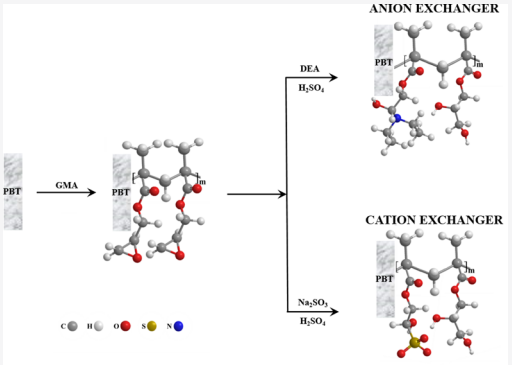
I&EC research
Industrial & Engineering Chemistry Research
pubs.acs.org/IECR
Article

Heat Induced Grafting of Poly(glycidyl methacrylate) on Polybutylene Terephthalate Nonwovens for Bioseparations

Michael Heller, Qian Li, Kellie Esinhart, Behnam Pourdeyhimi, Cristiana Boi,* and Ruben G. Carbonell*

Cite This: *Ind. Eng. Chem. Res.* 2020, 59, 5371−5380
Read Online

ABSTRACT: Polybutylene terephthalate (PBT) nonwovens were successfully grafted with poly(glycidyl methacrylate) (polyGMA) using a heat induced grafting approach with the thermal initiator benzoyl peroxide (Bz_2O_2). This grafting method resulted in complete, uniform, and conformal grafted layers around the PBT fibers that could be further functionalized as ion exchangers for protein capture. Protein binding capacities as high as 200 mg/g were achieved for ion exchange PBT nonwovens grafted to 20% weight gain using this heat induced grafting method. Compared to UV grafted polyGMA PBT nonwovens, the rates of protein adsorption are several times faster for the heat grafted polyGMA PBT nonwoven, reaching equilibrium within minutes; UV grafted polyGMA ion exchange PBT nonwovens require hours to reach equilibrium. This indicates that polyGMA grafts formed by heat induced grafting are thinner, and therefore more dense, than UV grafted layers with the same % weight gain. To further investigate the structural differences between the two grafting methods, targets of various molecular weights (ATP, lysozyme, BSA, hIgG) were adsorbed to the materials. Increasing the target size resulted in a decrease of target molecules bound for both grafting methods. However, the heat grafted nonwovens exhibited a much stronger dependence of protein molecular weight on protein capture, indicating that heat induced grafting results in a polyGMA layer that has a smaller free volume between chains available for protein binding compared to the UV grafting method. Protein adsorption isotherms for the two grafting methods confirmed that both methods resulted in similar strengths of protein binding, with dissociation constants on the order of $K_d = 10^{-6}$ M which is consistent with ion exchange binding on polymer brush networks. Heat grafted polyGMA ion exchange PBT nonwovens showed excellent protein binding and elution.



1. INTRODUCTION

Nonwovens fabric membranes, broadly defined as web structures in which the random fibers and filaments are bonded together mechanically, thermally, or chemically, are used in numerous applications from medicine to electronics.[1] Surface initiated polymerization of nonwoven fabric membranes is a useful technique for introducing a thin polymer layer with substantially different surface properties on the material being grafted.[2,3] In bioseparations, polymer grafting has been used to functionalize polyolefin and polyester based nonwoven fabric membranes to create high capacity protein capture devices.[4−10] Polyolefin and polyester are inexpensive thermoplastic polymers commonly used in high-rate nonwoven manufacturing technologies. These nonwoven fabric membranes are particularly attractive as platforms for bioseparations since they are highly engineered to exhibit controllable porosities, fiber diameters, and thicknesses with sufficient mechanical integrity for filtration applications.[11] On the other hand, these materials suffer from low specific surface areas, are chemically inert making functionalization difficult, and are hydrophobic in nature making them not ideal for protein capture from aqueous solutions.[12] The grafting of polymer brushes to the surface of the nonwoven fibers offers the potential to create a three-dimensional environment that is hydrophilic and can be chemically modified for protein adsorption resulting in protein capture devices with high capacities.[4−8]

Zheng et al. successfully grafted uniform and conformal poly(glycidyl methacrylate) (polyGMA) layers to polypropylene (PP) nonwoven and functionalized the material to be a weak anion exchanger. This resulted in bovine serum albumin (BSA) capture with a binding capacity of 120 mg/g of membrane.[9] Similarly, Liu et al. grafted complete and conformal polyGMA layers to polybutylene terephthalate (PBT) and then functionalized it to be a completely hydrophilic membrane that significantly reduced nonspecific protein adsorption to negligible amounts.[10] Liu et al. also investigated polyGMA grafted PBT, functionalized as a weak

Special Issue: Donald R. Paul Festschrift

Received: September 4, 2019
Revised: January 29, 2020
Accepted: February 28, 2020
Published: February 28, 2020

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

anion exchanger for capture of BSA. In this study it was determined that the equilibrium binding capacity was dependent on the degree of GMA polymerization around the PBT fibers due to an increase in the size of the three-dimensional binding volume of the grafted GMA brush layer around the fibers.[13] Zheng et al. and Liu et al. both applied a UV-light induced radical polymerization to graft a GMA monomer from the surface of the polyolefin and polyester, respectively.[9,10] UV-light radiation based polymerization is a more robust radical vinyl polymerization used heavily in commercial applications, as for heat grafting; this type of polymerization requires a photoinitiator that undergoes homolysis in the presence of UV-light creating a radical that can abstract a proton from a polymeric nonwoven surface, forming an initiation site for vinyl polymerization.[4,9,10,14] The issue with UV-light polymerization is that it is limited to the penetration depth of the light source. As a result, nonwoven samples that are sufficiently thick or do not have a large enough pore structure for light penetration will result in insufficient graft coverage in the depth of the material due to a lack of UV-light penetration.

There is a need to develop a polymerization technique that can graft polymer layers on nonwoven membranes regardless of the pore structure, thickness, and shape of the material. Thermally induced surface polymerization is a process that offers the potential to be robust and facile at creating a polymer grafted layer on the surface of nonwoven fiber that is independent of the morphology of the membrane being grafted. Heat induced vinyl polymerization is commonly used industrially to create bulk polymerizations in solution but has found limited application for surface modification of membranes.[14] A few studies have been conducted investigating thermal grafting of vinyl monomers on polyethylene terephthalate (PET) and PP.[15−17] Arslan has successfully grafted polyGMA onto PET and then further functionalized the material for removal of chromium from aqueous solutions.[15] Carroll et al. grafted acrylic acid, 2-(*N*,*N*-dimethyl amino)ethyl methacrylate, and poly(ethylene glycol) monomethyl ether monoacrylate to PP to reduce membrane fouling by organic waste.[16] However, little work has been done on the thermal grafting of polyGMA onto PBT nonwovens for applications in bioseparations.

This paper describes a methodology for creating highly uniform and conformal polyGMA grafts on PBT nonwovens using a heat induced radical polymerization method initiated by benzoyl peroxide (Bz_2O_2) at elevated temperatures. Bz_2O_2 is a thermal initiator very similar in structure and function to the photoinitiator benzophenone used for photoinduced grafting of polyGMA onto PBT nonwovens. Additionally, Bz_2O_2 has been shown to initiate heat grafting on polyester fabrics,[15,17,18] and the homolysis of Bz_2O_2 at elevated temperatures has the potential to create radical polymerization sites on the PBT surface for polyGMA propagation. The epoxy end groups in polyGMA introduce chemical functionality to the PBT surface that enables the covalent attachment of amines, thiols, and hydroxyl groups.[19,20] Of course, the heat-induced-grafting method requires heating as well as solvents that could be problematic, but it does allow for grafting materials that are thicker and more complex as stated previously.

