Surface Modification of Nylon Membrane by Glycidyl Methacrylate Graft Copolymerization for Antibody Immobilization

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ABSTRACT: Surface of nylon membrane was modified by the graft copolymerization of glycidyl methacrylate (GMA) using persulfate and thiosulfate as redox initiator system. Effect of various reaction parameters such as initiator concentration, monomer concentration, polymerization time, and temperature on degree of grafting was also studied. Maximum grafting of 100% was achieved by using equimolar concentration (0.008*M*) of redox initiator and 0.5*M* of GMA monomer at 70°C in 60 min. Grafted nylon membranes with various graft levels of GMA were characterized by various techniques such as fourier transform infrared spectroscopy, thermo gravimetric analysis, and scanning electron microscopy. The GMA grafted nylon (NyM-*g*-GMA) membranes with different graft levels were evaluated as a support for immobilization of rabbit anti goat antibody (RAG IgG). Antibody (Ab) immobilized NyM-*g*-GMA membranes were evaluated using ELISA and Bradford protein estimation method. Nylon membrane with 60% graft level showed optimum immobilization of Ab at RAG IgG conc. of 0.625 μg/mL with low nonspecific binding. Maximum immobilization efficiency (I.E.%) of 56% was observed for membrane with 60% graft level at 50 μg/mL of RAG IgG in PBS (pH 7.4). Ab immobilized NyM-*g*-GMA discs were found to be stable up to 6 weeks at 4°C and 2 days at 37°C. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1700–1709, 2010

Key words: copolymerization; FTIR; modification; nylon membrane; proteins

INTRODUCTION

Immobilization of biomolecules on to various polymeric matrices is of immense importance for various biomedical applications like diagnostics, drug delivery, microarray, and biosensor development.[1–4] Immobilization of biomolecules onto the matrix is generally carried out by means of entrapment, physical adsorption, copolymerization, or covalent attachment.[5] Biomolecule immobilization depends on the method used for immobilization, which in turn influences parameters such as shelf life and surface regeneration of matrix. Immobilization methods based on adsorption, entrapment, crosslinking, and electrostatic interactions result in randomly immobilized biomolecules, sometimes partially hindering their biological activity. Covalent binding enables oriented biomolecule immobilization, providing controlled, reproducible, highly active modified surfaces.[6] Moreover, biomolecules bound covalently to the support show better shelf life and operational stability as compared to the nonspecifically bound molecules, which leak out during storage and/or assay.[7]

Various technologies such as organosilanization, ionized gas treatment, and grafting have been directed to the introduction of new reactive functionalities for enabling covalent binding of biomolecules onto polymer surface.[1] Grafting has advantages over other surface modification methods because of easy and controlled introduction of graft chains with a high density and better localization of functional groups on polymer surface.[8] Of the various techniques used for grafting, the two most widely used are radiation and chemical initiated grafting; wherein, the later has ease of operation in laboratory conditions and also does not require any specialized or expensive equipment.[9] Surface modification of various solid matrices like polypropylene, polyethylene, polystyrene, cellulose, and nylon has been achieved by chemical grafting of glycidylmethacrylate (GMA).[10–14] Pendant epoxide groups of GMA on polymer surface directly react with amino,

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sulfhydryl, and carboxyl groups of biomolecules to form stable covalent bonds with them.[15,16] Such epoxy modified polymer surfaces are stable during long storage periods and are resistant against hydrolysis.[12] GMA grafted surfaces have been used for immobilization of biomolecules like antibody, enzymes by covalent coupling at both laboratory and industrial scale.[17] A number of researchers have utilized antibody immobilized polymeric surfaces for various applications including biosensor development and diagnostics. Immobilization of antibody on solid support has been the subject of attention owing to the high cost of antibodies and their availability in limited amounts.[2,18–20]

Out of the various polymeric matrices that have been used in diagnostics for immobilization of biomolecules, nylon membranes are a good choice because of being biocompatible, economic, and easy to be surface modified by various methods.[14,21–24] However, the use of GMA chemical grafting for surface modification of porous nylon membrane, and its subsequent utilization as matrix for antibody immobilization has not been reported so far. Therefore, the aim of this study was to surface modify porous nylon membrane by chemical grafting of GMA to be later used as a solid matrix for immobilization of antibody. The influence of various grafting parameters on the yield of grafting on nylon membrane was also investigated in this study.

EXPERIMENTAL

Materials

GMA and Bradford reagent were purchased from Sigma–Aldrich Chemical Company (St. Louis, USA). Nylon-6,6 membrane (Immobilon^{+}, 0.45 μm, Millipore, India) was used for grafting. Analytical grade potassium persulfate ($K_2S_2O_8$), sodium thiosulfate ($Na_2S_2O_3$), and copper acetate were purchased from CDH, India. Absolute alcohol was purchased from Merck, India. Rabbit anti-goat IgG (RAG), Goat anti-rabbit (GAR IgG-HRP) antibody conjugate, and substrate (TMB/H_2O_2) were received from Bangalore Genei, India and were used as received. Microtitre plates were obtained from Greiner bio-one, Germany.

