Immobilized Metal Ions Cellophane–PGMA-Grafted Membranes for Affinity Separation of β-Galactosidase Enzyme. I. Preparation and Characterization

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ABSTRACT: Immobilized Cu^{2+} ions affinity cellophane–poly(glycidyl methacrylate) (PGMA)-grafted membranes have been prepared through three steps. The first step was introducing of epoxy groups to its chemical structure through grafting process with PGMA. Factors affecting the grafting process have been studied and grafting percentage (GP) up to 233% has been obtained. The second step was converting the introduced epoxy groups to sulfonic ones. It was found that maximum amount of sulfonic groups (2.7 mmol/g) was obtained with minimum GP (46.08%). The third and last step was the immobilization of Cu^{2+} ions into sulfonated grafted membranes obtained from the previous step. Maximum amount of immobilized Cu^{2+} ions was found to be 60.9 ppm per gram of polymer. The verification of the grafting and sulfonation steps has been performed through characterization of the obtained membranes using FTIR, TGA, and EDAX analysis. Finally, Cu^{2+}-immobilized membranes have been evaluated in separation of β-galactosidase (β-Gal) enzyme from its mixture with bovine serum albumin (BSA) in different pH medium. Maximum protein adsorption, for both proteins, has been obtained at pH range 4–4.5; as 90 and 45% for β-Gal and BSA, respectively. The results showed high affinity toward β-Gal separation although BSA concentration (0.5%) is 20-folds of β-Gal (0.025%). © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2647–2656, 2009

Key words: chromatography; proteins; enzymes; separation techniques; graft copolymers

INTRODUCTION

In general, membrane materials could be divided into two categories: inorganic and organic.[1,2] Inorganic materials usually show better performance in mechanical strength, thermal stability, and chemical resistance than organic materials. But on the other hand, their pore properties, cost, capability for surface modification may not be competitive. Accordingly, inorganic materials are infrequently adopted as the affinity membrane supports. The inorganic substrates found in the literature are titanium oxide modified to form anion exchange membranes[3] and glass hollow fibers used for immobilized metal affinity membranes (IMAMs).[4] Organic membranes are commonly made of natural or synthetic polymer. The materials include cellulose (cellulose acetate, cellulose nitrate, cellulose ester, regenerated cellulose, etc.), hydrocarbon polymers (polyethylene, polypropylene, etc.), aromatic copolymers (polycarbonate, polysulfone, polyethersulfone, etc.), aliphatic polyamides (nylon-6, nylon-66, etc.), polyvinyl alcohol, synthetic copolymer, and so on. These organic materials and their properties have been thoroughly evaluated in several of previous review papers.[1,2] The IMAMs are one of the most popular affinity membranes (others include dye affinity and protein A/G affinity membranes). By far, there are a lot of published research papers regarding this topic.[1,2,4–9] Their designs basically follow the IMA chromatographic systems developed since 1970s, and hence, the properties and applications are very similar. IMA method generally adopts the chelators coupled on the supporting matrix to immobilize metal ions (as electron-pair acceptors), which could specifically interact with the exposed electron-donating amino acid residues (such as histidine, cysteine, tryptophan, tyrosine, aspartic acid, or glutamic acid) on biomolecule surface through nonbonding lone pair electron coordination.[4–11] In determining the immobilized metal ion capacity, the functional groups on the base membrane material, the nature of chelating agent, immobilization method, types of metal ions, and metal ion concentration all play an important role. Among these factors, chelating agent and metal ions have particularly significant influences

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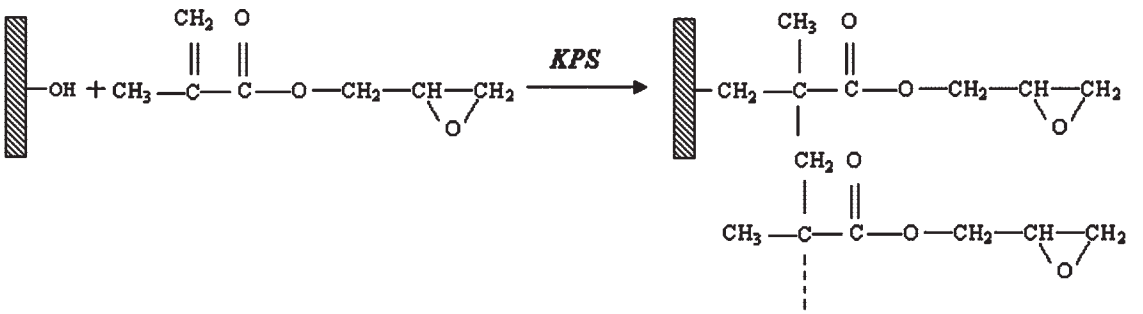


