signals at 1618 cm⁻¹, 1501 cm⁻¹, 1184 cm⁻¹, 1012 cm⁻¹ and(882 cm⁻¹& 748 cm⁻¹) are attributed to the (C=C) stretching vibration of furan ring stretching, ether (C-O) stretching, furan ring breathing, (C-H) out of plane vibration of furan ring respectively.^{45,46} After Diels-Alder (D.A.)

cycloaddition reaction of PVBDF with maleimide (in PVBDM), the appearance of a much broad signal at 3430cm⁻¹ and a shoulder at 1804 cm⁻¹ indicates (N-H) stretching (H-bonded) and (C=O) stretching vibrations respectively due to the fused succinimide rings. The broad signal observed at 1624 cm⁻¹ is attributed to the (C=C) stretching vibrations corresponding to conjugated double bonds of furan rings of PFMA chains and isolated double bond in the (3,4) position of the furan rings in D.A. adduct of PFMAM chains. This is further supported by the observed blue shift in signal position compared to PVBDF. The shoulder observed at 1596 cm⁻¹ might indicate typical coupled vibration [between C=O (stretch.) and N-H (def.)] of the imide groups. Apart from these, the strong signal observed at 1731 cm⁻¹ in cases of PVBDF and PVBDFM both is attributed to the ester carbonyl stretching vibrations corresponding to the different methacrylate repeating units present in them. In Figure 4.5a signals observed at (533 cm⁻¹, 615 cm⁻¹, 765 cm⁻¹, 796 cm⁻¹) and (510 cm⁻¹, 840 cm⁻¹, 1279 cm⁻¹) signify the coexistence of both α - and β -polymorphic forms respectively in PVDF (obtained after evaporation of DMF from its solution).^{20,42,47} Interestingly, after attachment of the polymethacrylate graft chains (with PVDF) in PVBDF, signals corresponding to the apolymorph decrease in intensity or disappear while signals corresponding to the βpolymorphic form are retained and become more intense (Figure 4.5b).



Figure 4.5: FTIR spectra of a) PVDF, b) PVBDF and c) PVBDFM. In all cases samples are obtained after completely evaporating DMF from their respective DMF solutions.

This is attributed to the nucleation of PVDF chains in an 'all-anti' form on the surface of polymethacrylate chains to maximize specific interactions between ester carbonyl (>C=O) groups present in the graft chains and (>CF₂) groups of PVDF.^{20,42} In our previous reports it is demonstrated that specific interactions leading to the β -phase stabilization occurs for PDEGMEM rather than PBMA graft chains, which might be due to the more polar and flexible ethylene oxide side chains of the former, allowing better cohesion with the PVDF chains.^{42,43} However, the signals corresponding to the β -polymorphic form (at 840 cm⁻¹ and 1279 cm⁻¹) becomes less intense and broad in PVBDFM (**Figure 4.5c**), which may be attributed to the loss in overall degree of crystallization of PVDF chains due to the steric congestion imposed after formation of bulky D.A. adduct. A similar set of observations are found from the analysis of FTIR spectra of PVOF and PVOFM samples (**Figure 4.6**).



Figure 4.6: FTIR spectra for a) PVDF, b) PVOF and c) PVOFM taken from the solid residues obtained after evaporation of DMF from their respective solutions.

Furthermore, likewise the previous system, disappearance of the α -phase and retention of the β -phase is observed here with the growth of the polymethacrylate graft chains (random copolymer of POEGMA and PFMA) on PVDF. However, in contrast to PVBDFM, in case of PVOFM characteristic signals for α -phase redevelops with poor intensity, retaining the relatively intense signals for the β -phase. This is presumably due to the competitive

interaction of oligoethyleneglycol chains with PVDF chains and *IDC* moieties of fused succinimide rings that allows PVDF chains somewhat free to crystallize in α -polymorph. The red shift in the (C=O) stretching frequency of fused succinimide rings at 1776 cm⁻¹ in PVOFM compared to PVBDFM (1804 cm⁻¹) perhaps indicates the said interaction. However this effect is not observed in case of PVBDFM due to the presence of flexible polymethacrylate chains in much higher proportion (72% w/w in PVBDFM compared to 36% w/w in PVOFM).