Graft copolymerization

The grafting of GMA onto nylon membrane was carried out by free radical chemical initiation method using persulfate and thiosulfate as redox initiator system. Grafting was done in a glass ampoule with the ethanol : distilled water (1 : 1) as a solvent. $K_2S_2O_8$ and $Na_2S_2O_3$ (0.002*M* to 0.016*M*) were added to solvent and stirred well on magnetic stirrer at room temperature (RT) for the complete solubilization of initiator. Reaction initiation enhancer copper acetate (0.004%, w/v) was then added and mixed well followed by the addition of monomer (0.1*M* to 0.7*M*). Preweighed nylon membrane (5 cm × 2 cm in size) was immersed in the grafting mixture and nitrogen was purged into the ampoule to remove air from grafting solution. The reaction ampoule was placed in a temperature controlled oil bath and grafting was carried out for desired time period under constant magnetic stirring at different temperatures (50–70°C). GMA grafted nylon membrane was taken out from glass ampoule, immersed in methylethyl ketone in a conical flask, and placed on mechanical shaker for 16 h with change of solvent at 4 h interval to remove polyglycidylmethacrylate (PGMA) homopolymer and unreacted monomer from the membrane. GMA grafted nylon membrane (NyM-*g*-GMA) was dried at 40°C for overnight and weighed. The grafting percentage or percent graft level (%*G*) and grafting efficiency (%G.E.) were measured as:

$$(\%G) = \frac{W_a - W_b}{W_b} \times 100 \quad (1)$$

$$(\%\,\mathrm{G.E.}) = \frac{W_a - W_b}{(W_a - W_b) + W_b} \times 100 \quad (2)$$

where W_b and W_a are weight of the nylon membrane (NyM) before and after the grafting process, respectively, and W_h is the weight of homopolymer.

Circular discs of 0.5 cm diameter were punched out from grafted nylon membrane using a standard paper punch for characterization and Ab immobilization studies.

Characterization of GMA grafted nylon membranes

ATR-FTIR spectroscopy

Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectral analyses of the ungrafted and GMA grafted nylon membranes were recorded with a Perkin–Elmer-spectrum one spectrophotometer. Punched discs with different percent graft level were placed directly under the probe of ATR and spectra were recorded at a rate of 16 scans/min.

Thermo gravimetric analysis

TA 2100 thermal analyzer having a 951 TG module was used for the thermal characterization of various grafted nylon membrane. Thermogravimetric analysis of sample was done in the range of 50–750°C in nitrogen atmosphere (flow rate = 60 cm^3/min) at a heating rate of 20°C/min.

Scanning electron microscopy

Scanning electron micrographs of the modified and unmodified nylon membrane discs were obtained using STEREOSCAN 360 (Cambridge Scientific Industries) scanning electron microscope, after sputtering the sample with silver using silver sputtering instrument (BioRad Polaran Sputter, Model 450X). Two cycles of sputtering (2 min each) was used to have a coating thickness of about 100 Å on nylon discs to provide conduction.

Immobilization of antibody on NyM-*g*-GMA

NyM-*g*-GMA discs were equilibrated in phosphate buffer solution (50 m*M*, pH 7.4) for 30 min and then transferred to microcentrifuge tubes having antibody solution of different concentration (5–0.156 μg/mL). Immobilization was carried out at 4°C for 16 h with occasional shaking. After this period, antibody immobilized discs were removed from the antibody solution and washed with phosphate buffer saline (PBS) for three times. Discs were then stored at 4°C in fresh buffer until use.

Evaluation of antibody immobilized NyM-*g*-GMA discs using ELISA

Antibody immobilized NyM-*g*-GMA discs were evaluated using Disc ELISA to determine the minimum amount of Ab sufficient for immobilization and corresponding percent graft level of the nylon disc showing least nonspecific binding (NSB). Nylon discs of different percent graft level (10%G–100%G) were immobilized with different concentrations (5–0.156 μg/mL) of RAG IgG as described earlier. Discs were washed with PBS/Tween (PBS-T) and blocked with 2% skimmed milk (200 μL) for 1 h at 37°C. Blocked discs were washed with PBS-T and incubated with GAR-HRP IgG conjugate (1 : 16000) for 1 h at RT. Discs were again washed with PBS-T and transferred to 96-well microtiter plate. TMB/H_2O_2 was added and kept at RT for 7 min to determine the bound enzyme activity on the discs. The enzyme–substrate reaction was terminated by addition of 100 μL of 0.5*M* H_2SO_4 to give a yellow color product. The discs were removed from the wells and the color intensity of the solution was measured at 450 nm with reference filter of 650 nm using a BioRad ELISA microplate reader (Model 480). A set of the negative controls (discs without RAG IgG immobilization) was also kept for nylon membranes of all graft levels for determining NSB values and was given the same treatment as the test discs.

Evaluation of antibody immobilized NyM-*g*-GMA discs using Bradford assay

To determine the immobilization efficiency and amount of antibody immobilized per disc (Ab loading), antibody immobilized NyM-*g*-GMA discs with different GMA graft levels were evaluated using Bradford assay. Amount of Ab in the immobilization solution was determined using Commassie Brilliant blue method as described by Bradford,[25] at wavelength 595 nm. Amount of protein (antibody) immobilized on grafted nylon disc was determined by measuring the initial and final concentrations of protein in the immobilization solution and was calculated as:

$$Q = \{C_i - (C_t + \Sigma C_W)\} \times V \tag{3}$$

where, Q is the amount of antibody immobilized/disc (μg) or Ab loading (μg/disc), C_i and C_t are the concentrations of the antibody (μg/mL) in the initial solution and in the solution after immobilization, respectively. $\sum C_w$ is the total concentration of antibody (μg/mL) lost in three washings with PBS-Tween and V is the volume of aqueous phase (mL).