In this work, we successfully converted PBT nonwovens heat grafted with polyGMA into anion and cation exchangers for bioseparations by covalently attaching diethylamine (DEA) and sodium sulfite (Na_2SO_3) via the epoxy groups of polyGMA, respectively. A direct comparison is drawn between the heat induced and the UV induced polyGMA grafting of PBT nonwovens since UV grafting is the primary methodology for vinyl grafting of polyester and polyolefin membranes and has been investigated extensively for grafting of polyGMA onto PBT nonwovens.[4,9,10,13] For these reasons, in this investigation, it is the benchmark for comparison of the heat grafted PBT nonwovens regarding graft morphology, chemical functionalization, protein binding capacity, thermodynamics of protein adsorption, and binding kinetics.

2. EXPERIMENTAL SECTION

2.1. Materials and Reagents. Macopharma (Tourcoing, France) provided commercially available meltblown PBT nonwovens with thickness, average fiber diameter, basis weight (grams per square meter (GSM), mean pore diameter, and porosity of 200 μm, 3.0 μm, 56 g/m^2, 15 μm, and 79%, respectively. Glycidyl methacrylate (GMA) was purchased from Pflatz & Bauer (Waterbury, CT, USA). Inhibitors in GMA were removed through a prepacked inhibitor removal column to remove hydroquinone and monomethyl ether hydroquinone (Sigma-Aldrich, St. Louis, MO, USA). Benzoyl peroxide (70% wt) (Bz_2O_2), *N*,*N*-dimethylformamide (DMF), sodium hydroxide, 1-butanol, isopropyl alcohol, Tris base, hydrochloric acid, sodium chloride, and sodium acetate trihydrate were purchased from Fisher Scientific (Fairlawn, NJ, USA). Tetrahydrofuran (THF), methanol, sulfuric acid, and acetic acid were purchased from BDH (West Chester, PA, USA). Diethylamine (DEA) was purchased from Alfa Aesar (Ward Hill, MA, USA). Sodium sulfite (Na_2SO_3) was purchased from Acros Organics (Fairlawn, NJ, USA). Solid phase extraction tubes were purchased from Supelco (Bellefonte, PA, USA). Benzophenone (BP), albumin from bovine serum (BSA), egg white lysozyme, and adenosine 5′-triphosphate (ATP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Human immunoglobulin G (hIgG) was purchased from Equitek-Bio, Inc. (Kerrville, TX, USA).

2.2. PolyGMA Grafting on PBT Nonwovens. Nonwoven PBT fabric was cut into 75 × 50 mm size samples and weighed prior to the grafting process; sample weight was approximately 200 mg. PolyGMA grafting was performed with the two different techniques: (i) heat induced polyGMA grafting on PBT nonwovens and (ii) UV induced polyGMA grafting on PBT nonwovens.

i) Heat induced grafting. The sample was immersed in 20 mL of a thermal initiator solution containing 75 mM Bz_2O_2 in DMF at room temperature for 1 h to allow Bz_2O_2 to adsorb to the surface of PBT. It was then removed from the initiator solution and laid across a paper towel to wick excess solution from the pores of the nonwoven. The sample was then placed into 20 mL of the thermal grafting solution at a specific polymerization temperature and allowed to graft for a given amount of time. The effects of GMA monomer concentration, polymerization temperature, and time were investigated. In particular, GMA concentrations of 5, 10, 20, 30, and 40% (v/v) in DMF were tested, while the polymerization temperatures were kept constant at 70, 80, or 90 °C using a water bath (Isotemp 115, Fisher Scientific, Fairlawn, NJ, USA) for times ranging from 30 min to 6 h. After polyGMA grafting, the sample was placed in a flask containing 25 mL of THF and sonicated for 30 min

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

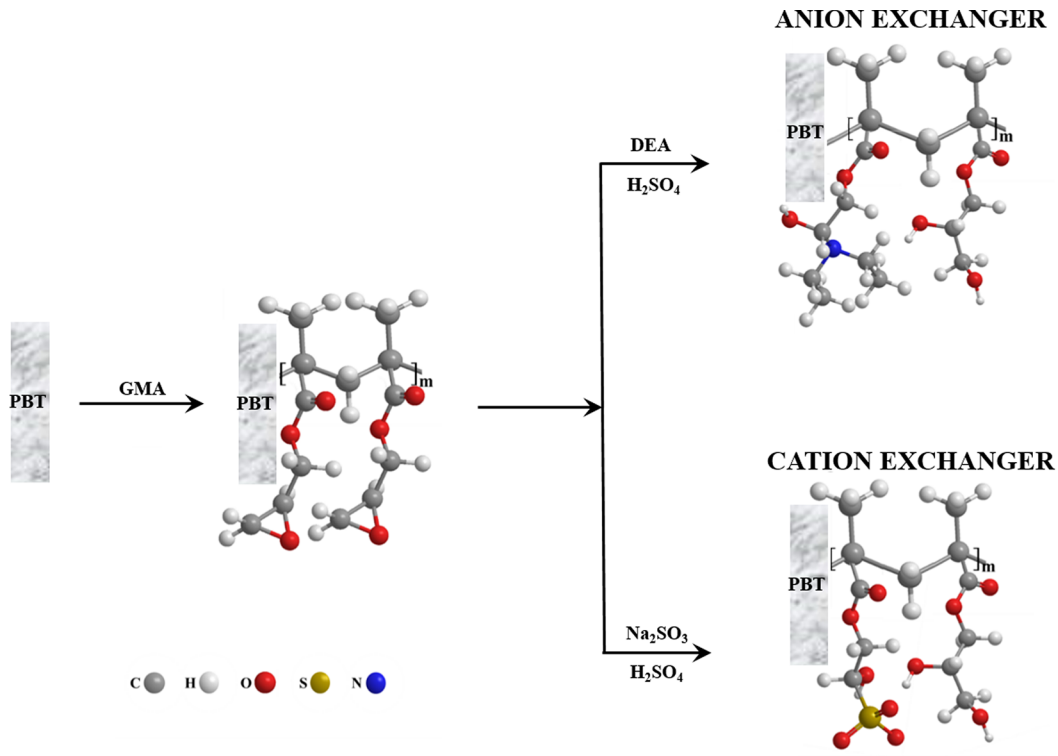


Figure 1. Process of polyGMA grafting and functionalization of PBT nonwovens.

to remove any unreacted grafting solution or untethered polyGMA (Bransonic 3510R-MT, Branson Ultrasonics Corporation, Danbury, CT, USA). THF was replaced once after 15 min of sonication.

ii) UV induced grafting. The GMA grafting solution consisted of 20% v/v GMA monomer in 1-butanol as the solvent. The photoinitiator benzophenone (BP) was added into the grafting solution with a BP:GMA ratio of 1:20 (mol:mol). The nonwoven PBT sample was placed onto a borosilicate glass microscope slide and wet by 1 mL of grafting solution that was evenly distributed onto the membrane using a syringe, and a second borosilicate glass slide was placed on top of the nonwoven. The nonwoven sample was placed 3 mm below the light source, consisting of a UV lamp (model EN-180L, Spectronics Corporation, Westbury, NY, USA) with a wavelength of 365 nm, an intensity of 5 mW/cm^2. Samples were irradiated at various exposure times to achieve different % weight gain of grafted polyGMA. After grafting, the sample was placed in a flask containing 25 mL of THF, and the flask was sonicated with the ultrasonic bath for 30 min to remove any unreacted grafting solution or untethered polyGMA.

In both cases, following the THF wash the samples were placed in a flask containing 25 mL of methanol that was sonicated with the ultrasonic bath for 10 min to remove THF from the nonwovens. Finally, the samples were removed from the flask and allowed to dry in air overnight. The final weight of the nonwovens was measured, and the degree of polyGMA grafting was determined using eq 1 in terms of % weight gain

$$\text{Degree of grafting (\% weight gain)} = \frac{W_f - W_i}{W_i} \times 100\% \quad (1)$$

where w_i is the initial nonwoven weight prior to grafting, and w_f is the final nonwoven weight after grafting.

