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Research Article

Chemical Vapor Deposition of Poly(hydroxyethyl methacrylate-glycidyl methacrylate) Thin Film Coatings for Immobilization of Human Serum Albumin

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Abstract

In this study, poly(2-hydroxyethyl methacrylate-glycidyl methacrylate) (P(HEMA-GMA) thin films were deposited on crosslinked polyvinyl alcohol (PVA) supports by initiated chemical vapor deposition (iCVD). Use of the tert-butyl peroxide as an initiator allowed very high deposition rates up to 125 nm/min at a filament temperature of 280 ^{o}C. FTIR and XPS analyses of the deposited films confirmed that the epoxide functionality of the copolymers increased with increasing glycidyl methacrylate fraction in the reactor inlet. The potential of the glycidyl methacrylate containing copolymer films to act as substrates for protein immobilization was revealed. The immobilization of Human Serum Albumin (HSA), which is the main protein to carry fatty acids and metals to target tissues, was carried out via solid phase extraction. The effects of film composition and thickness on binding capacities of the protein to the polymers were studied. The maximum protein binding for the iCVD synthesized copolymer films was found to be 223 µg.cm^{-2}. Protein binding was also clarified by FTIR, AFM, and SEM analyses. The mild immobilization conditions, easy and rapid membrane preparation, one-step protein binding at substantially higher levels and membrane reusability make iCVD deposited P(HEMA-GMA) films useful for biomolecules immobilization and for several biochemical processes.

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INTRODUCTION

Modification of the surface of supporting materials such as polymers, nanoparticles, nanocomposites and thin films have recently been of major interest for biological applications [1-4]. A number of surface modification techniques have widely been used in order to alter the chemical composition and lead to changes on the surface properties of the materials. Some of the methods are plasma-treatment techniques, binding of the specific ligands for targeting biomolecules, blending with other macromolecules and silanizatin techniques on the surface [5-6]. Many studies have been reported the adsorption or immobilization of the biomolecules such as DNA, oligonucleotides, different types of proteins, antibodies and enzymes on the polymer surfaces, depending on the type of molecules used and the functional groups on the surfaces such amine, epoxy and thiol groups [7-8]. Most commonly used protein in immobilization study is albumin which is the most abundant protein in the blood. Albumin which has a molar mass of 66.5 kDa is responsible for the transport fatty acids, metals, vitamins and the drugs to the target tissues [9].

In recent years, PHEMA and PHEMA based materials have been used for a variety of applications including immobilization of proteins and enzymes [10-11], controlled release of drugs[12-13], biosensors [13-15], separation of molecules and cells [16-17], and metal-ion adsorption[18-19]. PHEMA based hydrogel thin films can be prepared by various methods, such as, UV initiated graft-polymerization [20], surface-initiated atom transfer radical polymerization [21-22], electrospinning method [23], sol-gel technique [24] and chemical vapor deposition (CVD) [25-27]. CVD is an all-dry method to produce polymeric thin films with good control over film morphology and stoichiometry. In this method, the substrates with complex geometries can be coated with high uniformity and without solvent related damages, which are observed in conventional wet processes [28-29]. Initiated chemical vapor

deposition (iCVD) is a combination of all-dry CVD technique with free radical polymerization process. In iCVD, resistively heated filaments which are placed a few centimeters above the substrate surface provide the activation energy for polymerization reactions. The technique allows polymerization and surface modification to be completed in a single step and enables very high retention of the functional groups because of the low filament temperatures and the lack of the harmful plasma environment [30-31].

Due to their hydrophilic character, chemical stability, and biocompatibility, PHEMA based polymers and copolymers are attractive in biomaterials area for many years [32-33]. The physical and chemical properties of PHEMA can be modified by copolymerization. The introduction of functional epoxy groups on the PHEMA structure can be provided by copolymerization of HEMA with an epoxy group carrying monomer[34]. GMA is an excellent monomer having a reactive epoxy group, which can be directly coupled with a biological molecule via ring opening reactions or further modified into different functional groups. HEMA is able to form a hydrophilic environment on crosslinked PVA sheet (CPS) [35].

In this work, a copolymer thin film containing functional epoxide and hydroxyl groups was synthesized on a PVA support material using iCVD technique to prepare a PHEMA-based thin film hydrogel membrane. A series of P(HEMA-GMA) thin films with varying compositions were prepared and the as-deposited films were characterized chemically by FTIR and XPS methods. The obtained thin film hydrogel (TFH) membranes were used for the immobilization of human serum albumin. The effects of film thickness and composition on immobilization efficiency were examined.

EXPERIMENTAL

Preparation of Crosslinked PVA Sheets

Polyvinyl alcohol (Alfa Aesar, MW=88000-97000 g/mol, degree of hydrolysis= 98-99%) was dissolved in distilled water and stirred at 95 ^{o}C for at least 4 h to obtain 2,0 wt % homogeneous aqueous PVA solution. Then the solution was cooled down to room temperature. 1,0 M hydrochloric acid (Aldrich, HCl, % 37) solution was added dropwise to adjust the pH level of the PVA solution (the target pH is about 1). A glutaraldehyde (Merck, GA, 25 wt% in water) solution was subsequently added to initiate the cross-linking reaction. 4 mL of the PVA/GA solution was cast onto the petri dishes of 6 cm diameter. To complete the cross-linking reaction, the PVA sheet were kept in a humid chamber for 6 h and then washed with distilled water.