Immobilization efficiency (I.E.%) was also calculated for different concentration of immobilized antibody on nylon discs as:

$$\text{I.E.\%} = \frac{\text{Absorbance of immobilized Ab}}{\text{Absorbance of Ab in original solution}} \times 100 \tag{4}$$

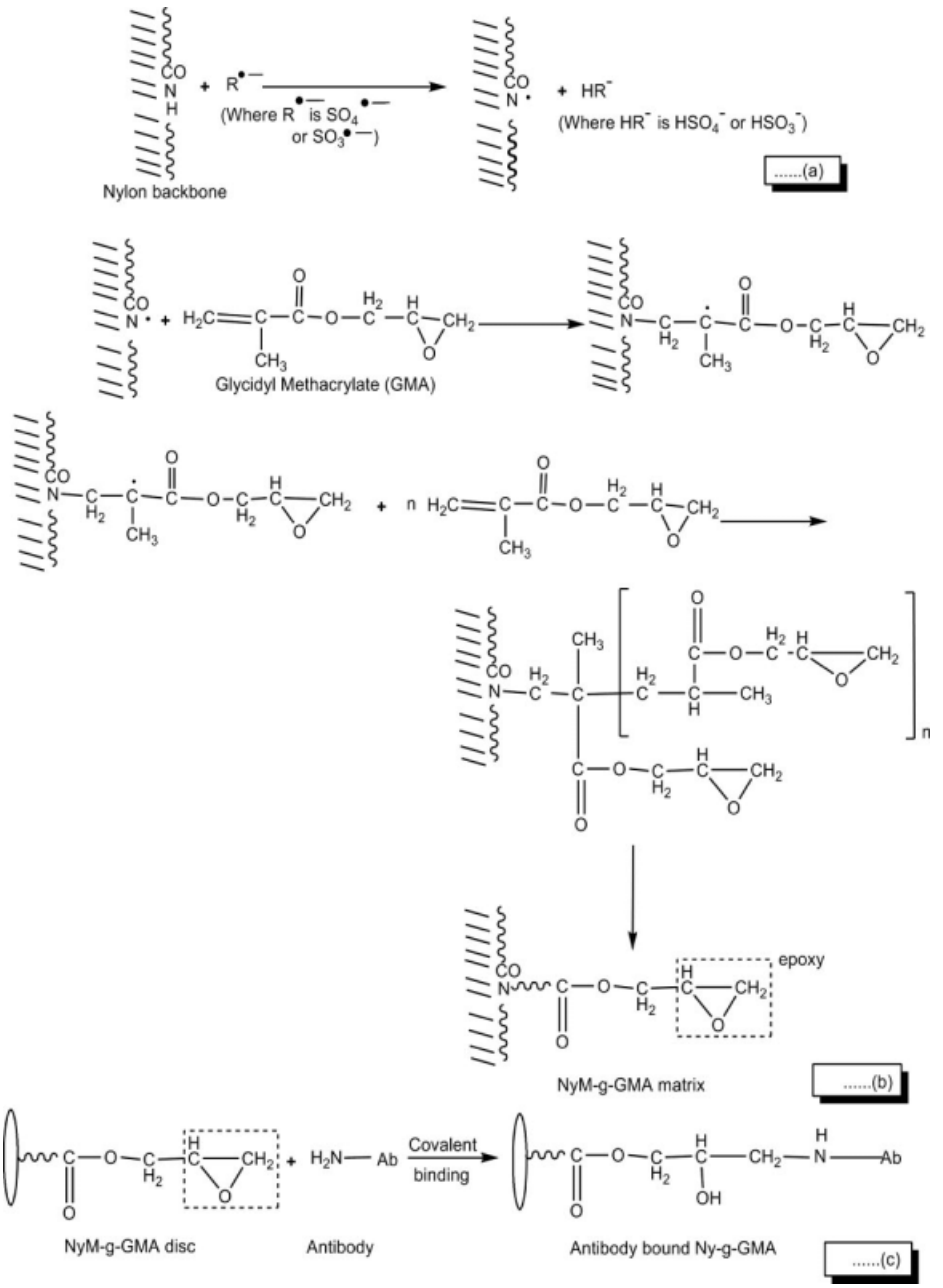
Stability studies of antibody immobilized nylon discs on storage

Stability studies of the antibody immobilized grafted nylon discs were also carried out over a period of time. RAG IgG (0.625 μg/mL) immobilized nylon discs (60%G) were kept for 8 weeks at 4°C and evaluated by ELISA at regular interval of 1 week for their stability. Similarly, another set of discs was also kept for 1 week at 37°C and evaluated at regular interval of 1 day.

RESULTS AND DISCUSSION

Graft copolymerization

Grafting of GMA onto nylon membrane was carried out by free radical redox polymerization method. The redox initiator, viz. persulfate and thiosulfate, used in graft copolymerization are soluble in water and the monomer GMA is reported to be soluble in presence of alcohol (methanol or ethanol)[21] in aqueous bath. Therefore, in this work, aqueous-alcohol was used as grafting medium. GMA was chosen as



Scheme 1 Reaction scheme for grafting of GMA on nylon membrane and the immobilization of antibody.

monomer because of its dual functionality of an acrylic double bond and an epoxy group in the same molecule. The probable mechanism (Scheme 1) of $K_2S_2O_8/Na_2S_2O_3$ initiated graft copolymerization of GMA onto nylon membrane involves $HSO_4^{\bullet -}/SO_3^{\bullet -}$ radicals generated from the decomposition of redox initiator. Anion radical thus formed abstracts H atom from the polyamide backbone and creates free radical active centers on the nylon surface[14,26] Once the active centers are formed, polymer chain starts to grow on them, resulting in chain branches to produce graft copolymer with the glycidyl pendant groups [eq. (b)]. Termination of the graft copolymer takes place through the combination of radicals.

To optimize the conditions of grafting, effects of various parameters such as monomer and initiator concentration, reaction time, and reaction temperature on the graft level were studied. Grafting reactions were carried out in duplicate and variation of ±2% was observed during grafting studies.