4.4.4. Characterization by Wide Angle X-ray Scattering (WAXS):

Figure 4.7: WAXS analysis of a) (i) PVDF, (ii) PVBDF and (iii) PVBDFM; b) (i) PVDF, (ii) PVOF and (iii) PVOFM. The samples used are the solid residues obtained after evaporation of DMF from their respective solutions.

The WAXS analysis of the DMF re-crystallized PVDF shows coexistence of α and β polymorphic forms, where peaks at 17.74°, 18.33°, 20.01° and 26.69° correspond to (100), (020), (110) and (021) planes of the α phase and right hand shoulder of $\alpha(110)$ peak at 20.6° corresponds to total diffraction of (200) and (110) planes corresponding to the β phase (**Figure 4.7**).^{48,49} In PVBDF, after grafting of significant amount of amorphous polymethacrylate chains (72% w/w, **Table 4.1**), PVDF crystallization is found to be significantly affected by the appearance of a broad nearly amorphous hallow in the region 17.4°-20.1°.^{42,43,47,50} The $\alpha(110)$ and $\alpha(021)$ peaks disappear with the rise of $\beta(200/110)$ peak at 20.53°. The situation remains nearly similar in case of PVBDFM where the $\beta(200/110)$ peak appears at 20.27° (**Figure 4.7a**). In case of PVOF similar disappearance of the α polymorphic peaks and the development of relatively sharp $\beta(200/110)$ peak occurs at 20.6° (perhaps due to much lower polymethacrylate graft chain concentration of 36% w/w, **Table 4.1**). The situation remains almost comparable in PVOFM excepting somewhat shift in the $\beta(200/110)$ peak to 20.4° (**Figure 4.7b**). Therefore, it is apparent that in both of the graft

copolymers after grafting of polymethacrylate chains, suppression of α phase and arise of the β phase occurs which is in conformity with the FTIR analysis. The signal observed at $2\theta = 7.6^{\circ}$ in cases of PVBDF or PVBDFM is attributed to the long range ordering in PBMA part, which has also been reported previously.⁴³

4.4.5. Morphological Characterization:

4.4.5.1. FESEM Analysis:

The FESEM images of PVDF and different graft copolymers from their films casted from DMF at room temperature under air are presented in **Figure 4.8**. PVDF exhibits array of uniformly distributed large spherulites having diameter $\sim 3 \ \mu m$ (**Figure 4.8a**). Dimethyl formamide (DMF) being a good solvent for PVDF, chains remain in expanded form thus, fewer nucleation sites being formed, in addition slower rate of solvent evaporation also help large crystallite formation.⁵¹



Figure 4.8: FESEM images of the graft copolymers casted from DMF solution: a) PVDF, b) PVBDF, c) PVBDFM and d) PVOFM

The image also shows a large number of relatively small sized ($\sim 1 \mu m$) spherulites are formed in the surface of large spherulites. A possible reason behind their formation should be condensation of moisture during DMF evaporation, which triggers local coiling of PVDF chains thus, a large number of nucleating sites being produced leading to smaller crystallites.

A significant change is observed in the morphology of PVBDF, where relatively small sized apparently embedded in the matrix of amorphous **PVDF** spherulites grafted polymethacrylates are abundantly distributed (Figure 4.8b). The reduced sizes of the spherulites are attributed to the grafting of long polymethacrylate chains which only allow the non-grafted segments of PVDF to crystallize. Furthermore, the presence of high T_g amorphous chains and possible interactions of PDEGMEM chains with PVDF might also inhibit crystallization of PVDF. The appearance of amorphous polymethacrylate coated PVDF spherulites further advocates towards specific interactions between PVDF and PDEGMEM chains in their interfaces.⁴² Relatively large sized, spherical/elliptical domains, visible in PVBDFM, are possibly generated via fusion of the discrete domains present in PVBDF (Figure 4.8c). The observed morphology probably results from improved mixing between various polymethacrylate chains and PVDF/polymethacrylates at the core-shell interfaces led by imidodicarbonyl (-CONHCO-) (IDC) groups of PFMAM (PFMA + maleimide adduct) chain segments.

4.4.5.2. AFM Analysis:



Figure 4.9: Tapping mode AFM images of DMF casted samples: a) PVDF, b) PVBDF, c) PVBDFM and d) PVOFM.