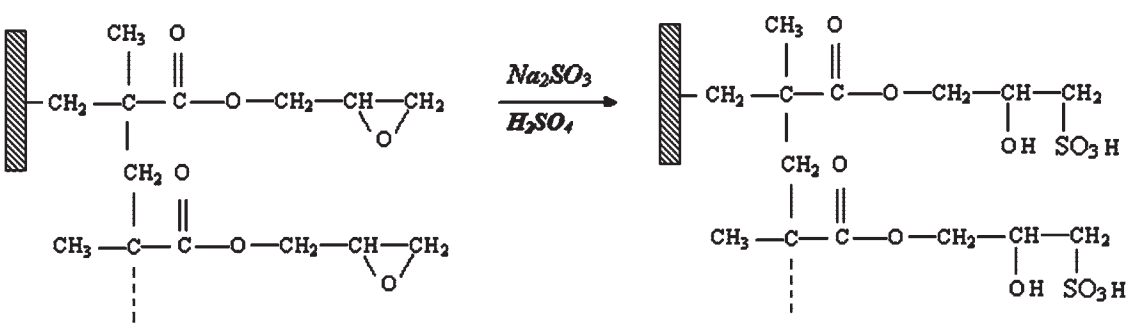
Scheme 1 Mechanism of cellophane grafting membranes.

and are most commonly evaluated in the related chromatographic literature. Immobilized-metal affinity membrane chromatography (IMAMC) is a separation technique that uses covalently bound chelating compounds on membrane supports to immobilize metal ions, which serve as affinity ligands for various proteins.[12,13] In the past decade, applying affinity membranes for biomolecules separation has gained much attention owing to the lower mass-transfer limitations revealed for the membrane process than conventional column chromatography.[1,2,14,15] The most used technique in the literature to prepare IMAMs from regenerated cellulose is inducing chelating agents into its structure.[1,16] One of the main drawbacks of using this method is the leakage of metal ions through the elution step,[17,18] which was mainly explained by insufficient chelating bonds with metal ions. Grafting technique has been persecuted as one of the solutions where unlimited numbers of functional groups with metals chelating character could be induced to have higher amounts and more stable immobilized metal ions.[1,16] IMAMC has many advantages over typical methods in affinity chromatography: different metal ions can be immobilized on the same chelating medium and can be easily removed for regeneration by a stronger chelator such as EDTA without any detectable loss of metal chelating properties of the membrane matrices.[19] Copolymer of acrylates can meet most of these requirements; they are very component of the network. They also possess sufficient mechanical strength and also GMA monomer can provide a reactive epoxy groups for covalent attachment of various ligand molecules, and thus, the support activation procedure can be eliminated.[20–22] IMAC has been used to examine the relationship between amino acid side chain surface topography of proteins and binding selectivity.[23] One of the more recent applications of IMAC is the purification of recombinant proteins containing histidine tags.[23] Up till now, $Cu^{2+}$and Ni^{2+} are the most commonly used metal ions for IMA method in the literature and the commercial chromatographic systems. The amino acid side chain interaction with the chromatographic adsorbents immobilized with these two metal ions could refer to the literature. In summary, most of the membranes employ IDA and copper ions[2,4–6] and high-percentage chelator utilization could be achieved. In this work, cellophane membranes have been functionalized with sulfonic groups, which are known as strong cations exchanger to immobilized Cu^{2+} metal ions. The immobilized Cu^{2+} ions membranes have been used in the affinity separation of β-galactosidase (β-Gal) form protein mixture with BSA protein.

EXPERIMENTAL

Materials

- Glycidyl methacrylate (GMA) (purity 97%) is obtained from Sigma-Aldrich Chemicals, (Switzerland).
- Potassium persulphate (KPS) (purity 99%, M. wt. 270.31),



Scheme 2 Mechanism of sulfonation of PGMA-*g*-cellophane membranes.