2.3. Functionalization of PolyGMA Grafted PBT Nonwovens. PolyGMA grafted PBT nonwovens using both heat and UV induced methods were functionalized to produce anion and cation exchangers. In these cases, samples of approximately 100 mg (35 × 50 mm) were modified.

2.3.1. Anion Exchanger. The sample was immersed in 20 mL of a 50% v/v aqueous diethyl amine (DEA) solution, thus creating a tertiary amine on the polyGMA brushes. The reaction was kept at a constant 30 °C with agitation at 100 rpm using an incubation shaker contained in an incubation hood (Certomat RM and Certomat HK, B. Braun Biotech International, Melsungen, Germany) for 16 h.

2.3.2. Cation Exchanger. The sample was immersed in 20 mL of sodium sulfite solution containing sodium sulfite, isopropyl alcohol (IPA), and water (Na_2SO_3:IPA:water = 10:15:75% wt). The reaction was incubated at 80 °C for 7 h in the water bath as to create cation exchangers by attaching sulfonic acid groups to the polyGMA brushes.

2.3.3. Hydrolysis. After functionalization in DEA or sodium sulfite solution, the sample was placed in a flask containing 20 mL of DI water, and the flask was placed in the ultrasonic bath for 5 min to remove excess DEA or sodium sulfite. Following sonication, the DI water was replaced with fresh DI water, and the process was repeated until a neutral pH of 7.0 was reached and verified with pH testing paper. Ten washes ensured that all DEA or sodium sulfite had been removed from the nonwoven. Any unreacted epoxy groups were hydrolyzed by immersion of the sample in 20 mL of 100 mM sulfuric acid overnight. The sample was then placed in a flask containing 20 mL of DI water and sonicated for 5 min to remove excess sulfuric acid. Finally, the sample was washed by DI water 10 times and dried in air overnight.

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

A graphic schematization of the process of polyGMA grafting and functionalization from PBT nonwovens is shown in Figure 1.

2.4. Material Characterization. The morphology of the nonwoven membrane was observed using a variable pressure scanning electron microscope (VPSEM, Hitachi S-3200N, Hitachi High Technologies America, Inc., Schaumburg, IL, USA) at magnification of 4,000× and 10,000×. Grafted and functionalized nonwoven samples were fixed onto a sample holder by carbon tape and sputtered with Pd/Au alloy in argon gas for 5 min. Images were captured using the microscope with an accelerating voltage of 5 kV at a working distance of 15 mm.

The surface chemical composition of PBT nonwoven membranes after polyGMA grafting was characterized by ATR-FTIR using a Nicolet iS10 FT-IR spectrometer with a diamond HATR crystal (Thermo Fisher Scientific, Waltham, MA, USA). Each spectrum was collected with 64 scans at a resolution of 4 cm^{-1}. The beam radius was 5 mm with a range of inverse wavelengths of 4000−675 cm^{-1}, and the analysis depth of penetration was ∼0.67 μm at 2000 cm^{-1}.

The amount of tertiary ammine on nonwovens was analyzed on a CHN Elemental Analyzer (Model 2400 CHN Elemental Analyzer series II, PerkinElmer Inc., Waltham, MA, USA). The measure of total nitrogen content provided a direct quantification of DEA ligand density.

The sulfur was determined by an Ion-coupled Plasma Spectrometer (ICP, 8000 ICP-OES, PerkinElmer Inc., Waltham, MA, USA). The measure of total sulfur content provided a direct quantification of Na_2SO_3 ligand density.

2.5. Static Binding Capacity. Experiments to determine the kinetics of protein adsorption and equilibrium binding capacity were performed in batch on the functionalized PBT nonwovens. Moreover, various target proteins were used to study the effect of molecule size on binding. The protein properties, the type of exchanger, and equilibration buffer used are reported in Table 1.

Table 1. Proteins, Membranes, and Buffers Used in Batch Experiments[21,22]

protein	molecular weight	isoelectric point	exchanger	equilibration buffer
BSA	66.5 kDa	4.7	anion (DEA)	20 mM Tris HCl pH 7.0
ATP	507 Da	6.5 (pK_a)	anion (DEA)	20 mM Tris HCl pH 7.0
hIgG	150 kDa	7−9	cation (Na_2SO_3)	20 mM acetate pH 5.5
lysozyme	14.3 kDa	11.35	cation (Na_2SO_3)	20 mM acetate pH 5.5

Approximately 20 mg (25 × 15 mm) of nonwoven sample was placed in a 3 mL solid phase extraction (SPE) tube and washed with 3 mL of equilibration buffer 5 times. The sample was equilibrated for at least 2 h in fresh buffer on a rotator (Tissue culture rotator, Glas-col, Terre Haute, IN, USA) prior to protein binding. Once equilibrated, 3 mL of 0.03−10 mg/mL protein in binding buffer was added to each sample and allowed to bind from 5 min to 24 h at room temperature. The sample was washed with 3 mL of binding buffer 5 times to remove all the unbound protein, until a negligible amount of protein in the fifth and final wash was verified. Bound protein was eluted using 1 M NaCl in 3 mL of equilibration buffer. Unbound protein and elution fractions were collected and analyzed. Protein concentration was determined using UV−vis spectroscopy (Agilent Technologies, G1103A, Santa Clara, CA, USA) at 280 for BSA, hIgG, and lysozyme and at 256 nm for ATP.

The static equilibrium binding capacity, q (mass of protein per mass of membrane), values were determined using eq 2:

$$q\left(\frac{\text{mg}}{\text{g}}\right) = \frac{c_P\left(\frac{\text{mg}}{\text{mL}}\right) \times V_{elu}\,(\text{mL})}{\text{mass of membrane (g)}} \quad (2)$$

where c_P is the protein concentration and V_{elu} is the volume of the eluted fraction.

The obtained data were fit to the Langmuir adsorption model, and the parameters were calculated by least-squares regression using the Solver tool implemented in the Excel software package in Microsoft Office Professional 2013

$$q = \frac{q_m C}{K_d + C} \quad (3)$$

where q is the amount of protein bound to the nonwoven sample (mg/g), q_m is the maximum binding capacity (mg/g), c is the free protein concentration (mg/mL), and K_d is the dissociation constant (mg/mL).

3. RESULTS AND DISCUSSION

3.1. Heat Induced Grafting of PolyGMA on PBT Nonwovens. To optimize the heat induced grafting of poly(GMA) onto commercial PBT using the thermal initiator Bz_2O_2, monomer concentrations ranging from 5% to 40% (v/v GMA in DMF) were investigated with polymerization temperatures between 70 and 90 °C. The results of % weight gain over various polymerization times for the conditions tested are presented in Figure 2.

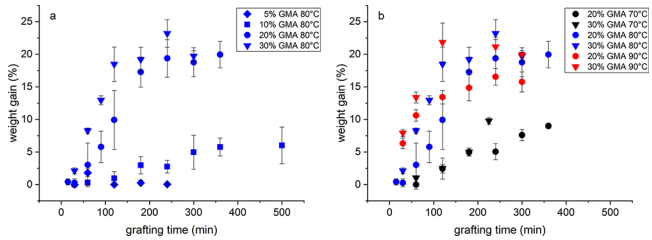


Figure 2. Heat induced grafting evaluated by % weight gain for a) different GMA concentrations (% v/v) at 80 °C and b) for 20 and 30% GMA at different polymerization temperatures over a range of polymerization times.