Effect of monomer concentration on graft copolymerization

The influence of monomer concentration on grafting reaction was studied using varied amounts of GMA (0.1*M* and 0.7*M*) at different temperatures (50–70°C), while other reaction parameters were kept constant.

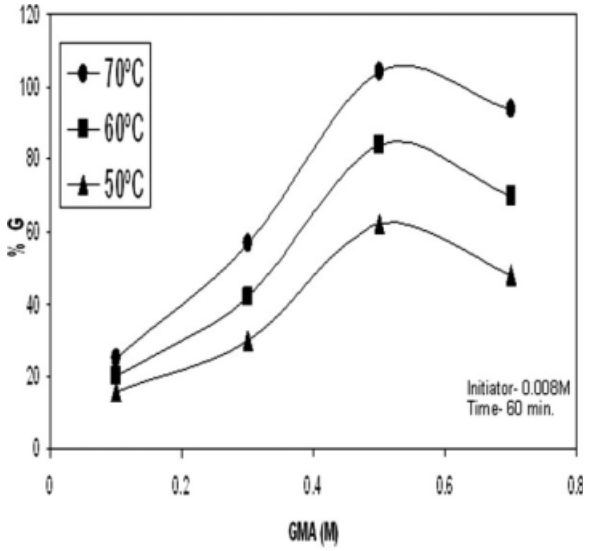


Figure 1 Effect of monomer concentration on grafting.

Figure 1 showed the effect of monomer concentration on graft copolymerization with the increase in concentration of GMA. Percent graft level (%G) increased with the increase in monomer concentration, reached maximum at 0.5*M* concentration of GMA and then decreased. This trend remained same at all the temperatures range under investigation but grafting increased with the increase in temperature from 50 to 70°C. The GMA concentration of 0.5*M* was therefore recognized as an optimum monomer concentration for grafting reaction. The enhancement in the rate of grafting on increasing the monomer concentration to an optimum value could be ascribed to the greater availability of monomer molecules to the free radicals (grafting sites) on the nylon membrane. After certain limit, the increase in GMA concentration accelerates the homopolymerization reaction rather than graft copolymerization due to the excess of the monomer. Other researchers have also witnessed similar observations when studying the effect of monomer concentration on grafting. Wang et al.[27]) studied the grafting of *N*-isopropylacrylamide into tubular type porous polyethylene membranes. They also reported that the grafting increased with monomer concentration up to a certain limit and afterward showed decreasing trend. Xu et al.[28] also reported similar observation for acrylic acid grafting onto microporous polypropylene hollow fiber membrane.

Effect of initiator concentration on graft copolymerization

The effect of redox initiator concentration on graft copolymerization was studied at various temperatures. The concentration of GMA used was 0.5*M* in the reaction for polymerization time of 60 min. The result obtained by changing initiator concentration from 0.004*M* to 0.016*M* is shown in Figure 2. The percent grafting reached a maximum value at the critical initiator concentration of 0.008*M* for all the temperatures studied. Any further increase in initiator concentration was accompanied by a decrease in grafting yield. Concentration of free radicals that were formed through decomposition of the initiator increased with the increase in initiator concentration. These radicals probably interacted directly with the surface to form active sites. A further increase in the amount of the initiators over 0.008*M* resulted in enormous primary radicals (surface radicals) and growing macroradicals of the side chains, which may interact with each other, resulting in a termination of reactive sites and, hence, a reduction in the percent grafting. Similar observation were also recorded by Sun et al.[29] and Gaffar et al.[30] for surface modification of chitosan by grafting of methacrylic acid using ammonium persulfate and cyclodextrin itaconate (CDI) using ceric ammonium nitrate, respectively.

Effect of time and temperature on graft copolymerization

The influence of polymerization time on percent grafting is shown in Figure 3. With an increase in polymerization time, the graft level increased initially and reached a plateau in 60 min as no further increase was observed after this polymerization period. When the reaction time was increased, more time was available to the free radicals for reaction, and it, therefore, resulted in higher percent grafting. After sometime, all the initiator and

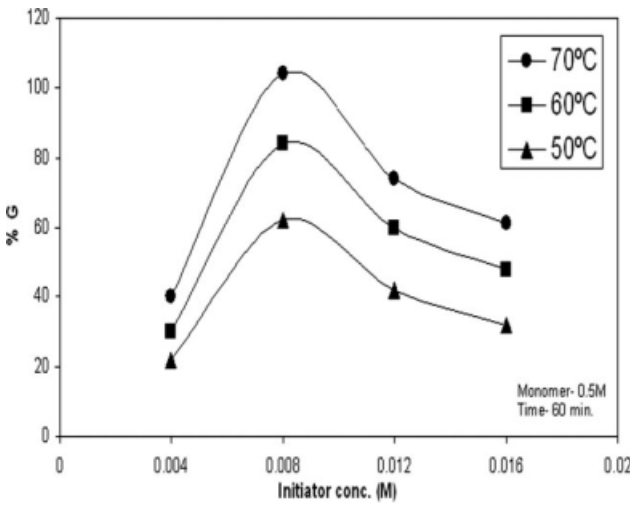


Figure 2 Effect of initiator concentration on grafting.