- Sulfuric acid (purity 95–97%),
- 2-Propanol (purity 99.8%),
- Bovine serum albumin (BSA fraction V, minimum 96% electrophoresis, nitrogen content 16.2%),
- Sodium chloride (purity 99.5%, M. wt. 58.44), and
- Acetic acid (purity 99.8%, M. wt. 60.05) are obtained from Sigma-Aldrich Chemicals, (Germany).
- β-Galactosidase (from *Aspergillus oryzae*) is obtained from Sigma-Aldrich Chemicals, (USA).
- Ethyl alcohol absolute (purity 99.9%),
- Sodium sulfite anhydrous (SS) (purity 95%),
- Methyl alcohol (pure reagent for analysis),
- Copper sulfate (purity 98%, M. wt. 249.68),
- Lactose (Pure Lab. Chemicals M. wt. 360.31), and
- Sodium acetate trihydrate (purity 99%, M. wt. 136.08) are obtained from El-Nasr Pharmaceutical Co. For Chemicals, (Egypt).
- Glucose kit (enzymatic colorimetric method) and
- Total protein kit (colorimetric method) are obtained from Diamond Diagnostics Co. for Modern Laboratory Chemicals, (Egypt).
- Cellophane sheets type, uncoated; dimensions, 80 cm × 117 cm; cellulose content, 80% (W %) regenerated cellulose; additives content, 20% (glycerol and Na2SiO3) is obtained from Misr Rayon Co. Kafr El-Dawar, (Egypt). The additives were removed by extraction with hot distilled water, then the films were cut with dimensions 5 cm × 5 cm.

Methods

Membrane preparation

Grafting process. Grafting is carried out using different monomer concentrations (2 : 12% v/v) dissolving in KPS solution (0.004 : 0.012*M*) with different composition of ethanol/water. Grafting process was conducted in different temperatures (35 : 65°C) in a water bath for different time intervals (1 : 6 h) (Scheme 1).

The grafting process was monitored through evaluation of different grafting parameters namely,

Grafting percent (GP %), grafting efficiency (GE %), and weight conversion (WC %). These parameters were calculated using the following equations[24]:

$$\text{GP } \% = \frac{\text{Wt. of grafted membrane} - \text{Wt. of original membrane}}{\text{Wt. of original membranes}} \times 100 \quad (1)$$

$$\text{GE } \% = \frac{\text{Wt. of grafted membrane after ext.} - \text{Wt. of original membrane}}{\text{Total Wt. of converted polymer}} \times 100 \quad (2)$$

$$\text{WC}\% = \frac{\text{Wt. of grafted polymer} + \text{Wt. of homopolymer}}{\text{Wt. of used monomer}} \times 100 \quad (3)$$

Sulfonation process. The epoxy groups of the PGMA chains were reacted with sodium sulfite (SS) (Na_2SO_3) dissolved in alcohol (ethyl alcohol, methyl alcohol, isopropyl alcohol) and water in different ratios using water bath at different temperatures (30 : 80°C) and for different time intervals (15 : 120 min) (Scheme 2). The amount of sulfonic acid groups (—SO_3H) was determined by titration using developed procedures from one described in the literature.[25–27] The membrane was equilibrated in 20 mL of NaOH 0.05*M* for 1 h at room temperature, washed with distilled water 30 mL. The total volume solution (50 mL) was then titrated using phenolphthalein indicator to end-point against 0.05*M* HCl.

The SO_3H group density and its conversion by sulfonation were calculated as follows[28]:

$$SO_3H \text{ group density (m mol/g)} = \frac{\text{Amount of So}_3\text{H groups}}{\text{Wt. of sulfonated grafted film}} \quad (4)$$

$$\text{Conversion, \%} = \frac{\text{Amount of } SO_3H \text{ groups}}{\text{Amount of epoxy groups before sulfonation}} \times 100 \quad (5)$$

Immobilization of Cu^{2+} ions. The cellophane sulfonated-grafted membrane was immersed in 20 mL of copper sulfate solution (5 mmol) in water bath for temperatures (60°C) of pH (5.4) at 2 h. The amount of copper remaining in solution was then measured using atomic absorption spectrophotometer (Perkins-Elmer Analyst 300, USA).[17,18]

Protein adsorption

The membrane immobilized with Cu^{2+} ions was immersed in protein solution, pH (2.6 : 5.2), containing β-Gal (0.025%) and BSA (0.5%) in a water bath at a temperature of 30°C for 2 h.

Determination of β-galactosidase enzyme amount

One milliliter of protein solution, adsorbing and/or eluting, was mixed with 100 mmol lactose solution, pH 4.4, at 250 rpm for 30 min in room temperature. Samples were taken every 5 min to assess the glucose production using glucose kit. Enzyme activity is given by the angular coefficient of the linear plot of the glucose production as a function of time.