From Figure 2a, it is apparent that increasing the monomer concentration results in an increase in the rate of grafting and the overall extent of grafting at given polymerization time. At a GMA monomer concentration of 5% (v/v), effectively no grafting was observed even at grafting times of up to 4 h. At a GMA monomer concentration of 10% (v/v), poly(GMA) grafting was observed; however, the overall extent of grafting even after 6 h of polymerization was very low only reaching a 5% weight gain of poly(GMA). Increasing the monomer concentration to 30% (v/v) resulted in grafting as high as nearly 20% weight gain after 2 h. At a GMA monomer concentration of 40% (v/v), a rapid uncontrolled bulk polymerization occurred resulting in the complete solidification of the grafting solution that was not observed for monomer concentrations at, or below, 30% (v/v). The same

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

phenomena has been reported in other investigations of vinyl polymer grafting onto polymeric substrates using heat induced polymerization where, past a threshold monomer concentration, polymerization in solution outcompetes polymerization onto the polymeric surface.[15,17,18] For these reasons, GMA monomer concentrations were kept between 20% and 30% (v/v) for this polymerization scheme, which is the preferred range of monomer concentration to achieve efficient grafting. At a given temperature, a 30% (v/v) GMA monomer concentration demonstrated a faster rate of polymerization compared to a 20% monomer concentration, for polymerization times less than 2 h, as can be seen in Figure 2b. The extent of grafting over time for polymerization with 20% and 30% GMA (v/v) at polymerization times up to 2 h demonstrated a linear relationship, indicating a first order rate of grafting with respect to monomer concentration.[14,18] The kinetic rate constants, calculated at times up to 2 h, confirm the above considerations, as can be observed from their values, reported in Table 2, together with the values of R^2 for the linear interpolation.

Table 2. Kinetic Rate of Polymerization at Different GMA Concentrations and Temperature

GMA monomer (%v/v)	polymerization T (°C)	k ($\times 10^4$ min^{-1})	R^2
20	70	2.33	0.95
30	70	2.44	0.95
10	80	1.45	0.94
20	80	7.89	0.94
30	80	13.6	0.98
20	90	12.2	0.80
30	90	17.7	0.95

For polymerization times longer than 2 h, a plateau in the extent of grafting is observed for 20% and 30% (v/v) GMA, at polymerization temperatures at or above 80 °C as shown in Figure 2a. This is a common phenomenon observed for grafting of vinyl polymers onto polyester substrates using the thermal initiator Bz_2O_2.[15,17,18] There are a number of potential reasons for this, including a depletion of available initiator, a reduction in the available active sites on the PBT fiber, the development of a diffusion barrier due to an increased viscosity of polyGMA in solution, or an increased termination rate of the polyGMA grafting compared to initiation.

From the results reported in Figure 2, it is apparent that temperature has a significant influence on the polymerization rate, with higher temperatures resulting in higher % weight gains at shorter polymerization times. The polymerization temperature affects the decomposition rate of Bz_2O_2 into its radical form that is capable of initiating polymerization at the PBT surface. Increasing the temperature results in an increased rate of decomposition of Bz_2O_2; indeed, the rates of decomposition of Bz_2O_2 in benzene at 60, 78, and 100 °C are 2×10^{-6}, 2.3×10^{-5}, and 5×10^{-4} s^{-1}, respectively.[23] Therefore, every 20 °C increase in the polymerization temperature results in an order of magnitude increase in the rate of radical formation and therefore in a faster initiation. It is observed in Figure 2b that at 70 °C grafting proceeds very slowly with 8% weight gain being the highest achieved after 4 h of polymerization for 30% (v/v) GMA. On the other hand, at 80 and 90 °C polymerization proceeds substantially faster and is capable of achieving 20% weight gains of polyGMA coverage in approximately 3 h at 80 °C and 2 h at 90 °C. It should be noted that 80 °C is the recommended polymerization temperature for thermal initiation using Bz_2O_2.[14] Heat grafting conditions of 30% (v/v) GMA at 80 °C gave the most consistent and reproducible polyGMA grafting onto the PBT nonwovens.

Heat induced grafting of polyGMA onto nonwoven PBT resulted in complete, conformal, highly uniform polyGMA coverage around the exterior of the PBT fibers. This can be seen in the SEM images presented in Figure 3. Figure 3B–F

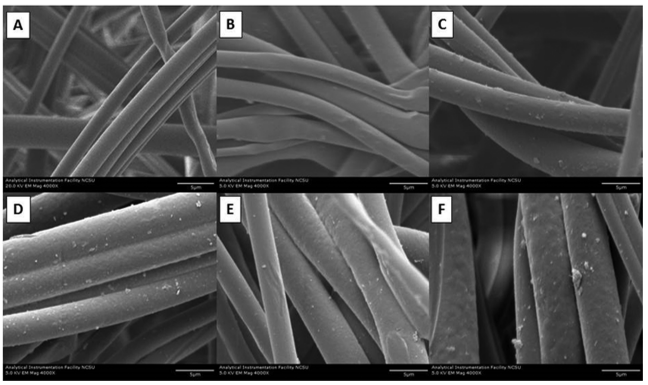


Figure 3. SEM images (4,000×) of heat induced grafting onto PBT nonwovens with various % weight gain: A) blank PBT nonwoven, B) 1.5% weight gain, C) 7.5% weight gain, D) 11.5% weight gain, E) 16% weight gain, and F) 19% weight gain.

displays a visible surface roughness that is attributed to a polyGMA grafted layer that is not present on the blank PBT nonwoven shown in Figure 3A. Increased polyGMA graft coverage results in an increase in surface roughness of the fibers as can be seen comparing PBT nonwovens grafted at low weight gains (1.5% weight gain, Figure 3B) to PBT nonwovens grafted at high weight gains (19% weight gain, Figure 3F). It is also important to note that this method of heat grafting is capable of grafting to the entirety of the surface of PBT fibers without any pore blockage resulting in highly uniform, conformal, discretely grafted fibers.

In addition, the presence of viable epoxy pendant groups that are capable of further functionalization was demonstrated with ATR-FTIR analysis, and the results are reported in Figure S1.

3.2. Functionalization of PolyGMA Grafted PBT Nonwovens. The activations of epoxy groups on the polyGMA grafted PBT nonwovens were achieved by reaction with DEA and Na_2SO_3 to form tertiary amine and sulfonate functional groups on PBT nonwovens to obtain anion exchangers and cation exchangers, respectively. Elemental analyses on N and S were performed on the heat grafted PBT nonwovens functionalized as anion and cation exchangers to determine the ligand density of membranes grafted under various conditions. The results of the ligand density as a function of % weight gain for heat and UV induced polyGMA grafted PBT nonwovens are presented in Figure 4.

It is apparent that the ligand density increases with the extent of polyGMA grafting for all of the grafted membranes. The linear nature of the data indicates that ligand density is directly proportional with the amount of polyGMA coverage. In Figure 4a, a comparison of the ligand density for nonwovens grafted at monomer concentrations of 20% and 30% (v/v) GMA for polymerization temperatures between 70 and 90 °C

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

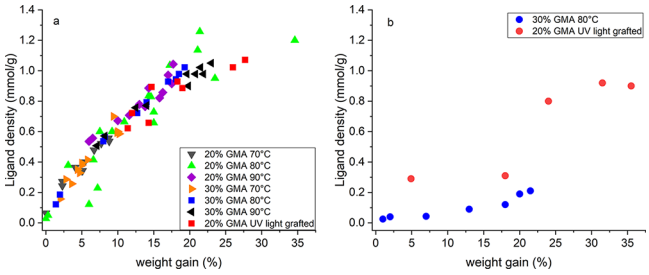


Figure 4. Ligand density of a) DEA and b) Na_2SO_3 functionalized polyGMA grafted nonwovens.

demonstrates that there is no observable difference in DEA ligand density for any of these conditions over the entire range of polyGMA graft coverage, and there is no difference in ligand density between the heat grafted nonwovens and the UV grafted nonwovens over the entire range of polyGMA graft coverage. This is a strong indication that for all of the conditions evaluated for the heat grafted and UV grafted nonwovens there are the same number of available epoxy groups that can be readily functionalized to become anion exchange binding sites. However, as shown in Figure 4b, the Na_2SO_3 ligand density is lower than DEA ligand density at a similar % weight gain, especially for heat induced grafting.