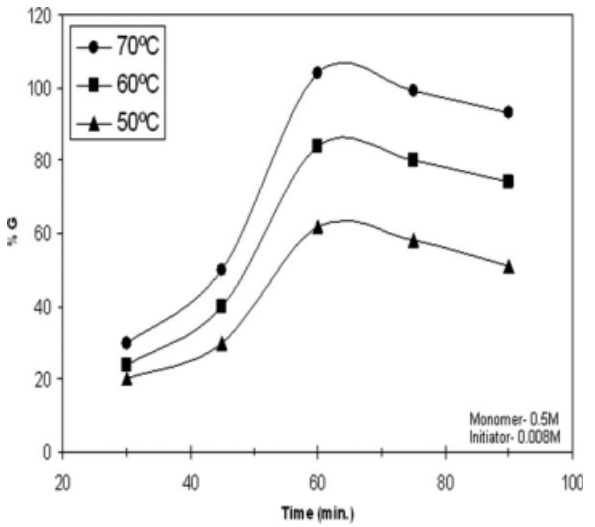


Figure 3 Effect of polymerization time concentration on grafting.

monomer molecules were consumed, and, hence, there was no further change in the percent graft level observed. Thus, the optimum polymerization time recorded was 60 min. Mosleh also reported similar observation for graft copolymerization of 2-ethyl methacrylate phosphoric acid (EMPA) onto nylon 6 fabric was carried out using the $K_2S_2O_8$/$CuSO_4$ system as reaction initiator.[31] Figure 4 shows the effect of temperature on percent of grafting and grafting efficiency for various grafting reactions. Maximum %G (100%) was obtained at 70°C that decreased with further increase in temperature at fixed monomer concentration (0.5*M*) and initiator concentration (0.008*M*) for 60 min. The dependence of percent graft level (%G) on temperature could be ascribed to higher rate of dissociation of initiator as well as the diffusion and mobility of monomer from the aqueous phase to nylon matrix, resulting in considerable improvement in grafting yield. The grafting efficiency (%GE) reached a maximum value of 82% at 70°C. With further increase of temperature beyond 70°C, chain radical termination by disproportionation and chain transfer might be accelerated, leading to decrease of %G as well as %GE. These findings were found to be in agreement to those reported in literature.[32]

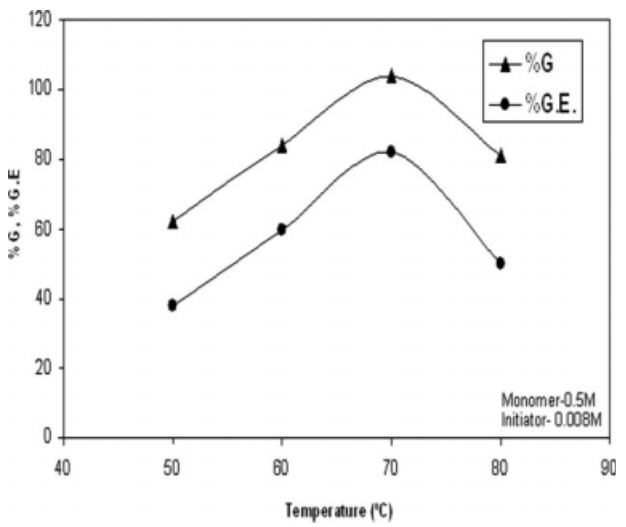


Figure 4 Effect of polymerization temperature on percent of grafting (%G) and grafting efficiency (%G.E.).

Characterization studies of GMA grafted nylon membranes

ATR-FTIR spectroscopy

FTIR spectra of ungrafted and grafted nylon membrane samples are presented in Figure 5. FTIR spectrum of nylon membrane exhibits characteristic absorption bands at 3300, 1633 and 1536 cm^{-1} because of *N*—H bending vibrations in primary amine/amide, C—O stretching of amides and combination absorbance of *N*—H and C—N of amidos of nylon membrane, respectively[23] [Fig. 5(a)]. Grafted surface exhibited prominent peaks at 906 and 845 cm^{-1} due to epoxide groups of grafted GMA. Absorbance at 1728 and 1171 cm^{-1} increased with the increase in grafting percentage of the membrane that corresponds to the carbonyl functional group and to the vibration of ester (C—O—C) of the grafted GMA, respectively. These results confirmed the

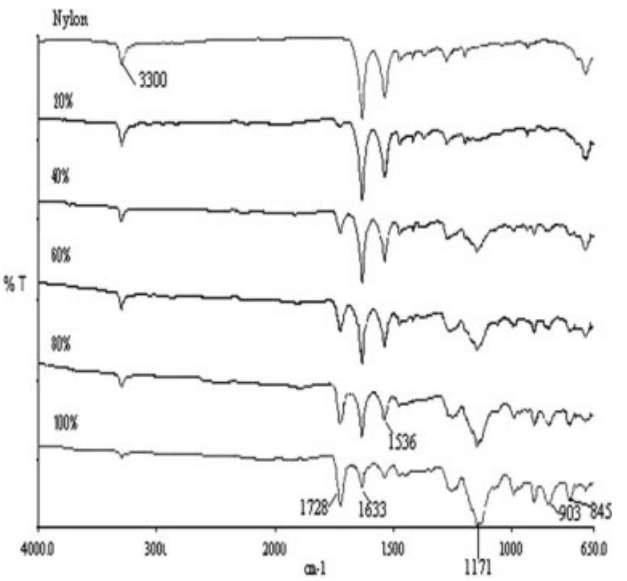


Figure 5 FTIR spectra of (a) ungrafted nylon and (b–f) GMA-grafted nylon membrane with varying percent of GMA grafting.