Preparation of Poly(2-hydroxyethyl methacrylate-co-glycidyl methacrylate) Film

Films were deposited on silicon wafer (100, p type) and crosslinked poly(vinyl alcohol) (PVA) sheets by initiated chemical vapor deposition method in a custom-built vacuum reactor. The schematic representation and details of reactor set-up is given elsewhere [36]. In this reactor, the reactant gases were thermally activated by heating a tungsten filament array (Alfa Aesar) resistively. The filament array containing 12 parallel tungsten filaments was placed 22 mm above the substrate surface and resistively heated to 210, 240 and 280 ^{o}C. The substrates are placed on a backside cooled stage maintained at 27 ^{o}C. The filament temperature was measured by directly attaching a K-type thermocouple (Omega) to one of the filaments. Reactor feed consisted of glycidyl methacrylate (GMA, 97%), 2-hydroxyethyl methacrylate (HEMA, 95%), and di-tert-butyl peroxide (TBPO, 98%), which were purchased from Sigma Aldrich, and used as-received. Monomers GMA and HEMA were vaporized in separate jars at 65^{o}C and 67 ^{o}C, respectively, and metered into the reactor using needle valves. The initiator TBPO was vaporized in a different jar at room temperature and fed to the reactor

by using a mass flow controller (MKS). The flowrates of TBPO and GMA were kept constants at 1 and 2 sccm, respectively during all experiments, while the flow rate of HEMA was varied between 0 and 2 sccm to synthesize copolymer films of varying compositions. Reactor pressure was kept at 300 militorrs, which was measured and controlled by a capacitance manometer (MKS Baratron) and a downstream butterfly valve operated by a PID pressure controller (MKS), respectively. The reactor was equipped by a laser interferometry system for real time monitoring of the deposition thickness. During the depositions, silicon wafers were placed next to the crosslinked PVA sheets, for characterization purposes, and for interferometric thickness determination. The chemical structures of the resultant PHEMA and PGMA homopolymers and P(HEMA-GMA) copolymer is given in Figure 1.

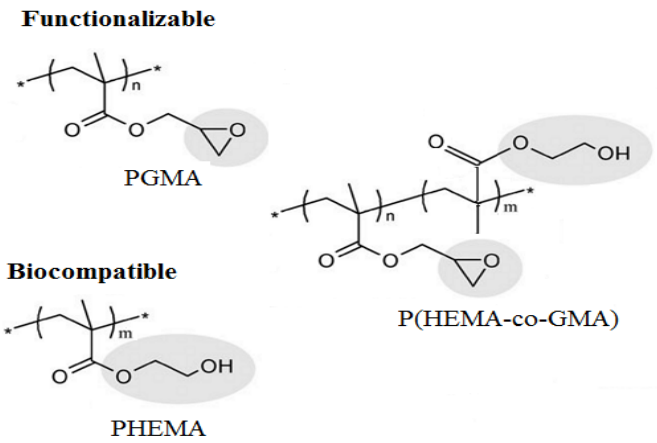

Figure 1. Chemical structures of PGMA, PHEMA, and P(HEMA-GMA).

In the series of iCVD experiments, the only variables were the filament temperature and flow rate of HEMA. For the homopolymers (PHEMA and PGMA, denoted F1-F2 in Table I and copolymer experiments (P(HEMA-GMA), denoted F3 through F7 in Table I, substrate temperature, reactor pressure and flow rates of GMA and TBPO were all kept constant. All runs were carried out to produce films with thickness of 0.5 μm. After iCVD deposition of thin films on crosslinked PVA sheet, the resultant TFH membrane sheets were

cut into 0.6 cm diameter circular discs to be used in the further protein immobilization experiments.

Determination of the swelling rate of the prepared films

The prepared films were allowed to soak in distilled water for 24 h, swollen film were weighed after removing the excess water, dried in vacuum oven at 50 °C for 24 h until constant weight. The swelling rates of the films were calculated as following equation:

Table I. Experimental condition for iCVD of PDPAEMA thin films

	temperature (^{o}C)		flow rate (sccm)			reactor pressure (Torr)	deposition rate (nm/min)
sample	Filament	substrate	HEMA	GMA	TBPO		
F1	280	27	2		1	0.3	10
F2	280	27		2	1	0.3	25
F3	280	27	0.5	2	1	0.3	54
F4	280	27	1	2	1	0.3	96
F5	280	27	2	2	1	0.3	125
F6	240	27	2	2	1	0.3	78
F7	210	27	2	2	1	0.3	54

Swelling rate% = (Ws − Wd)/Wd× 100 (1)

where Ws and Wd are the weights of swollen and dry film, respectively

Characterisation of poly(HEMA–GMA) films

Chemical analysis of the as-deposited thin films and TFH membranes was carried out using Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Vertex 70) and X-ray photoelectron spectroscopy (XPS) (Specs spectrophotometer with a monocromatized Al source). FTIR spectra were obtained using a reflectance accessory (Bruker optics) over a spectral range of 800-4000 cm^{-1} at 4 cm^{-1} resolution. All FTIR spectra were baseline corrected and thickness normalized. Film thicknesses during the depositions were monitored by laser interferometry, in which the reflectance of a 633 nm HeNe laser beam (JDS Uniphase) off the polished silicon substrate was monitored by a laser powermeter. In order to check the accuracy of the interferometry, the film thicknesses were also measured ex-situ by a mechanical profiler (AES Nano 500). Surface morphologies of the dried thin films were obtained using scanning electron microscope (JEOL) and atomic force microscope (NtMdt). Contact angle measurements were carried out on a Kruss Easy Drop contact angle goniometer system at ambient temperature. Pure water with a pH close to 7.0 at room temperature was used in static contact angle measurements. A pH-meter (Orion) equipped with a combined glass-Ag/AgCl was used for pH measurements.