3.3. Equilibrium Protein Binding Capacity. A study with BSA binding on anion exchangers was performed as to optimize the grafting conditions with respect to protein binding. Although from Figure 4a it was demonstrated that DEA ligand density is almost solely dependent on the extent of polyGMA grafting, the results of BSA binding gave different indications. Indeed, BSA binding to PBT heat grafted at various conditions apparently indicates that equilibrium protein binding capacity increases with weight gain and with an initial GMA monomer concentration in the grafting solution; however, the results obtained and shown in Figure S2 are within experimental errors. This is an indication that the structure of the polyGMA and consequently the accessibility of protein binding sites are largely dependent on the grafting conditions even though similar % weight gains may be achieved.[14,24−26] This finding is consistent with previous investigations that determined equilibrium protein binding on ion exchange functionalized polyGMA grafted PBT nonwovens using UV light was dependent on the extent of grafting.[10,13] Heat grafted membranes with a monomer concentration of 30% (v/v) GMA at a temperature of 80 °C achieved the highest average overall protein binding capacity (Figure S2) and were primarily used for all subsequent investigations unless otherwise stated.

BSA and hIgG were chosen as model proteins for anion and cation exchangers, respectively, to compare the equilibrium protein binding capacities for ion exchange functionalized PBT nonwovens grafted using heat and UV induced grafting approaches. Figure 5 shows how the equilibrium binding capacity is directly proportional to the extent of grafting for both heat and UV grafted PBT nonwovens. However, the observed equilibrium protein binding capacities are on average 4.8 and 6.7 times higher for the UV grafted nonwovens functionalized as anion and cation exchangers, respectively, compared to their heat grafted counterparts. This observation reinforces that the structure of polyGMA grafts is dependent on the grafting conditions and methodology.

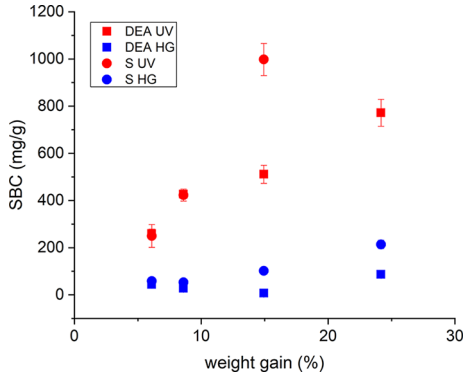


Figure 5. Equilibrium protein binding capacity of PBT nonwovens grafted by heat (30% (v/v) GMA, 80 °C) and UV light (20% (v/v) GMA) functionalized as anion (DEA) and cation (S) exchangers for capture of BSA and hIgG, respectively.

It is obvious from Figure 5 that UV grafting creates a polyGMA structure that can accommodate more protein binding than the polyGMA structure obtained using a heat induced grafting approach.

A visual comparison of PBT fiber cross sections grafted with UV light and grafted thermally are presented in Figure 6A and

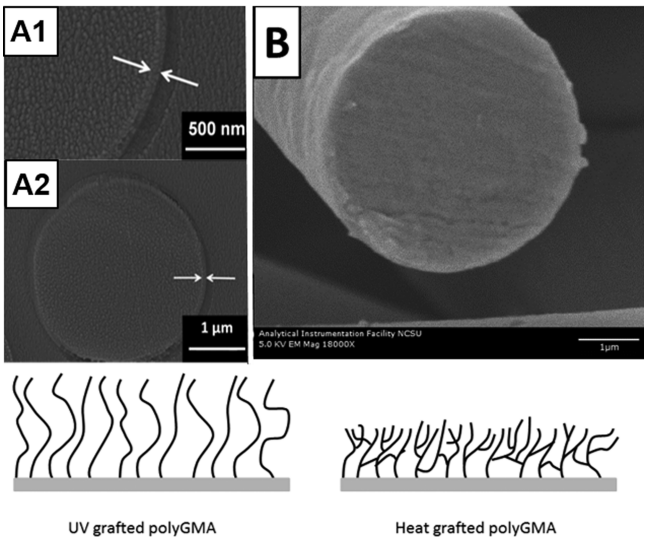


Figure 6. SEM images for PBT fiber cross sections grafted with A) UV (A1 and A2 are images with different magnifications of the same fiber)[13] and B) heat induced grafting. Schematic representation of polyGMA grafted layers resulting from UV and heat induced grafting.

B, respectively. In Figure 6A, there is a visible distinction between the polyGMA grafted layer and the PBT fiber for the UV grafted nonwoven. This distinction is not present in Figure 6B for the heat grafted PBT nonwoven. It is possible that the density of heat grafted polyGMA layer is close to that of PBT and therefore unable to be resolved using SEM microscopy. Vinyl grafting onto polymeric supports by radiation based free radical polymerization is known to create vinyl polymer brushes that are anchored to the polymeric surface.[4−10] These polymeric brushes tend to be tentacle in nature being highly linear and flexible.[6,14,27] This results in a three-dimensional binding environment where protein can pack efficiently throughout the entire volume of the grafted layer due to the rearrangement capabilities of the polymer brushes.[28] On the

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

other hand, vinyl grafting by heat induced free radical polymerization is far less controlled. Thermal based polymerizations result in higher rates of chain transfer compared to polymerizations by UV light.[14,24−26] High rates of chain transfer result in highly branched polymer chains, as well as highly cross-linked polymer networks, both of which would have significant influences on the density of the grafted polyGMA layer. A visual schematic representation of the proposed differences in the structures of the polyGMA matrix that result from UV light induced grafting and heat induced grafting is presented in Figure 6.

This occurrence would limit the grafted layers ability to bind protein in two ways: first, a grafted polyGMA layer with a higher observed density would have a smaller volume to accommodate proteins for a specific % weight gain; second, a highly cross-linked polymer network would be substantially more rigid in nature resulting in protein diffusion issues into the depth of the grafted layer due to size exclusion and an inability of grafted polymer rearrangement to accommodate more protein. Indeed, chain transfer rates are a function of temperature; this is likely why there was an observed decrease in protein binding for increasing polymerization temperatures as shown in the Supporting Information. PolyGMA grafts synthesized at 90 °C are more likely to be highly branched and cross-linked than polyGMA grafts synthesized at 70 °C.

3.4. Effect of Molecule Size on Binding Capacity. Target molecules with varying molecular weights were bound to the heat and UV grafted ion exchange nonwovens to investigate and compare the binding environment between the two grafting methods. ATP, having the lowest molecular weight of 0.5 kDa, was bound to anion exchange functionalized nonwovens. Lysozyme, having the second lowest molecular weight of 14.3 kDa, was bound to cation exchange functionalized nonwovens. BSA having the second largest molecular weight of 66.5 kDa was bound to anion exchange functionalized nonwovens, and hIgG having the largest molecular weight of 150 kDa was bound to cation exchange functionalized nonwovens. The results for equilibrium binding (mg/g) of these molecules for various extents of polyGMA grafting are presented in Figure 7a for both heat grafted PBT nonwovens and UV grafted nonwovens. From Figure 7a it is evident that the heat grafted nonwovens are capable of binding BSA and lysozyme with similar equilibrium capacities in terms of mass bound. The heat grafted nonwovens bound hIgG and ATP with similar capacities; both molecules bound more than BSA and lysozyme. It is interesting that ATP is 3 orders of magnitude smaller than hIgG but bound almost the same amount on a per mass basis. BSA and lysozyme have molecular weights in between ATP and hIgG but bound less on a per mass basis. On the other hand, for the UV grafted nonwovens an increasing molecular weight results in an increase in the binding capacity on a per mass basis.