The AFM images nicely reiterate the morphologies of PVDF to PVBDFM observed in FESEM images. Array of uniformly sized distinct large spherical shape morphology is observed for PVDF in **Figure 4.9a.** In case of PVBDF much small sized spheres composed of PVDF crystallites are dispersed in the fibrous domains of amorphous polymethacrylates (**Figure 4.9b**). Finally, a fluffy morphology of the spherical aggregates having hairy surfaces observed in case of PVBDFM (**Figure 4.9c**). A comparison of FESEM images of PVDF and PVOFM shows the basic spherical shapes of the PVDF spherulites are retained (**Figure 4.9d**) in the latter, although the uniformity in the shapes and sizes is affected. This might be attributed to the restrictions imposed in PVDF crystallization however, in smaller degree compared to in PVBDFM due to i) much less proportion of grafted polymethacrylate concentration (36% w/w, **Table 4.1**) and ii) competitive interaction of *IDC* groups of PFMAM segments between PVDF chains and very much polar POEGMA side chains. The genesis of the abundant small sized spherulites on the surface of the large spherulites is similarly explained due to moisture condensation as in pure PVDF (**Figure 4.8a**).

4.4.6. Thermal Characterization:

4.4.6.1. TGA Analysis:

The overlay of the various thermo gravimetric analysis (TGA) plots (upto 500°C under N₂ atmosphere) corresponding to the synthesized graft copolymers along with PVDF are presented in **Figure 4.10(a-f**). The TGA profiles (**Figure 4.10a and b**) and corresponding derivatograms (**Figure 4.10c and d**) make it apparent that PVBDF/PVOF show three steps of degradations occurring at 240°C-350°C (Step-I), 362°C-452°C (Step-II) and 455°C-500°C (Step-III). The Step-I degradation has been attributed to the side chain degradations of both PFMA and PBMA in case of PVBDF and PFMA side chain degradation only for PVOF occurring at 286°C.⁵² Reconvolution of Step-I signal in the derivatogram of PVBDF (**Figure 4.10e**) shows a couple of signals arising at 286°C and 313°C, which are therefore attributed to PFMA and PBMA side chain degradations respectively.^{43,52} The reconvolution of Step-II for PVBDF generates a couple of peaks at 409°C and 435°C, which may be attributed to the complete degradation of PDEGMEM and degradation of residual backbone for (PFMA+PBMA) respectively.⁴² Similarly reconvolution of Stage-II degradation of POEGMA and residual backbone degradation of PFMA respectively (**Figure 4.10f**).^{52,53}

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Figure 4.10: TGA thermogram of a) PVDF, PVBDF and PVBDFM; b) PVDF, PVOF and PVOFM; c) derivative plot corresponding to plot a, d) derivative plot corresponding to plot b; e) reconvolution of the signals of plot c and f) reconvolution of the signals of plot d.

The above assignments are pretty consistent considering the degradation step sizes with respective weight percentages of the component polymers and their literature reported degradation pattern. The degradation temperature of PFMA side chains observed both in cases of PVBDF and PVOF is in conformity with the literature reported degradation temperature of PFMA homopolymer side chain (~289°C).⁵² Therefore, it may be assumed that in both of the graft copolymers, PFMA does not interact with any of the graft component hence remains completely phase separated. In comparison, degradation of PBMA side chain at 313°C and single step degradation of PDEGMEM at 409°C (in Step-II of PVBDF) (Figure **4.10e**) show large shift of ~130°C and ~61°C compared to their literature reported respective degradation temperatures in the homopolymers.^{42,43} The observed increase in degradation temperatures are attributed to the increased stabilization due to supramolecular interaction between their ester carbonyl groups or polar ethyleneoxy (-CH₂CH₂O-) groups with the (>CF₂) groups of backbone PVDF chains.^{42,43} The additional step of degradation observed between (145-160°C), both in cases of PVBDFM and PVOFM, is attributed to the retro-D.A. reaction leading to the maleimide elimination (Figure 4.10a-d). An agreement between the observed step sizes and the weight percentages of PFMAM moieties present in the different graft copolymers indicate complete removal of maleimide moieties. In sharp contrast with PVBDF (295°C), the Step-I maxima in case of PVBDFM shows an appreciable shift to 338°C. Furthermore, reconvolution of this signal shows three distinct peaks at 289°C, 334°C and 353°C (Figure 4.10e). A possible explanation behind this may be put forward considering different morphologies of i) phase separated unconverted PFMA chains undergoing side chain degradation at 289°C and ii) derived PFMA chains from PFMAM, after maleimide elimination, undergoing side chain degradation at 353°C. At this stage it may be argued that the morphology of PVBDFM/PVOFM should be quite different than PVBDF/PVOF owing to the supramolecular interactions between IDC moieties of PFMAM with the backbone ($>CF_2$) groups and various polar groups of polymethacrylate chains. This further indicates that PFMA chains generated after retro-D.A. reaction should remain entangled with the backbone PVDF or with the other copolymeric grafted chains. Therefore, relatively higher energy (hence temperature) should be required for the attainment of favorable orientation by the PFMA chains for undergoing side chain degradation after deentanglement. This perhaps pushes the side chain degradation temperature of the derived PFMA chains to 353°C. The above mentioned change in morphology is however much more