Protein Immobilization Experiments

The prepared TFH membranes were mixed with a concentration of 0.15 $mg.mL^{-1}$ of human serum albumin (HSA) in 20 mM×Tris, pH 7.4. Each mixture was thumbled for 2 h at $4^{o}C$ at 0,5654 cm^{-2} of a surface area of TFH membranes. Protein bounded copolymers were separated from supernatant. Albumin bounded particles were also washed with Tris buffer for

chemical characterization. Supernatant was kept at 4^{o}C for analysis of protein concentration. Binding of HSA to copolymers was also confirmed by using fluorescence spectroscopy. For this purpose, intrinsic fluorescence of protein was measured at 280 and 342 nm of excitation and emission wavelengths [37]. Unbounded protein concentration was determined from the equation of calibration curve (y=29050x+36.882, R=0.9943).

RESULTS AND DISCUSSION

Initiated Chemical Vapor Deposition of P(HEMA-GMA) Thin Films

The deposition rates observed for the PHEMA and PGMA homopolymers are 10 and 25 nm/min, respectively. For iCVD process, the polymerization mechanism is believed to be very similar to the solution phase free radical polymerization, and the polymerization kinetics depends strongly on the surface concentration of species. The fractional saturation ratio, which is the ratio of monomer partial pressure (Pm) to its saturation pressure (Psat) is an important factor to determine the deposition rates. At the given substrate temperature and reactor pressure, Pm/Psat is 0.33 and 0.8 for GMA and HEMA, respectively. The lower deposition rate of PHEMA even at such a high fractional saturation value could be attributed to its low reactivity at the given filament temperature of 280^{o}C. However, when both monomers are fed into the reactor simultaneously; the deposition rates were observed to increase significantly as compared with the homopolymer depositions under similar conditions. The maximum deposition rate for the copolymer is 125 nm/min, which is 12.5-fold higher than the deposition rate of HEMA only polymer. The observed increase in the deposition rate may be due to the interactions between the monomers that have different polarities, which were previously observed in free radical copolymerization reactions[38]. As can be seen in Figure 2, any increase in the HEMA flowrate resulted in higher deposition rates and this dependency is expected for free radical polymerizations.

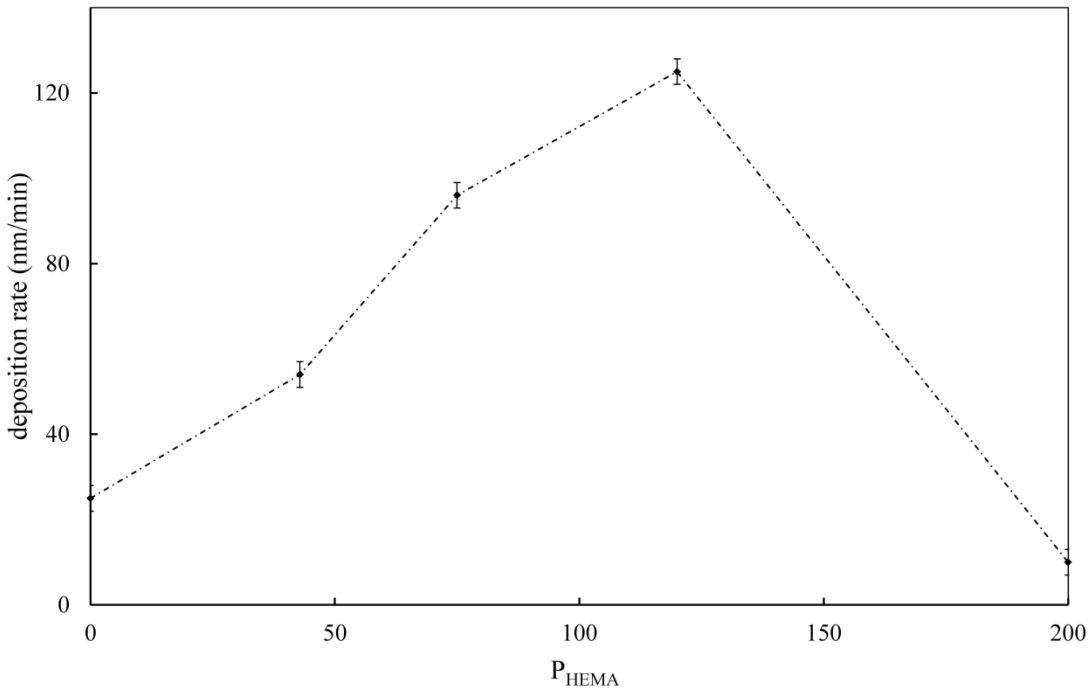

Figure 2. Deposition rate as a function of HEMA partial pressure (T_f=280 ^oC, P=300 mtorr, T_s=27 ^oC)

The effect of filament temperature on the deposition rates of copolymers was investigated by keeping any other variables constant in the iCVD depositions. Figure 3 gives the plot of the deposition rate as a function of temperature in an Arrhenius form in the temperature range of 210-280 °C. Apparent activation energy of 26.7 kJ/mol is calculated from the slope of the least squares linear regression to the data. This is a technique widely used in the iCVD literature to estimate apparent activation energy [30-31]. In this calculation the concentration of the monomers in the reactor was assumed to be constant considering low monomer consumption values in iCVD. The observed activation energy is almost 4-times lower than the activation energy observed for iCVD deposition of PGMA [30], meaning that deposition of copolymer is significantly less sensitive to the filament temperature.