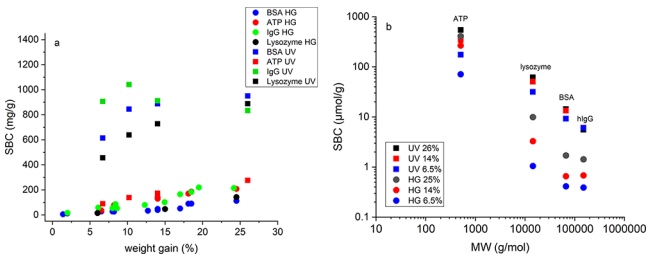


Figure 7. Equilibrium binding capacity of various target molecules onto heat and UV grafted ion exchange nonwovens: a) static binding capacity (SBC) in terms of mass adsorbed per mass of membrane as a function of % weight gain of polyGMA and b) static binding capacity on a molar basis as a function of the targets molecular weight for nonwovens grafted at 6.5%, 14%, and 25% weight gain.

To determine if the equilibrium binding capacity of polyGMA grafted nonwovens is limited by size exclusion and the volume of the polyGMA layer available for binding or is limited by the number of binding sites available for adsorption, the binding capacities of Figure 7a are reported on a molar basis in Figure 7b. Due to the order of magnitude differences in the targets' molecular weight, the data are presented on a log−log scale in Figure 7b. As it can be observed from Figure 7b, the UV grafted and heat grafted nonwoven ion exchangers show a strong dependence on the size of the target and the number of moles bound. hIgG is the largest target with the lowest binding capacity, and ATP is the smallest target with the highest capacity, the exception being the two largest targets tested, BSA and hIgG, which bound nearly the same number of molecules on the heat grafted membranes. The amount of protein bound (μmol/g) varies drastically between the UV grafted and the heat grafted nonwovens for the larger proteins tested, whereas UV and heat grafted nonwovens bound similar amounts of ATP for specific % weight gains. This is an indication that ATP is small enough that it can access the entire polyGMA binding layer for both materials and is therefore only dependent on the % weight gain. There might be size exclusion occurring in the heat grafted nonwovens that is creating the large discrepancy in protein binding when compared to the UV grafted nonwovens.

However, as the molecular weight of the target molecule increases, the number of molecules bound (μmol/g) diverges between the two grafting methods as can be seen in Figure 7b. The resulting divergence in binding capacity for larger targets indicates that the heat grafted nonwovens have either less available binding volume or that the polymer network is more size exclusive than the UV grafted nonwovens. This result further validates that grafting using a heat induced polymerization is likely to give a polyGMA network that is highly branched and cross-linked compared to a UV grafted polyGMA network, which is likely to have less volume to accommodate biomolecules and proteins due to its increased density. Additionally, a high degree of cross-linking is likely to make the matrix more rigid preventing polymer brush rearrangement to pack proteins efficiently and would also create pores that may be inaccessible to larger molecules.

3.5. Kinetics of Protein Adsorption. Ion exchange functionalized polyGMA UV-grafted PBT nonwovens exhibit very slow rates of protein adsorption that is a function of the polyGMA layer thickness.[13] Timed binding experiments were performed to investigate if the rates of protein adsorption are different for ion exchange functionalized nonwovens using a heat grafting method and a UV grafting method: anion exchange materials were exposed to BSA, while cation exchange materials were exposed to hIgG at varying contact times and evaluated for the amount of protein bound, and the results are presented in Figure 8.

It is shown that the UV grafted polyGMA anion exchange nonwovens exhibit extremely slow rates of BSA adsorption to reach equilibrium. The UV grafted polyGMA nonwoven grafted to 6% weight gain was able to reach equilibrium after about 4 h of protein contact time, and at 20% weight gain the

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

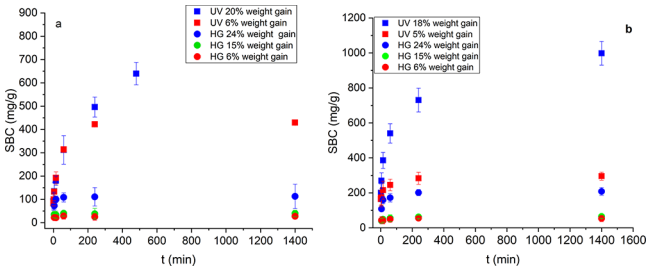


Figure 8. Static binding capacity at various contact times for ion exchange functionalized grafted nonwovens: a) BSA binding on anion exchange UV grafted at 20% and 6% weight gain, as well as heat grafted at 24%, 15%, and 6% weight gain and b) hIgG binding on cation exchange UV grafted at 18% and 5% weight gain, as well as heat grafted at 24%, 15%, and 6% weight gain.

sample required 8 h to reach equilibrium binding, Figure 8a. The heat grafted nonwovens functionalized as anion exchangers exhibited much faster binding kinetics compared to the UV grafted anion exchangers. At the lower degrees of polyGMA grafting, 6% and 15% weight gain, equilibrium binding was achieved after 5 min of protein exposure for the anion exchange functionalized heat grafted nonwovens. At a high degree of polyGMA grafting, 24% weight gain, equilibrium BSA binding is reached after 1 h, with over 60% of the equilibrium binding capacity reached after 5 min of protein exposure.

The kinetics of hIgG adsorption by cation exchange to polyGMA grafted nonwovens grafted using the UV method and the heat grafting method were also investigated, as shown in Figure 8b. Similar to the anion exchange functionalized nonwovens, the cation exchange functionalized nonwovens grafted by the UV method exhibited slower rates of hIgG adsorption compared to those grafted by the heat method. At 18% weight gain it takes nearly a full day to reach equilibrium for the cation exchange UV grafted polyGMA nonwovens. The UV grafted PBT nonwovens with a lower degree of coverage, 5% weight gain, reached hIgG binding equilibrium after 4 h with over 80% of the equilibrium capacity reached after 1 h, which is substantially faster than the UV grafted 18% weight gain nonwovens. The heat grafted polyGMA nonwovens functionalized as cation exchangers demonstrated faster rates of hIgG capture compared to the UV grafted nonwovens. The heat grafted nonwovens with 6% and 15% weight gain reached equilibrium after 5 min for hIgG binding. At a 24% weight gain, the heat grafted nonwovens reached equilibrium after 1 h with over 60% of equilibrium binding reached after 5 min of protein exposure.