β-Gal enzyme amount was determined as follows:

$$\text{Amount of } \beta - \text{Galactosidase (mg)} = \frac{\text{Measured protein solution activity}}{\text{Activity of 1 mg of free enzyme}} \times 1 \text{ mg} \quad (6)$$

Determination of bovine serum albumin amount

Using total protein kit, 20 μL of protein solution, adsorbing and/or eluting, was mixed with 1 mL reagent 2 [NaOH 0.2*N*, K-Na-tartarate 18 mmol/L, potassium iodide 12 mmol/L, cupric sulfate 6 mmol/L] (A sample) and 20 μL of reagent 1 [protein standard 6 g/dL] with 1 mL reagent 2 (A standard).

Mix and incubate for 5 min at 20–25°C. Measure the absorbance of sample (A sample) and standard (A standard) against reagent blank

$$\text{Bovine serum albumin amount (mg)} = \frac{\text{A sample}}{\text{A standard}} \times 6 \quad (7)$$

Membrane characterization

Water uptake (W %). For membrane previously immersed in distilled water at room temperature for 24 h, the surface was dried by wiping with filter paper and weighing. The obtained results are the average of three samples.[29]

$$W\% = \frac{\text{Wt. of wet membrane (gm)} - \text{Wt. of dry membrane (gm)}}{\text{Wt. of dry membrane (gm)}} \times 100 \quad (8)$$

Infrared spectrophotometric analysis. Analysis by I.R. spectroscopic investing anions for ungrafted and grafted membranes having different GPs was carried out using Fourier Transform Infrared Spectrophotometer (Shimadzu FTIR-8400 S, Japan).

Thermalgravimetric analysis. Analysis by TGA of ungrafted and grafted membranes having different GPs was carried out using Thermogravimetric Analyzer (Shimadzu TGA-50, Japan).

Energy dispersive analysis X-ray. Elemental analysis of ungrafted and grafted membranes having different GPs was carried out using Energy Dispersive Analysis X-ray (Joel Jsm 6360LA, Japan).

RESULTS AND DISCUSSION

Membrane preparation

Grafting process

Effect of monomer concentration on the process parameters. Diffusibility of monomers into the polymer matrix has a great influence on the grafting process. The effect of monomer concentration on the GP, GE, and total WC was investigated. Figure 1 shows that increasing the monomer concentration clearly increased the percentage of grafting and GE. Maximum GP was obtained at 10% GMA solution then tends to level off, whereas the GE reaches maximum at 8% GMA then tends to decrease slightly with further increase of GMA. These observations may be attributed to the following explanations.

1. Increasing the monomer concentration up to 10% facilitates the diffusibility of monomer toward the initiated sites on the cellulosic chains of cellophane membranes, which consequently increases the grafting yield.
2. At monomer concentrations higher than 10%, the rate of radical formation from the monomer becomes greater when compared with that of rate of diffusion through the polymer matrix, and a homopolymer is formed as a layer on the membrane's surface reducing, as a sequence, the diffusivity of GMA monomer; and hence, the GP and efficiency tend to decrease.

Effect of initiator concentration on the process parameters. Figure 2 shows the effect of KPS

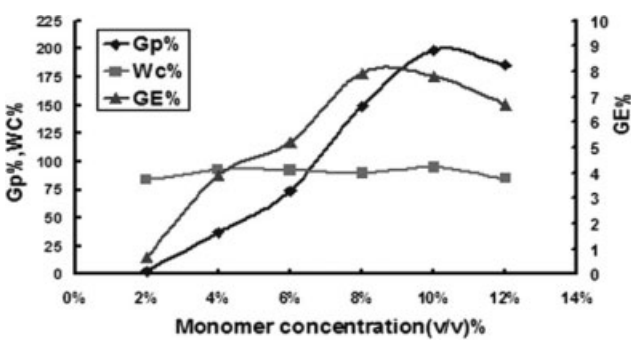


Figure 1 Effect of monomer concentration on the percentage of grafting, grafting efficiency, and weight conversion.

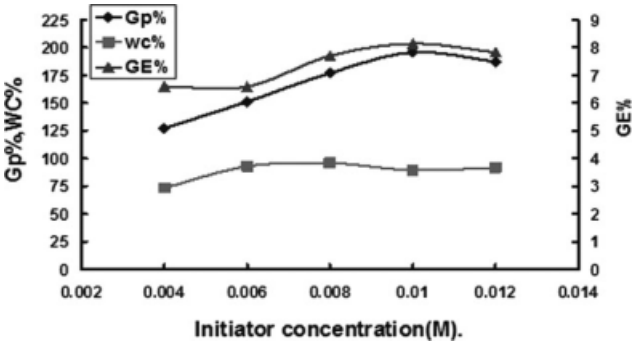


Figure 2 Effect of initiator concentration on the percentage of grafting, grafting efficiency, and weight conversion.