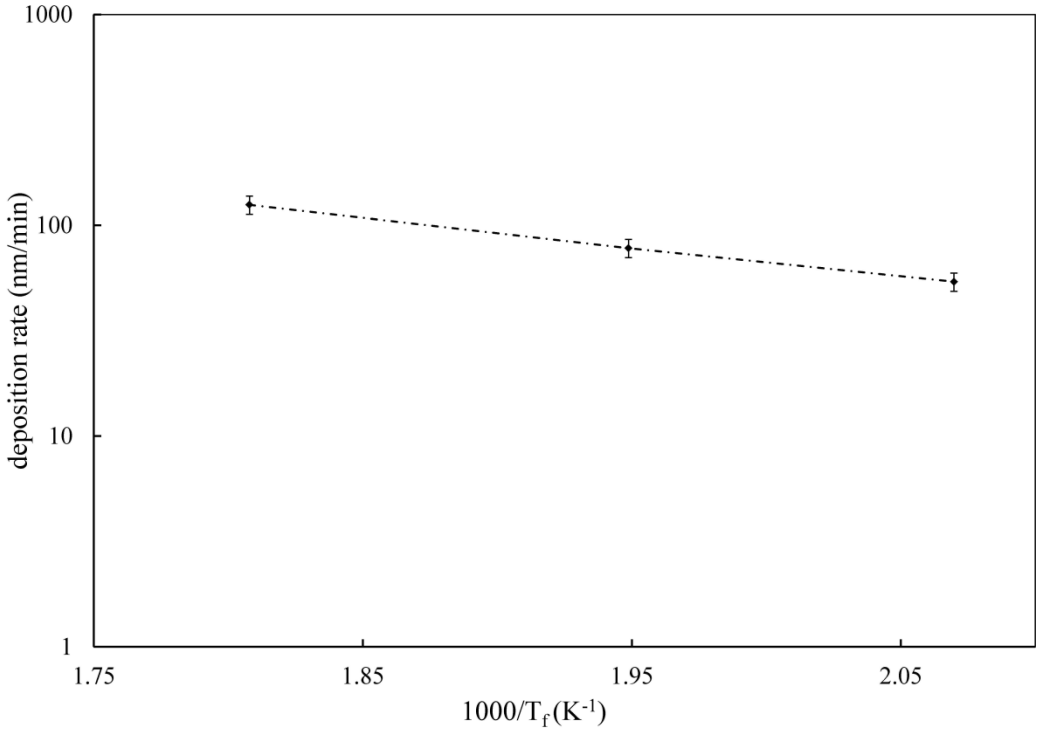

Figure 3. Deposition rate as a function of filament temperature (P=300 mtorr, T_s=27 ^oC)

Figure 4 (a-e) shows the FTIR spectra of homopolymers and copolymers with varying compositions. These spectra were thickness-normalized and baseline-corrected, and no other processing was performed. The spectra of iCVD-synthesized polymers do not involve peaks related with C=C bonds that exist only in the monomer spectrum, which proves that the polymerization proceeded through unsaturated C=C double bonds. In the spectrum of PHEMA (Figure 4 (e)), the broad peak between 3700 and 3050 cm^{-1} is related to the characteristic stretching vibration of -OH group. The other main vibrational modes are C-H stretching (3050-2700 cm^{-1}), C=O stretching (1750-1690 cm^{-1}), C-H bending (1500-1350 cm^{-1}), and C-O stretching (1300-1200 cm^{-1}). The existences of strong -OH and carbonyl peaks indicate that the chemical functionality of HEMA monomeris well preserved during iCVD polymerization.

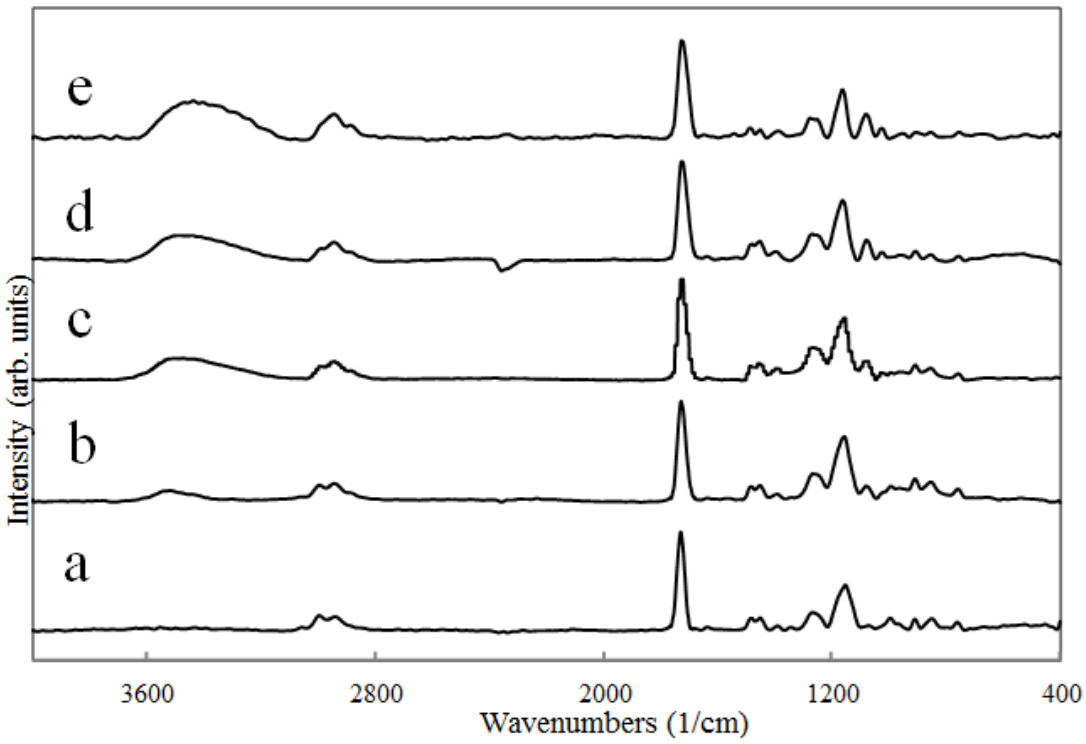

Figure 4. FTIR absorbance spectra of (a) PGMA, (e) PHEMA, and P(HEMA-GMA) with varying compositions ((b) F3, (c) F4 and (d) F5).