pronounced in case of PVBDFM than PVOFM due to the presence of much higher proportion of the grafted polymethacrylate chains in the former. Thus, shift in degradation temperature for the derived PFMA chains is also much less pronounced (~10°C) (**Figure 4.10d**) in PVOFM. The change in morphology of PVBDFM as stated above also shifts the side chain degradation temperature of PBMA to 334°C. The single step degradation of PVDF shows increase in both onset and degradation temperature by ~10°C in all of the graft copolymers compared to pure PVDF. This indicates PVDF chains may adopt favorable conformation for chain degradation only after complete removal of the grafted chains from the PVDF backbone.

4.4.6.2. DSC Analysis:

The analysis of the melting of PVDF crystallites from DSC thermogram of different samples has been pretty effective in determining interactions between various graft components hence, their morphology. Degree of PVDF crystallization in different samples has been calculated by comparing their normalized [with respect to the PVDF concentration (w/w) in the graft copolymers] enthalpy of fusion values with the enthalpy of fusion for pristine PVDF. In this regard, the enthalpy of fusion for α - and β -polymorphic forms of pristine PVDF has been considered to be equal $(\Delta H_0^{u} = 104 \text{ Jg}^{-1})$.⁴² The computed values are presented in Table 4.1. The DSC analysis for PVDF and all other graft copolymers has been carried out after recrystallization from DMF solution at room temperature (Figure 4.11). The thermogram corresponding to PVDF shows a broad endotherm, more precisely a double endotherm having melting points at 166.8°C and 170.3°C. This feature is often observed with semi crystalline materials like PVDF where the relatively low temperature endotherm may be attributed to the melting of imperfect crystalline region and high temperature endotherm for the melting of more compact crystalline phase.⁵⁴⁻⁵⁶ In cases of all graft copolymers two distinct endotherms are clearly visible. The low temperature endotherm may be attributed to the melting of imperfect crystalline phase consisting greater graft chain density and high temperature endotherm indicates melting of crystalline phase where graft chain density is low. In case of PVBDF, melting point of the imperfect crystalline phase shifts to relatively lower temperature (165.8°C) compared to pure PVDF (166.8°C). This may be attributed primarily to the steric obstruction induced by the grafted chains in backbone PVDF crystallization, as crystallization of the imperfect crystalline phase is only affected. However, some degree of interaction between the polar graft chains and backbone PVDF chains should

also be operative. In contrast, PVBDFM shows shift towards lower temperature for both of the imperfect crystalline phase and crystalline phase to 163.2°C and 168.7°C respectively.



Figure 4.11: DSC melting endotherm of PVDF and various other graft copolymers.

This might be attributed to appreciable specific interaction between the *IDC* moieties of the D.A. adduct with (>CF₂) groups of the PVDF chains, in addition with the obstruction in crystallization due to bulky D.A. adduct formation. The loss in crystallization is also apparent from the gradually decreasing value of degree of crystallization in cases of PVBDF (31%) and PVBDFM (28%) compared to pure PVDF (52%) (Table 4.1). In case of PVOF, both of the melting points have been shifted towards lower temperature (164.6°C and 168.8°C) indicating restriction in crystallization (compared to PVDF) despite much lower graft chain content (36% w/w compared to 72% w/w in case of PVBDF). This has been attributed to appreciably high specific interaction between flexible oligoethylene glycol chains with backbone PVDF thus, crystallization of PVDF chains is hindered.⁶In case of PVOFM, the melting point of the crystalline phase decreases further to 168.4°C indicating somewhat loosening in packing of PVDF chains, perhaps due to the increased bulk of the D.A. adduct. Interestingly, the melting point of the imperfect crystalline phase remains unaltered as in PVOF however; the corresponding melting endotherm becomes somewhat broader with associated increase in total enthalpy of fusion. This indicates improved ordering of PVDF chains of this region which might occur due to preferential interaction of the IDC moieties of PFMAM segment with polar and flexible oligoethylene glycol chains, leaving PVDF chains

free for crystallization. This is also reflected from the increased degree of crystallization of PVOFM (49%) compared to PVOF (33%) as presented in **Table 4.1**.