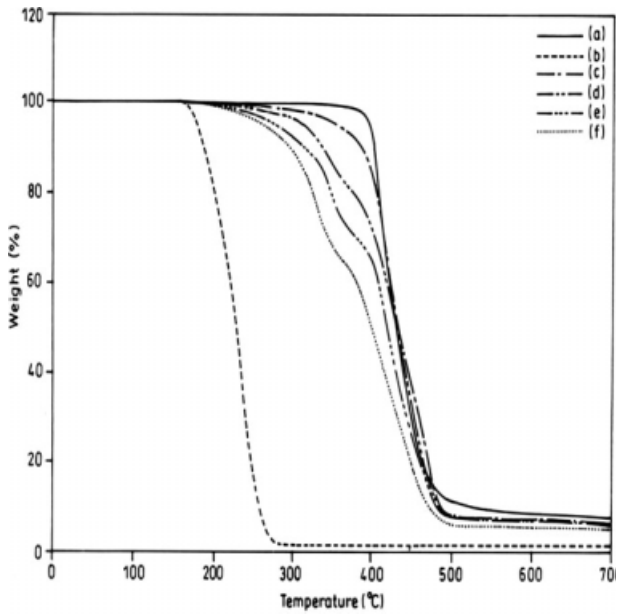


Figure 6 TGA thermograms of (a) ungrafted nylon membrane, (b) homopolymer polyGMA, and (c–f) GMA grafted nylon membrane with varying grafting percent [c(20%G), d(60%G), e(80%G), f(100%G)].

grafting of GMA onto the nylon membrane. Additionally, the intensity of these characteristic peaks, corresponding to those of GMA, increased with an increase in the graft level. GMA grafting on nylon membrane was further confirmed by the decreasing weak band intensity at 3300 cm^{-1} of NyM-*g*-GMA spectra as compared to the spectrum of the original nylon membrane. Weakening of the band was due to the utilization of some —NH groups of nylon during the formation of the graft copolymer as shown in Scheme 1.

Thermo gravimetric analysis

TGA thermograms of ungrafted and grafted nylon membrane samples were studied to understand their thermal behavior (Fig. 6). The ungrafted nylon membrane showed single stage of weight loss due to the decomposition of nylon backbone (399 to 500°C). The TG curve of PGMA homopolymer also showed single stage degradation due to random chain scission, which started at around 168°C and reached maximum at 280°C. Similar observation has also been reported by Caykara and Alaslan[33] and Tyagi et al.[13] The GMA grafted nylon samples, on the other hand, followed two step degradation patterns involving first stage loss from 168 to 340°C which may be related to the degradation of PGMA chains. The subsequent loss of nylon matrix started thereafter. The stage related to the degradation of GMA grafted surface showed an increase in weight loss with an increase in grafting level. This was expected because the PGMA component also increased along with an increase in percent grafting.

Scanning electron microscopy

The electron micrographs corresponding to modified and unmodified nylon membrane are depicted in Figure 7. Micrographs were studied to observe the changes in surface morphology of modified and unmodified nylon membrane. Before grafting, the nylon membrane surface had a smooth and relatively homogeneous appearance. Grafting of GMA developed roughness and introduced heterogeneity

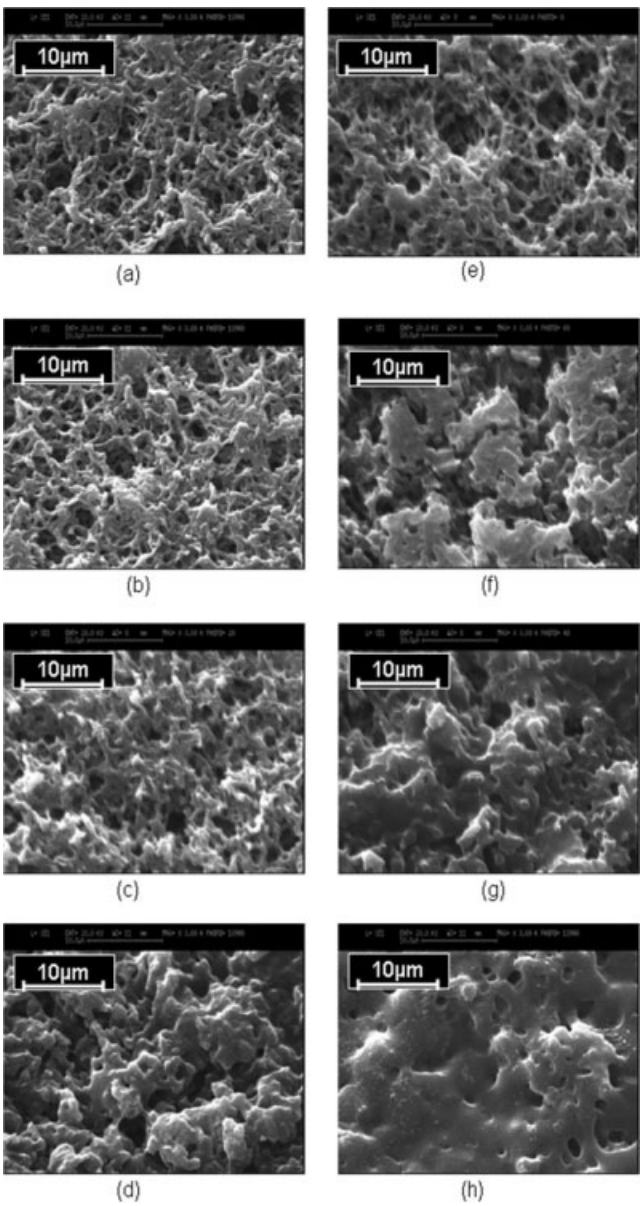


Figure 7 Scanning electron micrographs of (a) ungrafted nylon membrane, (b–d) GMA grafted nylon membrane with varying percent of GMA grafting [b(20%G), c(60%G), d(100%G)], and (e–h) membranes after antibody immobilization.

along and across the membrane that increased with the increase in degree of grafting [Fig. 7(b–d)]. This may be due to the attachment of the hydrophobic PGMA grafted chains onto the hydrophilic nylon membrane matrix. After the immobilization of antibody, again there were changes in the surface topography of modified membrane which became smooth and covered thereby proving the immobilization of biomolecule [Fig. 7(e–h)].