In the spectrum of PGMA (Figure 4(a)), the peaks at 907, 848, and 760 cm^{-1} are assigned to the characteristic epoxide group adsorption bands. In the case of spectra belonging to the copolymer films (Figure 4 (b-d)), it was observed that composition of a copolymer film can be tuned by changing the monomer feed ratios. As the flow rate of HEMA at the reactor inlet increases, the intensity of broad hydroxyl peak increases and that of epoxide peaks decrease. An increase in the flow rate of HEMA while keeping the flowrate of GMA constant during the depositions leads to increased partial pressure of HEMA with respect to GMA. Therefore, the concentration of HEMA monomer at the substrate surface becomes higher, which implies that more HEMA monomers can react with GMA monomers to form polymer films with higher hydroxyl functionality. The chemical structures of the copolymer films were also investigated by XPS analysis. According to the XPS survey scan of the copolymer film, for

which FTIR spectrum is given in Figure 4(d), carbon/oxygen atomic ratio was found as 2.25, which is in between the C/O atomic ratios found in GMA 2.33 and HEMA 2.00. High resolution C1s XPS scan was also obtained to investigate the chemical bonding states of the copolymer film.

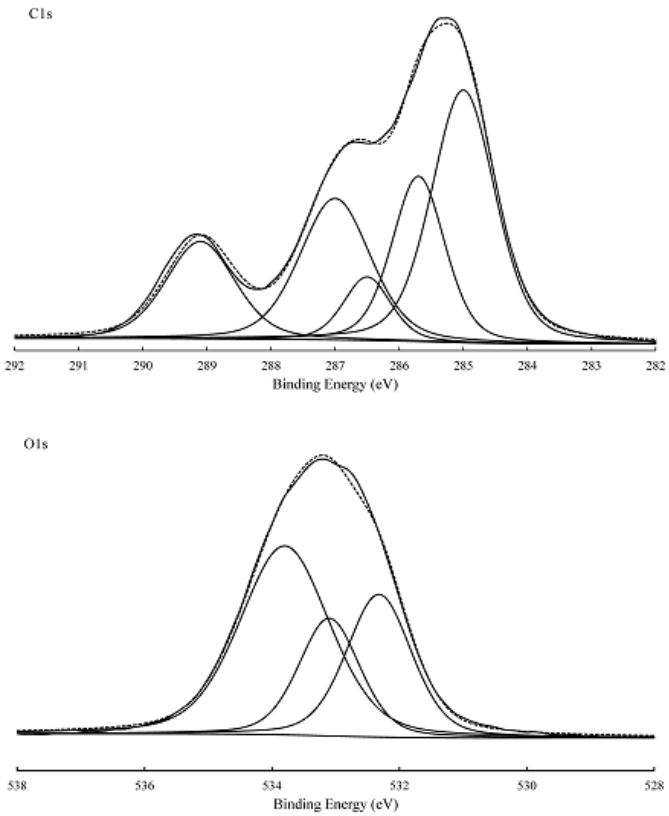

Figure 5. C1s and O1s high-resolution XPS scans of the P(HEMA-GMA) film deposited on PVA sheets by iCVD

Figure 5 shows the high resolution C1s and O1s spectra for P(GMA-HEMA) film together with spectrum deconvolutions. In both spectra, the fitted curves reproduce the experimental spectra with high accuracy. High resolution C1s and O1s spectra can be curve-fitted with five and three peak components, respectively, at binding energy values given in Table II. The peak positions are in good agreements with the previously synthesized PGMA and PHEMA films [21-24,26]. However, the areas of the peaks are different from HEMA or GMA only homopolymers, which can be expected with the copolymer films that contain

functional groups coming from both monomers. Both XPS and FTIR analyses confirm that iCVD produces copolymer thin films that retain essentially high fraction of hydroxide and epoxide functionality.

Table II. High-Resolution XPS Scan Data of the P(HEMA-GMA)

Core level	BE (eV)	Origin
C1s	285.0	-C*H$_3$, -C*H$_2$-
	285.7	-C*-(CH$_3$)-CO-
	286.5	-C*H$_2$-OH, -O-C*H$_2$
	287.0	-O-C*H$_2$, –C*H–C*H$_2$ (O)
	289.1	-C*=O
O1s	532.3	-C=O*
	533.1	-O*H , –C H–C H$_2$ (O)
	533.8	-CO-O*-CH$_2$-

FTIR spectra were also used to analyze the bulk compositions of the films by using the Beer-Lambert equation [39] assuming that the C=O bond oscillator coefficient is the same in the GMA and HEMA components. The HEMA mole fraction in the film was calculated using the following Equation :

$$f_{HEMA} = 1 - \left(\frac{A_{1730}}{A_{908}}\right)_{PGMA} \left(\frac{A_{908}}{A_{1730}}\right)_{P(GMA-co-HEMA)} \quad (2)$$

where A is the area under the peaks of epoxy (908 cm^{-1}) and C=O (1730 cm^{-1}) absorptions. The results were included in Table III, which show that depending on the partial pressures of HEMA and GMA monomers during the depositions the compositions of the copolymers can be well tuned.

Contact angle measurements were performed to test the hydrophilicity of the thin films synthesized by iCVD. The obtained polymeric thin films have different contact angles as regard to their hydrophilic characters. Compared to the bare or PGMA coated PVA sheets water contact angles of PHEMA and P(HEMA-GMA) coated surfaces were lower due to highly hydrophilic character of –OH functionality. The reported contact angle value of 15^o for PHEMA-only film is not stable in time as the linear PHEMA film dissolves gradually with the water droplet placed during the contact angle measurements. The copolymers, on the other hand, were stable. The results showed that the contact angle value is decreased with increasing mole fraction of HEMA, which enhances the hydrophilic character of the deposits.