4.4.7. Membrane Formation:

Graft modification of membranes for imparting antifouling properties or introducing functional groups capable of affinity interactions remain limited to membrane surface only instead of pore walls.⁸ Therefore, in the present work I have carried out homogeneous solution phase grafting on PVDF chains, followed by membrane fabrication to ensure the presence of desired functionalities both on the membrane surface as well as on the pore walls.





I have used *breath figure* and *immersion-precipitation* techniques on PVBDFM and PVOFM respectively for membrane fabrication. The former technique results a honey-comb like pore generation when film casting is carried out with a solution of water insoluble polymer in volatile solvent (usually water insoluble) under humid condition. The pore generation has been proposed to occur due to condensation of water droplets (as a result of evaporative cooling) and subsequently their encapsulation by the precipitated polymer, restricting coalescence (**Scheme 4.2**). The water droplets sink through the polymer/solvent matrix which results in formation of through pores after film drying.^{43,57-60} In the present work, PVBDFM has been found to be THF soluble despite the insolubility of PVDF, which is attributed to the grafting of THF soluble polymethacrylate chains in large proportion. Thus film casting is

carried out with THF solution of PVBDFM under a condition of steady air flow at 25°C and 85% humidity.

4.4.7.1. FESEM Analysis:



Figure 4.12: Fabrication of porous PVBDFM films by drop casting of THF solution of PVBDFM in air (85% humidity) using breath figure technique: a) THF solution of PVBDFM drop casted at 25° C; temperature dependent pore generation experiment carried out by immersing PVBDFM films generated via drop casting of its THF solution and subsequently immersing in b) water bath at 10° C, c) 35° C and d) 60° C; e) and f) represent porous PVOFM films prepared by immersion precipitation technique when a relatively concentrated solution (15% w/v) of PVOFM in NMP is immersed in water bath at room temperature followed by drying.

The FESEM image of the membrane surface is presented in Figure 4.12a, which shows ellipsoidal pores of various sizes instead of hexagonally arranged honey-comb pattern. The generation of hexagonally arranged spherical pores during breath figure mechanism requires relatively high value of interfacial tension between water/polymer solution interfaces. However, aqueous solubility of THF and immeasurably poor water/THF-solution interfacial tension leads to poorly controlled, ellipsoid shaped pore generation during THF solution casted polymer films in *breath figure* technique.⁵⁸ In this context, it would be very interesting to note the effect of temperature dependent hydrophilicity of PDEGMEM chains (LCST of 26°C in water)⁶¹ on the membrane morphology. In this purpose, three different membranes are casted similarly as above however, prior to complete drying they are immersed in different thermo stated water baths having temperatures10°C, 25°C and 60°C. The surface images of the corresponding films are presented in Figure 4.12(b-d). Apparently much greater ellipticity and randomness in pore sizes observed at 10°C compared to 30°C is attributed to the increased hydrophilicity of PDEGMEM chains below their LCST (26°C).⁶² This directs increased surface aggregation of PDEGMEM chains, which results in reduced interfacial tension. Furthermore, average pore sizes of the membrane immersed at 10°C water bath has been found to be relatively higher (~6.5 μ m) than the membrane immersed at 30°C (~2.5 µm). This may be attributed to the reduced solubility/precipitation of surface aggregated PDEGMEM chains over their LCST in water. On the other hand, developed hydrophobicity of the PDEGMEM chains at 60°C (which is much above the LCST of PDEGMEM) limit their interfacial aggregation moreover, ready miscibility of water and THF at this elevated temperature restricts any pore structure formation.⁵⁸

4.4.7.2. XPS Analysis:

In *breath figure* mechanism of pore formation, the water droplets behave as dynamic templates therefore; aggregation of hydrophilic segments in the interface towards the aqueous phase should leave a preponderance of PDEGMEM/PFMAM chains in the pore wall surface of the dried membranes. Insolubility of PVDF in THF results self-assembled aggregation of PVBDFM in casting solution where PVDF remains in the core thus, away from the water/THF solution interface. This leads to hierarchical structure of the membrane surface, where grafted polymethacrylates remain on the surface layer.⁶⁰ This is further supported from the absence of F atom in the XPS survey spectrum of PVBDFM (**Figure 4.13a**) despite ~ 7.6% of bulk F atom concentration (as obtained from the NMR analysis). Furthermore the

survey spectrum shows relative surface concentrations of O and N atoms to be ~ 22.5% (bulk concentration is 20.6%) and ~2.52% (bulk N atom concentration is 0.56%) respectively, which implies high degree of surface aggregation of polar grafted chains.