The kinetic rate constants of Figure 8 were calculated by fitting the experimental data at short times, and the obtained values confirm that the heat grafting of polyGMA onto nonwoven PBT results in overall faster rates of protein adsorption compared to UV grafting of polyGMA onto nonwoven PBT when functionalized as ion exchangers as Figure S3 demonstrates. However, equilibrium binding capacities are significantly lower for the ion exchange functionalized heat grafted nonwovens compared to the ion exchange functionalized UV grafted nonwovens. The structural differences of the polyGMA layer created by heat grafting and UV grafting are the cause of the observed differences in the rates of protein adsorption. The heat grafted polyGMA layer is denser and more rigid and contains inaccessible pores in the matrix compared to the UV grafted polyGMA layer, so there would be less protein diffusion and rearrangement to accommodate proteins than would have to occur in a UV grafted layer to reach equilibrium. However, although a smaller polyGMA volume available for binding would result in a lower overall binding capacity at a specific % weight gain, a protein would have a shorter distance to diffuse through that would result in shorter times to reach equilibrium binding.[29]

3.6. Protein Adsorption Isotherms. Adsorption isotherms for BSA binding on anion exchange nonwovens as well as hIgG binding on cation exchange nonwovens were performed for both grafting methods. All of the protein adsorption for both grafting methods and both ion exchange functionalities exhibit Langmuir behavior. Although polymer grafted nonwovens functionalized as ion exchangers exhibit multilayer binding[6,13,27,30,31] with respect to the surface they are grafted, the polymer layer itself behaves as a single site adsorbent where the number of binding sites is determined by the charge density.[30−32]

SBC experiments were performed with one membrane piece in a small vial; therefore, experimental errors due to membrane nonuniformity can be significant since there is a variation in the porosity of different small samples of nonwoven membranes. The apparent maximum binding capacity (q_m) and the dissociation constant (K_d) for each sample were calculated using eq 3. These values are presented in Table 3 for

Table 3. Apparent Dissociation Constant (K_d) and Maximum Binding Capacity (q_m) Obtained Using a Direct Fit of the Langmuir Model to the Isotherm Data for the Heat Grafted Nonwovens Functionalized as Ion Exchangers

degree polyGMA grafting (% weight gain)	ion exchange functionality: protein bound	K_d ($\times 10^{-6}$ M)	q_m (mg/g)	R^2
15	anion exchange: BSA	1.4	30	0.88
25	anion exchange: BSA	7.5	85	0.97
8	cation exchange: hIgG	2.8	40	0.92
15	cation exchange: hIgG	2.7	71	0.96
25	cation exchange: hIgG	9.7	202	0.98

Table 4. Apparent Dissociation Constant (K_d) and Maximum Binding Capacity (q_m) Obtained Using a Direct Fit of the Langmuir Model to the Isotherm Data for the UV Grafted Nonwovens Functionalized as Ion Exchangers

degree polyGMA grafting (% weight gain)	ion exchange functionality: protein bound	K_d ($\times 10^{-6}$ M)	q_m (mg/g)	R^2
11	anion exchange: BSA	2.6	467	0.96
14	anion exchange: BSA	3.0	771	0.96
18	anion exchange: BSA	6.7	833	0.93
11	cation exchange: hIgG	5.0	345	0.89
14	cation exchange: hIgG	6.8	339	0.87
19	cation exchange: hIgG	11.4	692	0.94

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

the samples grafted using the heat grafting method and Table 4 for the samples grafted using the UV grafting method. The calculated dissociation constants (K_d) are between 1.4 and 11.4 × 10^{-6} M for all of the samples tested including both methods of grafting and both ion exchange functionalities used for capture of BSA and hIgG. The values are in agreement with reported values for protein binding on ion exchange functionalized polymer brushes and ion exchange functionalized polymer networks that have dissociation constants on the order of 10^{-6} M.[30−32]

These types of binding environments exhibit strong protein-matrix interactions as can be seen from their low K_d values. However, the addition of salt as an eluent effectively disrupts protein binding with the ion exchange matrix and causes ion exchange polymer brushes to collapse forcing displacement of protein, resulting in 100% recovery of bound protein.[6]

A typical result is shown in Figure 9, where the adsorption isotherms for UV grafted membranes at 14% weight gain are compared to the adsorption isotherms for heat grafted membranes at 15% weight gain, while all other data are reported in Figure S4.

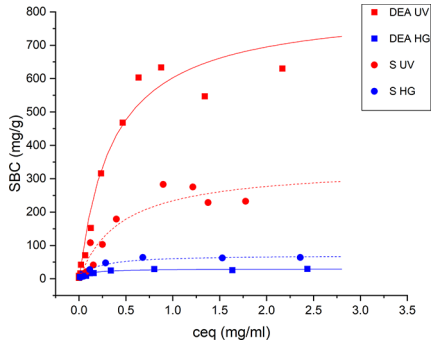


Figure 9. BSA and hIgG adsorption isotherms for UV grafted membranes at 14% weight gain are compared to the adsorption isotherms for heat grafted membranes at 15% weight gain. BSA isotherms are for binding on anion exchange (DEA) membranes, while hIgG isotherms are for binding on cation exchange (S) membranes.

4. CONCLUSIONS

PBT nonwovens were successfully grafted with polyGMA using a heat induced grafting method with the thermal initiator Bz_2O_2. The heat induced grafting results in a complete, uniform, and conformal polyGMA layer around discrete PBT fibers. Grafted polyGMA nonwovens using this method were readily functionalized with DEA to become anion exchangers or with sulfonic acid to become cation exchangers. Equilibrium binding results for BSA bound to anion exchange heat grafted nonwovens indicated that the initial thermal grafting conditions had an impact on the overall binding capacity of the material. An increasing initial monomer concentration for grafting results in a polyGMA grafted layer that binds more protein, and increasing polymerization temperature results in a polyGMA layer that binds less protein. Equilibrium binding capacities as high as 200 mg/g were observed for the heat grafted nonwovens grafted to 24% weight gain functionalized as a cation exchanger for binding of hIgG. The equilibrium binding capacities of the ion exchange heat grafted nonwovens were significantly lower than similar systems grafted using a UV induced radical polymerization for grafting. UV grafted polyGMA nonwovens functionalized as ion exchangers bound between 5 and 7 times more protein than the heat grafted polyGMA nonwovens, at a specific weight gain. However, kinetics of protein adsorption indicated that the heat grafted nonwovens were capable of achieving equilibrium binding on the order of minutes compared to the UV grafted nonwovens that required several hours to reach equilibrium. Ion exchange binding of biomolecules and proteins of varying molecular weights further reinforces the structural differences between the two grafting methods. For both grafting methods increasing molecular weight of the target protein results in a decrease in the number of molecules bound at a given degree of polyGMA coverage. However, this observation is more significant in the heat grafted polyGMA nonwoven samples indicating that the polymer matrix either has less available binding volume, a more rigid structure preventing efficient packing of proteins, or small pore structures that are inaccessible by larger proteins. These structural differences may be attributed to an increased degree of polymer branching and cross-linking that are not observed in the UV induced grafting method. Regardless of the proposed structural differences between the two grafting methods, they exhibited similar strengths of binding with dissociation constants calculated to be on the order of 10^{-6} M which is consistent for protein binding on ion exchange polymer networks. The large intrinsic binding capacities for protein binding for both UV- and heat-induced grafted nonwoven fabrics offer great potential for use as single-use product capture or polishing membranes for the production of biologics. Future work in this area will highlight the flow properties, dynamic binding capacities, and selectivity of these membranes in bioprocessing.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.9b04936.

ATR-FTIR spectra, kinetic rate constants, protein binding data, and adsorption isotherms (Figures S1−S4) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Ruben G. Carbonell − *Department of Chemical and Biomolecular Engineering and Golden LEAF Biomanufacturing Training and Education Center (BTEC), North Carolina State University, Raleigh, North Carolina 27695-7905, United States; National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL), Raleigh, North Carolina 27695, United States*; Phone: +1 919 515 5118; Email: rgcarbon@ncsu.edu; Fax: +1 919 515 3465

Cristiana Boi − *Department of Chemical and Biomolecular Engineering and Golden LEAF Biomanufacturing Training and Education Center (BTEC), North Carolina State University, Raleigh, North Carolina 27695-7905, United States; DICAM, Alma Mater Studiorum-Università di Bologna, Bologna 40131, Italy*; orcid.org/0000-0002-7264-9496; Phone: +39 051 209 0432; Email: cristiana.boi@unibo.it; Fax: +1 39 051 634 7788

https://dx.doi.org/10.1021/acs.iecr.9b04936
Ind. Eng. Chem. Res. 2020, 59, 5371−5380

Authors

Michael Heller − *Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, United States*

Qian Li − *Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, United States*

Kellie Esinhart − *Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, North Carolina 27695-7905, United States*

Behnam Pourdeyhimi − *The Nonwovens Institute, North Carolina State University, Raleigh, North Carolina 27695-8301, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.iecr.9b04936

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the Groz-Beckert Company and the Kenan Institute of Engineering, Technology and Science. Additionally, the authors are grateful for the nonwoven materials provided by The Nonwovens Institute (NCRC) located at the North Carolina State University, as well as facility use of the Golden LEAF Biomanufacturing Training and Education Center (BTEC) located at the North Carolina State University.