The swelling rate is of paramount importance when using supports for protein immobilization. The swelling rate of homo and copolymer thin films deposited on cross-linked PVA sheets was determined by weight difference between the water swollen and the dry TFH membrane. The effect of HEMA/GMA mole fraction on the swelling rate of the poly(HEMA–GMA) films given in Table III. Maximum swelling rate was observed in the PHEMA thin film with a swelling rate of 67%. As the molar fraction of HEMA in the structure of deposited copolymer decreased, the swelling rate decreased up to 21% as the amount of -OH groups decreased. Furthermore, considering that HEMA is a more polar

component than GMA, this result was found reasonable. Therefore, the addition of HEMA into the polymer structure caused a increase in the swelling capacity of the polymer.

Table III. Composition of the P(HEMA-GMA) films and amount of the immobilized HSA obtained with these film compositions

Film type	Mole fraction		Contact angle values	Water content (%)	Immobilized protein ($\mu g\ cm^{-2}$)
	HEMA	GMA			
F1	1		15 ^{o}	67	191
F2		1	45 ^{o}	21	219
F3	0.1204	0.8796	39 ^{o}	47	213
F4	0.2842	0.7158	31 ^{o}	51	223
F5	0.397	0.603	26 ^{o}	58	207

Protein Binding Studies

It is well-known that epoxy, amine and thiol groups on the surfaces of the supporting materials are the efficient functional groups for protein binding and enzyme immobilization due to electron donating character of oxygen and amine [40]. Immobilization mechanism may be covalent and non-covalent such as hydrophobic interactions, hydrogen bond and Van der Waals interactions. In this study, the addition of reactive epoxy group (GMA) in the polymer matrix can improve the covalent interactions with the proteins without the need of further modifications [41]. The epoxy group can combine with amino groups of proteins under neutral and alkaline conditions. The N–C or O–C bonds formed between epoxy containing support and proteins are extremely stable [42]. Hydroxyl group from HEMA have free

electron pairs that may be involved in hydrogen bonding with the ammonium ions in the protein [43-44]. Hydrogen bond contributes to protein immobilization on PVA/P(HEMA-GMA) along with covalent complexation. Some preliminary evaluations were performed to investigate binding efficiencies of the thin polymer films for HSA by using solid-phase extraction. Unbounded HSA was determined at the specific excitation and emission wavelengths of the protein by using fluorescence spectroscopy. After the removal of HSA from copolymers, immobilization amounts of HSA to PVA/P(HEMA-GMA) substrates were measured at 280 and 342 nm of excitation and emission wavelengths by using equation of calibration curve of the protein (Figure 6 (a)).

Determination of binding amount of the protein on all PVA/P(HEMA-GMA) substrates with different compositions was performed by using fluorescence measurements before and after treatment of the protein with copolymers (Figure 6(b)). According to the data, the binding amount of protein to PVA/P(HEMA-GMA) thin film with a GMA concentration of 60.3% (sample F5, Table III) was found to be 193 $\mu g.cm^{-2}$. Increase of concentration of GMA units in the polymer (samples F3 and F4-1, Table III) keeping the film thickness constant increased the binding amount up to 223 $\mu g.cm^{-2}$ due to introduction of more epoxy groups on the surface. The effects of film thickness on the binding amount was also investigated (samples F4-1, F4-2 and F4-3, Table III) and data showed that there is no significant difference in the immobilization capacity of the copolymers within a thickness range of 0.1-0.5 μm. The protein binding capacity of PHEMA coated PVA was found to be 198 $\mu g.cm^{-2}$. PGMA coating on PVA surface was observed to be more effective in protein binding with a capacity of 219 $\mu g.cm^{-2}$. The immobilization amounts of the protein HSA for both PGMA and P(HEMA-GMA) coatings were observed to be very close. This showed that the molecules having same functional epoxy groups didn't increase binding capacity by just increasing the ratio of the functional groups in the polymer due to stoichiometric ratio of

complexation between protein and the functional groups of copolymer. This data clarified that protein affinity depends on the variation of functional moiety on the surfaces of the polymer rather than molecular structures on the polymer film. However, the synthesis of the copolymer as a thin film provides new chemical properties for biological applications.