On the other hand, PVOFM is insoluble in THF due to the presence of much higher proportion of PVDF (64% w/w). Therefore, film fabrication with this polymer is carried out following *immersion-precipitation* technique from its relatively concentrated NMP solution (15% w/v), taking water as the precipitant. In this technique when a film of polymer solution is immersed in a non-solvent (precipitant) the system distributes in two phases in equilibrium; a solid (polymer rich) phase which forms the membrane structure and a liquid (polymer poor) phase, which constitutes the membrane pores filled with the precipitant. Thus, after drying the porous membrane structure is produced.³⁷ The FESEM images of the membrane surface (Figure 4.12e and f) show a combination of micro and nano pores which are essential for keeping a relatively high flux rate along with increased surface area necessary for affinity interactions.^{11,63} The hydrophilic POEGMA and PFMAM chains should aggregate in the polymer/water interface to lower the interfacial tension. The preponderance of the polar/hydrophilic graft chains in the PVOFM membrane surface is confirmed from the high O and N atom relative concentrations of ~ 22.97% (bulk atomic concentration of ~11%) and 5.90% (bulk atomic concentration 0.52%) respectively, as obtained from XPS survey spectrum (Figure 4.13b). On the other hand, much decreased relative surface atomic concentration of F atoms (~2.22 %) (bulk atomic concentration ~29.90%) indicate aggregation of hydrophobic PVDF chains away from the membrane surface. Relatively higher surface atomic concentration of N atoms in case of PVOFM compared to PVBDFM may be attributed to the preferential interaction of PFMAM chains with POEGMA chains as indicated previously from the thermal analysis (TGA and DSC).

4.4.8. Comparison of Membranes Formed by Different Techniques:

4.4.8.1. EDX Analysis:

At this stage a comparison between *breath figure* and *immersion-precipitation* techniques of membrane formation may be very interesting. It is apparent from the foregoing discussion that pore formation in both of the mechanisms relies on differential interaction of solvent/non solvent molecules with the graft copolymers. In the present work both of the graft copolymers are having non polar/hydrophobic PVDF backbone on which polar/hydrophilic graft chains are anchored therefore, the said differential solvent/non solvent interactions result in interesting membrane morphology where polar/hydrophilic moieties self-aggregate on the surface.



Figure 4.14: EDX analysis of PVBDFM a) inside the pores and b) outside the pores; PVOFM c) inside the pores and d) outside the pores.

Despite the similarities, the modalities of non-solvent invasion in the polymer/solvent matrix are different. In breath figure mechanism discrete water droplets behave as dynamic templates for pore generation whereas in *immersion-precipitation* technique water molecules 'flush flood' into the relatively concentrated polymer solution. Thus, in breath figure mechanism relatively better uniformity on the generated pore sizes compared to *immersion*precipitation technique is observed. Furthermore, during the entire path of sinking of water droplets, polar/hydrophilic graft chain moieties get scope for self-aggregation in the polymer solution (in THF)/water interface thus, aggregation of these moieties can occur through the entire pore surface. This is in contrast with the *immersion-precipitation* technique where composition in the membrane surface and the pore surface should be similar. This is indeed observed from the higher ratio of 'N' and 'O' atoms in the inner and outer pore surface of breath figure casted film ($N_{In}/N_{Out} = 1.23$ and $O_{In}/O_{Out} = 1.13$) than immersion-precipitation casted film ($N_{In}/N_{Out} = 0.82$ and $O_{In}/O_{Out} = 1.01$) (Figure 4.14). The higher proportion of polar/hydrophilic groups, particularly N atoms from IDC moieties should be beneficial for having anti fouling property as well as affinity interactions during convective flow through process.