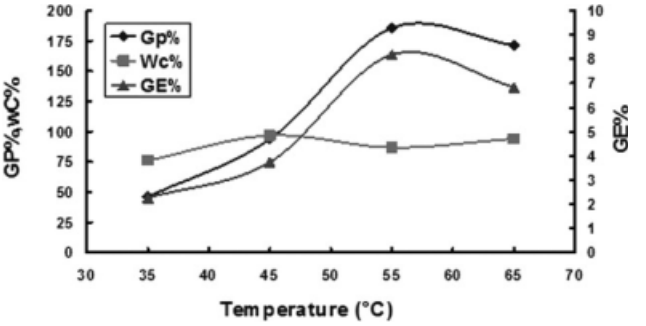


Figure 3 Effect of temperature on the percentage of grafting, grafting efficiency, and weight conversion.

concentration on the studied grafting parameters. It is clear that increasing the initiator concentration from 0.004 to 0.01*M* is accompanied by significant increase in the graft percentage. Further increase of the concentration beyond 0.01*M* results in the leveling-off of GP. On the other hand, the WC remains practically constant with increasing the initiator concentration beyond 0.006*M*. This may be due to the fact that at such initiator concentration complete conversion has been obtained. Further increase in the number of the formed free radicals, as a result of increment of initiator concentration, resulted in increase of diffusive numbers of formed free radicals inside the membranes in benefit of the GP. The GE of the system remains relatively unchanged, in spite of its low value.

Effect of grafting temperature on the process parameters. Effect of variation of the polymerization temperature on the grafting parameters is illustrated in Figure 3. A substantial increase in the GP and GE is observed with variation of the temperature from 35 to 55°C. This enhancement in grafting of GMA onto cellophane membranes on raising the polymerization temperature might be attributed to the following favorable effects of temperature on:

1. Diffusion of GMA from the solution phase to the swellable cellulose phase.
2. Increasing the solubility of the monomer.
3. Increasing the rate of thermal dissociation of KPS and hence the GMA rate of free radical formation on the membrane backbone.
4. Formation and propagation of grafted chains.

The net effect of all such factors leads to high grafting yield with increasing the polymerization temperature. Further increase of the polymerization temperature beyond 55°C resulted in decrease of both GP and GE. This could be attributed to the formation of homopolymer film of hydrophobic nature on the surface of cellophane membranes. Because poly(glycidyl methacrylate) (PGMA) did not dissolve in the monomer solution, it creates a diffusion barrier preventing the monomer from reaching to the cellulosic backbone, which leads to the formation of homopolymer on favorite of grafting process.

Effect of grafting time on the process parameters. The effect of variation grafting time on the grafting parameters is illustrated in Figure 4. The results reveal that increasing the grafting time from 1 to 5 h, increases the GP and GE in the same manner to reach maximum values at 5 h of grafting time. This kind of behavior may be referred to the combination of two processes. The first is increase the number of formed free radicals on both monomer solution phase and polymer solid phase. Simultaneously, in parallel with that, the amount of diffused monomer into the polymer phase increased during postgrafting time. The combination of the two processes leads finally to increase both GP and GE. On the other hand, further increase of the reaction time to 6 h affected negatively both GP and GE. This could be explained by the favorite of homopolymer formation over the grafting process. Because the homopolymer is hydrophobic, its precipitation on the surface of membrane creating diffusion barrier reduced the

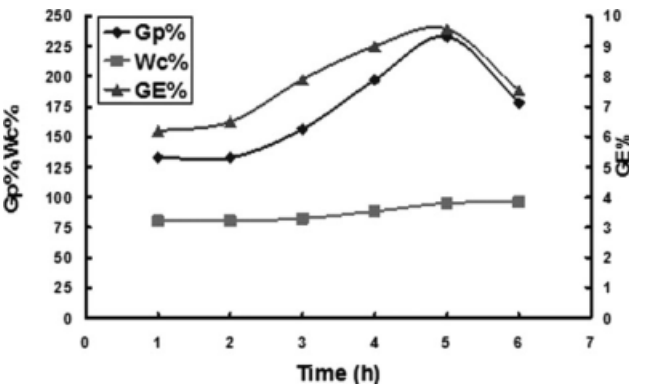


Figure 4 Effect of time on the percentage of grafting, grafting efficiency, and weight conversion.