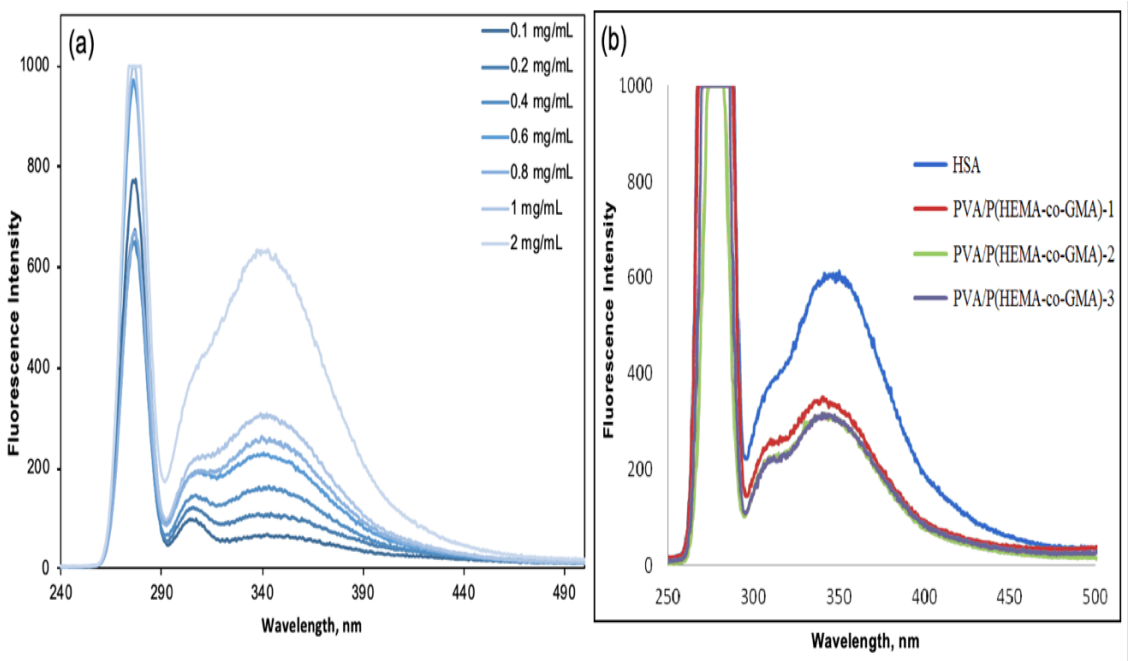

Figure 6. Fluorescence spectra of HSA at a concentration range of 0.001-0.02 µg mL^{-1} (a), Fluorescence spectra of HSA after binding on (PVA/P(HEMA-GMA) derivatives at 280 and 342 of emission wavelengths (b).

Figure 7 shows the SEM images of P(HEMA-GMA) surfaces before and after protein immobilization. Before the immobilization experiments, the surfaces are smooth and flat, however during the treatment the surface became highly porous which may provide a high internal surface area. After protein binding, globular structure of the protein is clearly seen on the surface. The protein may bind both the external surface of the P(HEMA-GMA) film and within the pore space near the surface. Therefore, this large surface contributes the reaction of substrate with the immobilized protein [45]. The increase in the surface roughness of the films

after the HSA immobilization was also verified by AFM (Figure 8 (a-b)). The RMS roughness value increased from 0.42 nm to 18.33 nm before and after the HSA bindings, respectively.

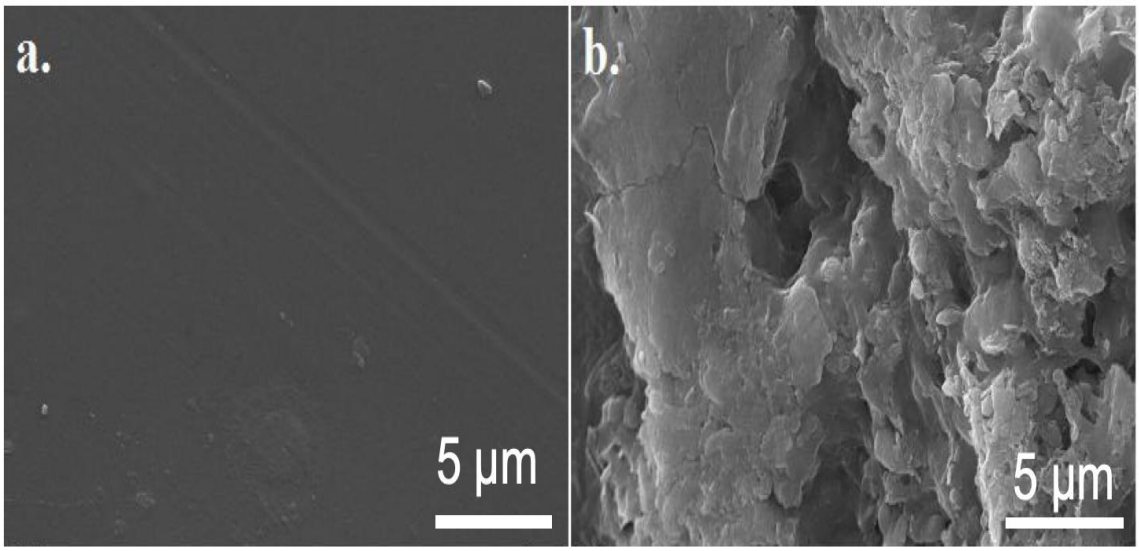

Figure 7. SEM images of PVA/P(HEMA-GMA) before (a) and after (b) HSA binding.