4.4.9. Antifouling Property of the Membranes:

Fouling in hydrophobic membranes originates from the attraction of organics (foulants) present in the feed water by hydrophobic membrane materials. Accumulation of foulants on the membrane surface or inside the pores has detrimental effect on membrane performance due to loss in membrane surface selectivity and pore clogging. An estimate says in ultra-filtration (UF) water treatment processes ~ 47% of the total process cost in involved behind membrane cleaning or replacement.⁸ In the present work assessment of the fouling properties of the PVBDFM or PVOFM membranes has been carried out using lysozyme as the model protein which emits at 350 nm in aqueous solution and compared their results with pristine PVDF membranes. In this respect, PVBDM, PVOPFM and PVDF membranes having normalized PVDF content is dipped in aqueous 5 μ M solution of lysozyme. Subsequently fluorescence spectra of the solution monitored time to time for assessment of the graft copolymeric membranes compared to PVDF membrane is apparent from the retention of much higher fluorescence intensity for PVBDFM or PVOFM membrane dipped solution (**Figure 4.15a**). This may be attributed to the increased polarity (hydrophilicity) of the graft

copolymeric membrane surfaces compared to PVDF membrane surface. In addition, plot of adsorption time dependent fluorescence intensity of lysozyme solution exhibits much reduced rate of lysozyme adsorption by the graft copolymers with the least rate being of PVBDFM membrane (**Figure 4.15b**). Improved anti fouling property of PVBDFM membrane compared to PVOFM may be attributed to the presence of higher proportion of grafted chains in the former, as well as their more effective aggregation towards the membrane surface or the pore wall surface during film fabrication following *breath figure* technique, as discussed previously.



Figure 4.15: a) Fluorescence spectra of aqueous solution of lysozyme after standing for 24 h under various conditions where the membranes are having normalized PVDF content. b) A comparison of the rate of lysozyme adsorption by various membranes having normalized PVDF content.

4.4.10. Immobilization of Nucleic Acids by the Membranes:

The study of the immobilization of adenine, melamine and uracil on PVBDFM surface from their respective aqueous solutions of different concentrations at 25°C are carried out by measuring their concentrations before and after incubation (15 min) with PVBDFM membranes by HPLC analysis. The adsorption of the above molecules on PVBDFM surface nicely fits with Langmuir type plot (**Figure 4.16a**). The binding constants determined from the analysis of the plots show higher value for adenine (28.8 M⁻¹) and melamine (15.3 M⁻¹) than uracil (8.8 M⁻¹). This may well be corroborated with the presence of complementary Hbonding sites between the guest molecules (adenine and melamine) and receptor moieties (*IDC* groups) present on the membrane surface, apart from the concurrently occurring π stacking and apolar interactions.^{36,64} It is indeed very difficult to trace the signals corresponding to the membrane adsorbed base moieties due to their low concentration and overlapping membrane signals however, the signals corresponding to adenine or melamine moieties have been detected by ATR-IR analysis of PVBDFM and PVOFM membrane surfaces after adsorption experiments (thoroughly washed by water and dried) (**Figure 4.16b** and **Figure 4.17**).



Figure 4.16: a) Langmuir-type plots for the adsorption of adenine, melamine and uracil (separately). The horizontal axis refers to the equilibrium concentration of the base in liquid phase and the vertical axis refers its ratio with the amount of adsorbed base (inset shows the Y-axis intercept); b) ATR-IR spectra of PVBDFM (i) before and after (thoroughly washed by water and dried) keeping in contact (15 min incubation time) with adenine (PVBDFM-A) (ii), melamine (PVBDFM-M) (iii) and uracil (PVBDFM-U) (iv), c) HPLC patterns for aqueous solution (6 mL) containing equimolar (1 mM) mixture of adenine, melamine and uracil before and after contact with PVBDFM membrane (100 mg); d) HPLC patterns for aqueous solution of RNA (5 μ M) before and after contact (for 15 min) with PVBDFM.