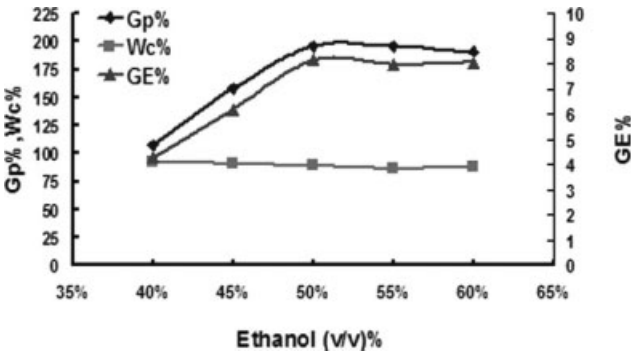


Figure 5 Effect of solvent composition (ethanol/water) on the percentage of grafting, grafting efficiency, and weight conversion.

amount of diffused monomer into the polymer matrix and hence the GP and GE.

Effect of solvent composition on the process parameters. The data illustrating the effect of solvent composition on the parameters of the grafting process are shown in Figure 5. It can be observed that the GP and GE increased linearly with ethanol percentage to reach maximum values in 50% ethanol aqueous solution then tend to level off. This could be explained by increasing the solubility of GMA which in turn facilitates its diffusion into the polymer matrix resulting in increase of both GP and GE. On the other hand, ethanol acts as a reducing agent, which facilitates the process of KPS oxidation to form free radicals.

Sulfonation process

The sulfonation process of the grafted membranes using SS alcoholic solution has been studied.[28] From the obtained data, it was found that increasing the GP over certain limit under any grafting conditions is generally accompanied by decrease in the amount of obtained sulfonic groups. The amount of sulfonic groups was found increased with GP increase up to 50%, which reaches its maximum value 2.7 mmol/g. The highest GP obtained (233%) has 1.02 mmol/g. This may be explained on the light of increasing the hydrophobic nature of the grafted membranes, with percentage of grafting, decreases the diffusibility of SS into the depth of polymeric matrix, and hence reduces the converted numbers of epoxy groups to sulfonic ones. On the other hand, the effect of the grafting conditions on the degree of sulfonation of four grafted membranes having almost the same GP (150, 150, 156, and 157%) but prepared under different conditions have the following amount of sulfonic groups; 1.5, 1, 1.1, and1.4 mmol/g, respectively. It is observed that how much the variation of the grafting conditions could affect the efficiency of the sulfonation process. This observation leads us to study the sulfonation process conditions in details.

Effect of sodium sulfite concentration on the amount of sulfonic group and conversion (%). Figure 6 shows the effect of SS concentration on the sulfonation parameters, amount of sulfonic group, and conversion %. It is clear that increasing the SS concentration from 0.25 to 1% increased sharply the obtained amount of sulfonic groups. Further increase of SS concentration to 2%, slightly increased the amount of sulfonic groups to reach its maximum. Increasing SS concentration up to 10% decreased the amount of sulfonic groups. The trend in the first part of the curve could be due to increasing the diffusive amount of SS into the polymeric matrix to react with the epoxy groups. On the other hand, the trend in the second part of the curve could be explained as following. Because the reaction between epoxy groups and SS carried out first on the surface creating clouds of sulfonated groups on the membrane surface, these clouds render the diffusion of further SS groups to both pores and bulk of polymer matrix leading consequently to reduce the amount of introduced sulfonated groups.

Effect of sulfonation temperature on the amount of sulfonic group and conversion (%). The effect of temperature variation on the sulfonation parameters has been studied. From the obtained results, it was found that on increasing the sulfonation temperature from 30 to 50°C, the amount of sulfonic groups increased by about 30%. The same behavior was noticed for the conversion percentage of the epoxy groups. The enhancement in the obtained amount of sulfonic groups on raising the sulfonation temperature might be attributed to the favorable effect of the temperature on diffusion of SS from the solution phase to the swellable cellulose phase and increasing the swellability of the grafting membranes. Further increase of the temperature was found to be of neglectable effect. This again confirmed the absence of mass diffusion problem, which is an advantage of using membrane form rather than bead one.