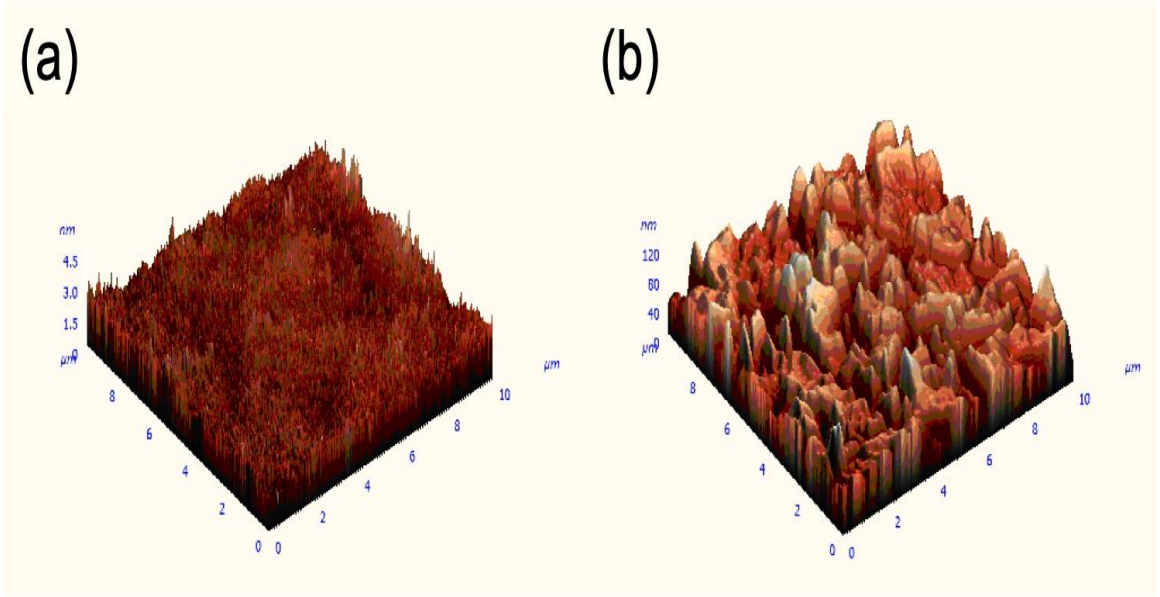

Figure 8. AFM images of PVA/P(HEMA-GMA) before (a) and after (b) HSA binding

Figure 9 (a-b) shows the FTIR spectra of the copolymer film (sample F4 in Table III) before and after the immobilization of HSA. The FTIR spectrum of PVA/P(HEMA-GMA) film have the characteristic stretching vibration band at around 1730 cm^{-1} from ester configuration of both HEMA and GMA. The intensity of that peak obviously decreased after formation of amide bond between ester and amine groups from amino acid residues and N-terminal of HSA, which led to new stretching vibration of Amide I at 1634 cm^{-1} and Amide II at 1526 cm^{-1}. The amide I vibration, absorbing near 1650 cm^{-1}, arises mainly from the C=O stretching vibration with minor contributions from the out-of-phase CN stretching vibration, the CCN deformation and the NH in-plane bending [44]. The amide II mode is the out-of-phase combination of the NH in plane bend and the CN stretching vibration with smaller contributions from the CO in plane bend and the CC and NC stretching vibrations [43-44]. The peak broading at 1415 cm^{-1}, 1341.5 cm^{-1} and 1057 cm^{-1} may arise from proline and tryptophan residues of the protein [44].

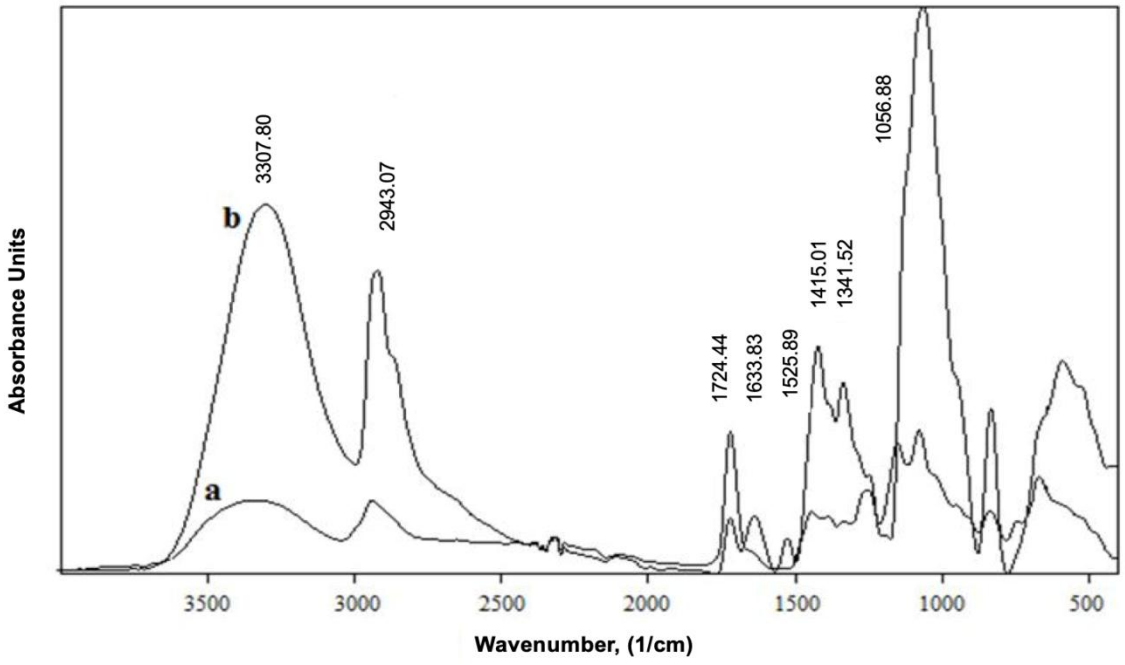

Figure 9. FTIR absorbance spectra of PVA/P(HEMA-GMA) before (a) and after (b) HSA binding

The change of morphology and chemical composition after the addition of HSA was also verified by contact angle measurements, and the results are given in Table III. The water contact angle values of HSA bonded film surfaces increased most probably due to the hydrophobic residues of the proteins such as tryptophan, valine, leucine and isoleucine on the surface. The surface roughness increase may be the other reason behind the contact angle increase.

CONCLUSIONS

P(HEMA-GMA) thin films can be synthesized by iCVD using HEMA and GMA as the monomers. The introduction of tert-butyl peroxide as an initiator allows very high film deposition rates at low filament temperatures. The iCVD, an all dry method, is able to functionalize fragile substrates such as PVA thin membrane sheets. The feasibility of the epoxy and hydroxyl functional groups containing copolymer films to act as substrates for protein immobilization was studied. The covalent protein binding was clarified by FTIR, AFM, and SEM analyses. The mild immobilization conditions, easy and rapid membrane preparation, one-step protein binding at substantially higher levels and membrane reusability make P(HEMA-GMA)/PVA TFH membran having abundant functional groups films useful for biomolecules immobilization and for several biochemical processes.

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