Immobilization of antibody on NyM-*g*-GMA discs

Nylon membrane was chosen as the matrix because of being porous, more aqueous stable, reasonable price, and easy availability. To impart functionality to the nylon surface, GMA with pendant reactive epoxide groups was grafted using redox free radical polymerization technique. Immobilization of antibody onto NyM-*g*-GMA discs was achieved by covalent coupling of epoxy groups of the GMA with amino groups of the antibody [eq. (c), Scheme 1].

Evaluation of antibody immobilized NyM-*g*-GMA discs using ELISA

The activity of immobilized antibody (RAG IgG) was determined by its binding to the antigen (complementary antibody, GAR-HRP IgG conjugate), and the event of Ag-Ab complex formation was determined by ELISA.[34] It was observed that antibody immobilization increased with the increase in percent graft level of nylon discs, as shown in Figure 8. It could be ascribed to the fact that with the increase in percent graft level more GMA was grafted onto

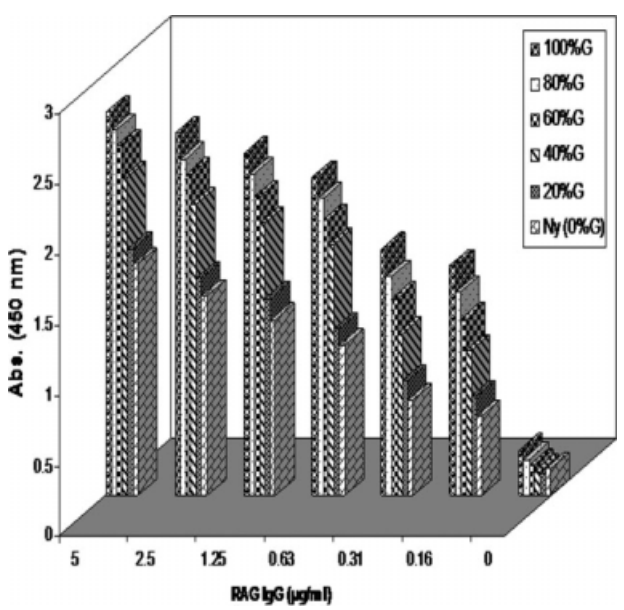


Figure 8 NyM-*g*-GMA Disc ELISA with different concentration of immobilization Ab solution (RAG IgG) at conjugate (GAR IgG-HRP) dilution of 1 : 16000.

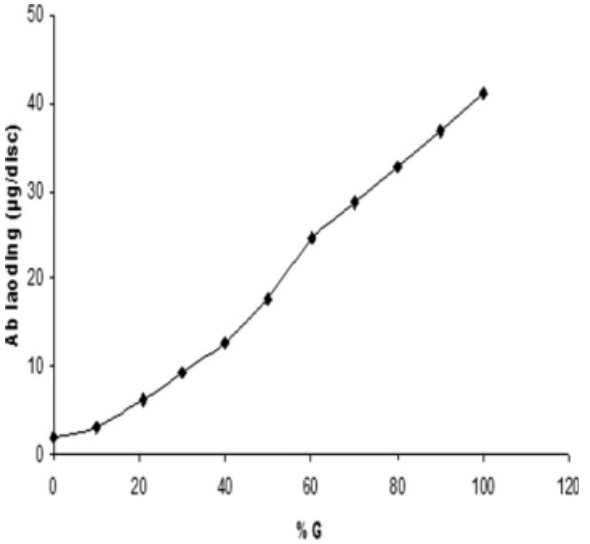


Figure 9 Effect of grafting percentage on antibody loading (µg/disc).

the surface and, consequently, more epoxy functionality of GMA became available for attachment of antibody. Disc with 100% graft level showed maximum binding but the value of NSB was also found to be quite high. Nylon membrane with 60% graft level showed good binding with lower NSB. Therefore, on the basis of optimum signal-to-noise ratio (i.e., binding to NSB ratio), nylon disc with 60% graft level was selected for further studies. Furthermore, it was seen that for any particular percent graft level, the Ab immobilization decreased with the decrease in the Ab concentration. The minimum Ab concentration that showed appreciable binding (absorbance) with Disc ELISA was found to be 0.625 µg/mL at 60% GMA graft level.

Evaluation of antibody immobilized NyM-*g*-GMA discs using Bradford assay

The NyM-*g*-GMA discs with different percent graft level (from 10%G to 100%G) were studied for determining amount of Ab loading per disc and efficiency of Ab immobilization. Results showed an increase in the amount of antibody bound, from 2 to 41 µg antibody/disc, with an increase in GMA content of the disc (Fig. 9). Ungrafted nylon showed the Ab loading of 2 µg/disc only, whereas Ab loading/disc increased to 41 µg/disc for NyM-*g*-GMA with 100% graft level. These findings further confirmed the ELISA results. It was also observed that with an increase in concentration of antibody in the immobilization medium ((1.25 to 100 µg/mL), there was an increase in the immobilization efficiency, which leveled off at an Ab concentration of 50 µg/mL (Fig. 10). This may be attributed to the limitation of available active epoxy groups and/or steric