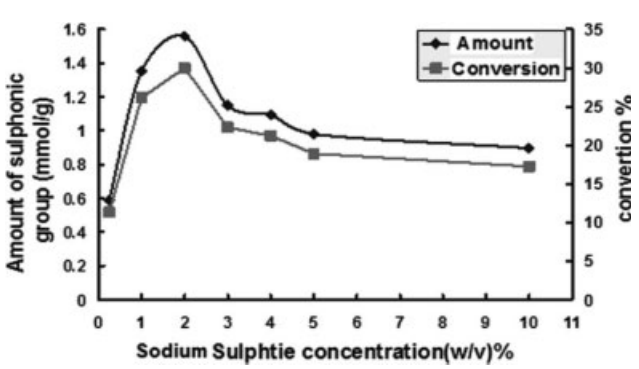


Figure 6 Effect of sodium sulfite concentration on the amount of sulfonic group and conversion, %.

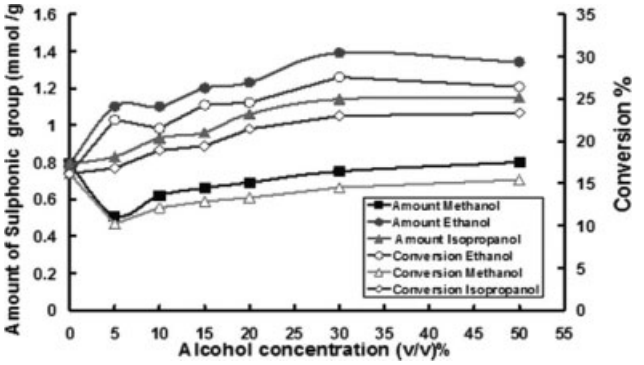


Figure 7 Effect of solvent composition used in the sulfonation process on amount of sulfonic group and conversion, %.

Effect of sulfonation time on the amount of sulfonic group and conversion (%). The dependence of the obtained amount of sulfonic groups and the conversion % on the sulfonation time has been studied. The amount of sulfonic groups increases slightly as the sulfonation time increased from 15 to 30 min. Further increase of the sulfonation time beyond 30 min resulted in insignificant effect on the obtained amount of sulfonic groups. This result confirmed the absence of diffusion limitation of SS, which presents as an advantage of membrane form over other form such as beads.[15,30]

Effect of solvent composition on the amount of sulfonic group and conversion (%). Based on the obtained results from studying the effect of sulfonation temperature and time, it was logic to study the effect of both solvent type and composition. Two different alcohols, methanol and ethanol, in addition to isopropanol were used, in different compositions with water, to accomplish this study (Fig. 7). In general, increasing the alcohol percentage relative to water (v/v) in the solvent mixture for SS increases the degree of sulfonation, which could be attributed to increasing the diffusive amount of SS as a result of increasing the swellability of the GMA-grafted membranes. It is obvious from the figure that the highest amounts of sulfonic groups and conversion % were obtained according to the following sequence of the used alcohol, ethanol, then isopropanol, and finally methanol. Different swelling powers of the alcohols are believed to be responsible for such observation.

Membrane characterization

Water uptake

Water absorption in cellulose films is attributed mostly to the hydroxyl groups located on polysaccharides units. Nearly one-third of the cellulose in cellophane membranes is amorphous in nature and is responsible for water absorption, while crystalline zones being impenetrable.

The data of water uptake of various grafted cellophane membranes together with that of ungrafted membrane are presented in (Table I). The data clearly indicate that the water uptake of the grafted cellophane membranes progressively decreased as the extent of grafting increased.

This may be due to the fact that partial blockage of the internal membranes structure by the hydrophobic PGMA graft side chains. These results are in agreement with the results obtained by the authors.[31] The effect of converting the epoxy groups, through sulfonation process, to sulfonated groups on the water uptake is also presented in the same table. Almost the same trend has been observed in comparison with the grafted membranes in spite of the hydrophilic nature of the sulfonic groups. The effect of the hydrophilic nature of sulfonic groups started to appear with membranes of GP higher than 70%. Immobilization of the sulfonic groups with Cu^{2+} ions implies different behavior of the water uptake. In general; the water uptake is higher than that of the sulfonated membranes. Maximum value was observed for membranes with GP from 22 to 35%. This behavior could be attributed to the amount of immobilized Cu^{2+} ions and its water chelating nature.