In case of PVBDFM-A (adenine adsorbed) (**Figure 4.16b**) the signals at 1650 cm⁻¹ and 1575 cm⁻¹ are due to the NH₂ bending and (C=N)/(C=C) stretching vibrations respectively for adenine moieties.⁶⁵ Similarly in case of PVBDFM-M (melamine adsorbed), signals at 1615 cm⁻¹ and 1550 cm⁻¹ have been attributed to the NH₂ bending and (C=N) (stretching) + NH₂ (bending) vibrations corresponding to melamine moieties.⁶⁶ In contrast, signals corresponding to uracil moieties are not detectable on the uracil base adsorbed membrane surface (PVBDFM-U). On the other hand, in **Figure 4.17d** for PVOFM-U, signal corresponding to characteristic stretching of uracil moiety has been detected. These observations are however expected due to the absence of potential H-bonding sites in uracil moieties with respect to the receptor *IDC* moieties of the membrane surface. Therefore, the adsorption of uracil observed during HPLC analysis (or ATR-IR analysis of PVOFM-U in **Figure 4.17**) may be attributed to the concurrent π -stacking and apolar interactions.



Figure 4.17: ATR-IR spectra of (a) PVOFM, PVOFM before and after (thoroughly washed by water and dried) keeping in contact (15 min incubation time) with (b) adenine (PVBDFM-A), (c) melamine (PVBDFM-M) and (d) uracil (PVBDF-U).

The greater selectivity in adsorption of adenine is further demonstrated by greater decrease in adenine concentration after 15 minutes of incubation of the aqueous solution of equimolar mixture (1 mM) of adenine, melamine and uracil with PVBDFM membrane (**Figure 4.16c**). Nevertheless, it is notable that, despite the possession of greater number of complimentary H-bonding moieties with respect to the *IDC* residues of receptor moieties, the immobilization of melamine on PVBDFM surface is poor than adenine. This is attributed to rather weak

interaction between melamine and succinimide moieties as the distance between 'O' and 'H' atoms in *IDC* units is higher due to the increased ring strain of the latter.⁶⁷ In view of the appreciable immobilization of the nucleobase (adenine), a filtration experiment is designed for testing RNA immobilization from its aqueous solution on PVBDFM surface. In this purpose, 5 ml of 5 μ M aqueous solution of RNA is passed through a permeation module (Millipore) fitted with PVBDFM membrane (**Figure 4.18**) for a duration of 5 minutes. The HPLC analysis of the solutions before and after filtration gives very much encouraging result of ~60% of RNA immobilization (**Figure 4.16d**).



4.18: Filtration module for testing RNA immobilization on PVBDFM surface.

4.5. Conclusion:

Synthesis of a couple of PVDF based graft copolymers PVDF-g-(PBMA-alt-PDEGMEM-alt-PFMA)(PVBDF) and PVDF-g-(POEGMA-alt-PFMA)(PVOF) has been successfully carried out by homogeneous solution phase ATRP after being initiated directly from PVDF macroinitiator. In a post polymerization modification step, Diels-Alder reaction of the pendant furan rings with maleimide carried out to prepare PVBDFM and PVOFM membrane materials respectively. The synthesized materials are casted into porous membranes by breath figure (from THF solution of PVBDFM) and immersion-precipitation techniques. A detailed

characterization of the polymerization kinetics, molecular weight, structure and morphology of the bulk graft copolymeric materials and membrane surfaces carried out by various spectroscopic, thermal and electron microscopic techniques. The polar/hydrophilic moiety enriched graft copolymer membrane surfaces (as confirmed from X-ray photo electron spectroscopy) resulted better anti fouling property than PVDF when tested with aqueous solution of lysozyme. Relatively higher population of polar residues in the pore wall compared to membrane surface of PVBDFM has been attributed to the dynamic template model of pore generation during breath figure mechanism. This feature is beneficial for having improved anti fouling property as well as affinity based immobilization during convective flow through process. Indeed PVBDFM has exhibited better anti fouling property than PVOFM, which has been attributed to higher graft copolymer concentration in PVBDFM and better aggregation of polar graft chains in the solvent-non solvent interface as THF is a selective solvent where PVDF chains are insoluble. Interestingly, PVBDFM membrane has shown adjustable pore sizes when breath figure casted freshly prepared membranes immersed in water baths having different temperatures. This has been attributed to the temperature dependent aqueous solubility of PDEGMEM block (LCST of 26°C). The graft copolymeric membranes have shown preferential immobilization of adenine and melamine over uracil, which is apparent from their higher binding constant determined from adsorption experiment on PVBDFM surface as well as higher immobilization (adenine > melamine >> uracil) under a competitive situation from aqueous solution of equimolar mixture of these three bases. In a filtration experiment PVBDFM membrane (100 mg) has shown ~ 60% RNA immobilization from 5 ml of its 5μ M aqueous solution.

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