■ REFERENCES

(1) Tuin, S. A.; Pourdeyhimi, B.; Loboa, E. G. Creating tissues from textiles: scalable nonwoven manufacturing techniques for fabrication of tissue engineering scaffolds. *Biomed Mater.* **2016**, *11*, 015017.

(2) Heller, M.; Wimbish, R.; Gurgel, P. V.; Pourdeyhimi, B.; Carbonell, R. G. Reducing diffusion limitations in Ion exchange grafted membranes using high surface area nonwovens. *J. Membr. Sci.* **2016**, *514*, 53−64.

(3) Li, R.; Wu, G.; Ye, Y. In vitro hemocompatibility of sulfonated polypropylene non-woven fabric prepared via a facile γ-ray pre-irradiation grafting method. *Appl. Surf. Sci.* **2015**, *356*, 1221−1228.

(4) Klein, E. Affinity membranes: a 10-year review. *J. Membr. Sci.* **2000**, *179*, 1−27.

(5) Muller, W. New ion exchangers for the chromatography of biopolymers. *J. Chromatogr.A* **1990**, *510*, 133−140.

(6) Tsuneda, S.; Kagawa, H.; Saito, K.; Sugo, T. Hydrodynamic evaluation of three-dimensional adsorption of protein to a polymer chain grafted onto a porous substrate. *J. Colloid Interface Sci.* **1995**, *176*, 95−100.

(7) Kobayashi, K.; Tsuneda, S.; Saito, K.; Yamagishi, H.; Furasaki, S.; Sugo, T. Preparation of microfiltration membranes containing anion exchange groups. *J. Membr. Sci.* **1993**, *76*, 209−218.

(8) Shinano, H.; Tsuneda, S.; Saito, K.; Furasaki, S.; Sugo, T. Ion exchange of lysozyme during permeation across a microporous sulfopropyl-group-containing hollow fiber. *Biotechnol. Prog.* **1993**, *9*, 193−198.

(9) Zheng, Y.; Liu, H.; Gurgel, P.; Carbonell, R. G. Polypropylene nonwoven fabrics with conformal grafting of poly(glycidyl methacrylate) for bioseparations. *J. Membr. Sci.* **2010**, *364*, 362−371.

(10) Liu, H.; Zheng, Y.; Gurgel, P.; Carbonell, R. G. Affinity membrane development from PBT nonwoven by photo-induced graft polymerization, hydrophilization and ligand attachment. *J. Membr. Sci.* **2013**, *428*, 562−575.

(11) Hutten, I. M. *Handbook of Nonwoven Filter Media*; Butterworth-Heinemann: Oxford, Burlington, MA, 2007.

(12) Przybycien, T.; Pujar, N.; Steele, L. Alternative bioseparation operations: life beyond packed-bed chromatography. *Curr. Opin. Biotechnol.* **2004**, *15*, 469−478.

(13) Liu, H. *Surface modified nonwoven membranes for bioseparations*, Ph.D. Thesis, North Carolina State Univ., Raleigh, NC, USA, 2012.

(14) Mishra, M.; Yagci, Y. *Handbook of Vinyl Polymers: Radical Polymerization, Process, and Technology*; CRC Press: Boca Raton, FL, 2008; DOI: 10.1201/9781420015133.

(15) Arslan, M. Preparation and Use of Amine-Functionalized Glycidyl Methacrylate-g-Poly(ethylene terephthalate) Fibers for Removal of Chromium(IV) from Aqueous Solution. *Fibers Polym.* **2010**, *11*, 325−330.

(16) Carroll, T.; Booker, N.; Meier-Haack, J. Polyelectrolyte-grafted microfiltration membranes to control fouling by natural organic matter in drinking water. *J. Membr. Sci.* **2002**, *203*, 3−13.

(17) Abdel-Fattah, S.; Shalaby, S.; Allam, E.; Hebeish, A. Benzoyl peroxide induced graft polymerization of 2-methyl-5-vinylpyridine onto polyester/wool blend. *J. Appl. Polym. Sci.* **1977**, *21*, 3355−3365.

(18) Okoniewski, M.; Sojka-Ledakowicz, J. Chemically induced graft copolymerization of acrylic acid on polyester fabrics. I. Kinetic investigation of grafting. *J. Appl. Polym. Sci.* **1988**, *35*, 1241−1249.

(19) Benes, M.; Horak, D.; Svec, F. Methacrylate-based chromatographic media. *J. Sep. Sci.* **2005**, *28*, 1855−1875.

(20) Vlakh, E. G.; Tennikova, T. B. Preparation of methacrylate monoliths. *J. Sep. Sci.* **2007**, *30*, 2801−2813.

(21) Bartolini, M.; Wainer, I.; Bertucci, C.; Adrisano, V. The rapid and direct determination of ATPase activity by ion exchange chromatography and the application to the activity of heat shock protein-90. *J. Pharm. Biomed. Anal.* **2013**, *73*, 77−81.

(22) Yun, J.; Shen, S.; Chen, F.; Yao, K. One-step isolation of adenosine triphosphate from crude fermentation broth of saccharomyces cerevisiae by anion-exchange chromatography using super-macroporous cryogel. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2007**, *860*, 57−62.

(23) Brandrup, J.; Immergut, E. H.; Grulke, E. A. *Polymer Handbook*, 4th ed.; John Wiley: NY, 1999.

(24) Allcock, H.; Lampe, F. *Contemporary Polymer Chemistry*, 2nd ed.; Prentice Hall: Englewood, NJ, 1990.

(25) Odian, G. *Principles of Polymerization*, 4th ed.; Wiley and Sons: Hoboken, NJ, 2004; DOI: 10.1002/047147875X.

(26) Wolf, C.; Burchard, W. Branching in free radical polymerization due to chain transfer. Application to poly(vinyl acetate). *Makromol. Chem.* **1976**, *177*, 2519−2538.

(27) Kato, K.; Uchida, E.; Kang, E.; Uyama, Y.; Ikada, Y. Polymer surface with graft chains. *Prog. Polym. Sci.* **2003**, *28*, 209−259.

(28) Bowes, B.; Lenhoff, A. Protein adsorption and transport in dextran-modified ion-exchange media. II. Intraparticle uptake and column breakthrough. *J. Chromatogr. A* **2011**, *1218*, 4698−4708.

(29) Heller, M. L. *Polymer Grafted Nonwoven Membranes for Bioseparations*, Ph.D. Thesis, North Carolina State University, 2015.

(30) Camperi, S.; Navarro, A.; Wolman, F.; Smolko, E.; Cascone, O.; Gruselli, M. Protein adsorption onto tentacle cation-exchange hollow fiber membranes. *Biotechnol. Prog.* **1999**, *15*, 500−505.

(31) Brown, G.; Muller, C.; Theodosia, E.; Franzreb, M.; Thomas, O. Multi-cycle recovery of lactoferrin and lactoperoxidase from crude whey using fimbriated high-capacity magnetic cation. exchangers and a novel "rotor-stator" high-gradient magnetic separator. *Biotechnol. Bioeng.* **2013**, *110*, 1714−1725.

(32) Skidmore, G.; Horstmann, B.; Chase, H. Modelling single-component protein adsorption to the cation exchanger S Sepharose FF. *J. Chromatogr. A* **1990**, *498*, 113−128.