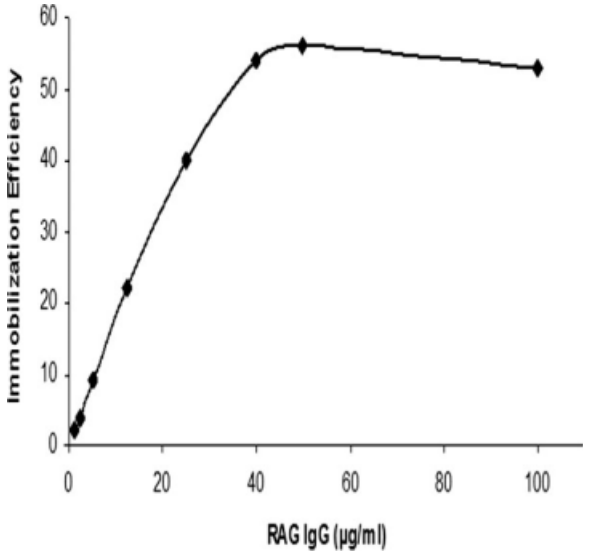


Figure 10 Effect of antibody concentration on the immobilization of RAG IgG on NyM-*g*-GMA discs (60% G).

hindrance of immobilized Ab at higher antibody concentration. Maximum I.E. % of 56% was observed for membrane with 60% GMA graft level at 50 µg/mL of RAG IgG in PBS (pH 7.4).

Stability studies of antibody immobilized NyM-*g*-GMA discs on storage

Ab immobilized NyM-*g*-GMA discs were found to be stable up to 6 weeks at 4°C (Fig. 11) and 2 days at 37°C (Fig. 12). It was observed that nylon membrane showed binding but the binding decreased gradually over a period of 6 weeks. NyM-*g*-GMA discs with 60% graft level showed not only

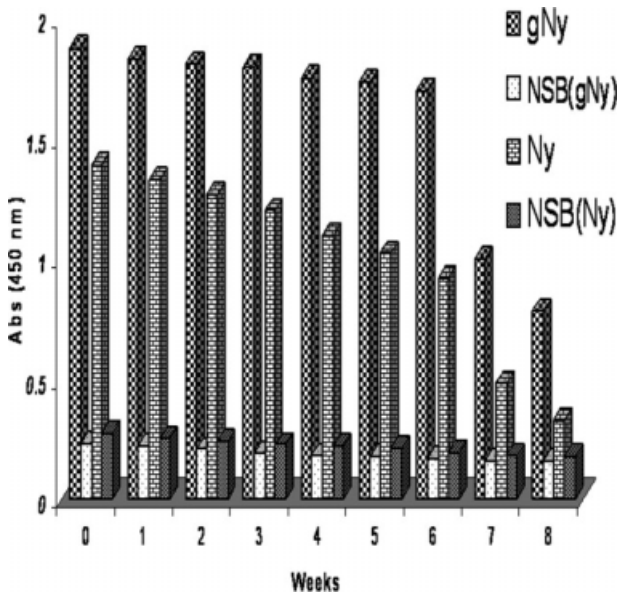


Figure 11 NyM-*g*-GMA Disc ELISA of immobilized RAG IgG (0.625 µg/mL) for storage stability study at 4°C.

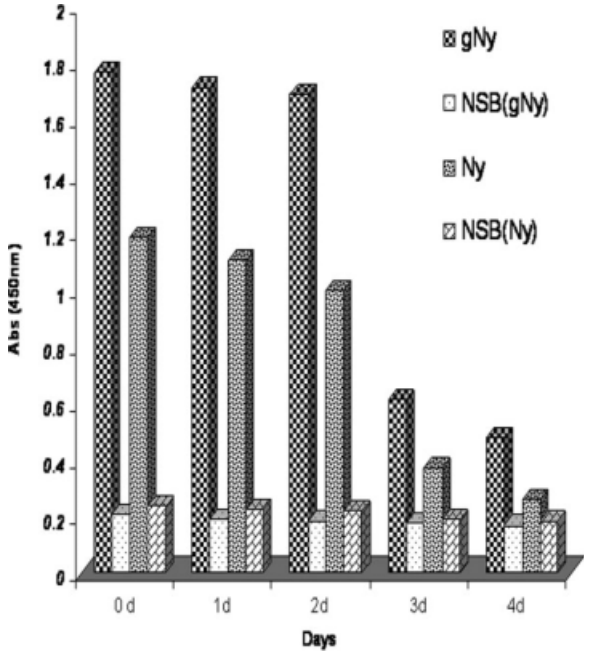


Figure 12 NyM-*g*-GMA Disc ELISA of immobilized RAG IgG (0.625 µg/mL) for storage stability study at 37°C.

higher binding but also it remained constant up to the entire period of 6 weeks. It could be attributed to the unstable ionic interaction of Ab with positively charged nylon surface that lead to the gradual leaching of the noncovalently bound Ab. Moreover, less binding of Ab was due to the lesser availability of reactive sites on ungrfated nylon surface for immobilization. Higher and constant binding in case of NyM-*g*-GMA was because of covalent attachment of antibody with epoxy groups of grafted GMA chains that prevented desorption of biomolecule from the matrix during storage.[35]

CONCLUSIONS

GMA was successfully grafted onto nylon membrane under an inert atmosphere, using redox initiator. Degree of grafting varied from 10 to 100% under different reaction conditions. Antibody (RAG IgG) was covalently immobilized onto the modified-nylon membrane surface via epoxy groups of grafted GMA. NyM-*g*-GMA with 60% graft level was found to be optimum in terms of maximum immobilization efficiency and lower NSB. Ungrafted nylon membrane was found to have less loading and unstable binding of Ab, whereas NyM-*g*-GMA showed higher loading and stable binding of Ab. The increased stability and covalent binding of biomolecules (Ab) on GMA grafted nylon discs make them promising candidate to evaluate them as matrices/platform for biosensors, well-plates for enzyme linked immunofiltration (ELIFA) systems, Disc ELISA for

the detection of various antigens including whole cell bacteria.

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