Infrared spectrophotometric analysis

Figure 8 illustrates the FTIR spectra for ungrafted, grafted, and sulfonated cellophane membranes. The

TABLE I
Water Uptake of Grafted, Sulfonated, and Metal's Immobilized Cellophane Membranes

GP (%)	Water uptake of grafted membranes (%)	Water uptake of sulfonated membranes (%)	Water uptake of immobilized Cu^{2+} ions membranes (%)
9.53	52.78	51.21	54.42
22.14	51.02	53.5	59.76
35.23	44.51	45.11	60.65
70.53	37.40	40.25	44.34
92.79	25.41	40.25	42.52

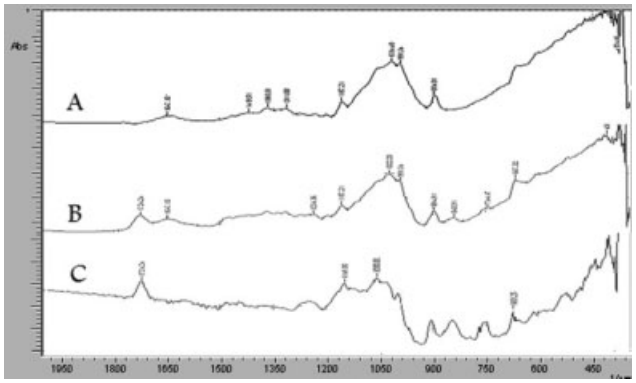


Figure 8 FTIR spectra of (A) ungrafted cellophane membranes, (B) 25% grafted cellophane membranes, and (C) 25% sulfonated cellophane membranes.

appearance of characteristic absorption band for —C=O at 1724 cm^{-1} and three characteristic bands for epoxy rings at 1238–1255, 840, and 750 cm^{-1}. Curve b provided evidence of occurring grafting process of PGMA. Opening of the epoxy rings through sulfonation process has been proved through appearance of three characteristic bands for sulfonic group at 1150, 1050, and 670 cm^{-1}. The remaining characteristic bands for epoxy rings are indicating incomplete sulfonation reaction (curve c).[32]

Thermal gravimetric analysis

Thermal analysis for PGMA-grafted and ungrafted cellophane membranes was carried out by thermogravimetric analyzer in nitrogen atmosphere at a heating rate of 20°C/min. It is clear from Figure 9 that weight-loss in case of ungrafted membranes occurs after 280°C at a relatively high rate up to 350°C, where the membrane lost about 50% of its original weight. Above this temperature the rate of weight loss becomes lower. On the other hand, grafted membranes show different behavior probably because of the gained thermal stability of the membrane when compared with the ungrafted case (curve B). The weight loss at 350°C has been reduced to reach 22% when compared with 68% in the case of ungrafted membranes. This behavior could be attributed to the formation of graft copolymer between PGMA and the cellulose backbone. The possibility of homopolymers purse has been eliminated depending on the absence of characteristic thermal beaks of PGMA (curve C).

EDAX analysis

The changes in the elemental composition as a result of the sulfonation and metal immobilization process have been proved. The changes in S/Cu ratio as a result of sulfonation and Cu^{2+} immobilization processes have confirmed occurrence of the sulfonation and Cu^{2+} ions immobilization processes and illustrate that direct relation between the amount of sulfonic groups and the immobilized Cu^{2+} ions. Increase the amount of sulfonic groups from 1.76 to 1.9 mmol/g increase the Cu/S ratio from 31 : 69 to 35 : 65.

Proteins separation

Membranes with the highest amount of immobilized Cu^{2+} ions were used in separation of β-Gal from its mixture with BSA. This protein mixture has been chosen based on the fact that PI of both proteins, PI = 4.7 for BSA and 4.61 for β-Gal, is very close. This choice eliminates the interference of the PI in the separation process. The membrane used in β-Gal separation step has been selected based on the following factors: maximum immobilized Cu^{2+} ions, minimum GP, and maximum water-uptake. The mechanism of proteins adsorption on the immobilized Cu^{2+} ions on the sulfonated grafted cellophane membranes is shown in Scheme 3.

To verify the affinity of the IMAC membranes, the BSA concentration was made 20 times higher than that of β-Gal and the separation process was examined under pH range from 2.6 to 5.2 (Figure 10). The percent adsorption results show that BSA and β-Gal adsorption increased with increasing pH from 2.6 to 4.4 and then decreased slightly followed by sharp decrease, respectively, where the pH was raised further up to 5.2. The figure also reveals that the percentage of adsorbed β-Gal is double when compared with that of BSA although the initial concentration of the latest is 20 times higher. Such behavior could be explained in the light of the higher concentration of the incorporated amino acids located on the surface of β-Gal when compared with BSA protein. In addition, it is worthy to mention here that isoelectric point of both proteins is almost

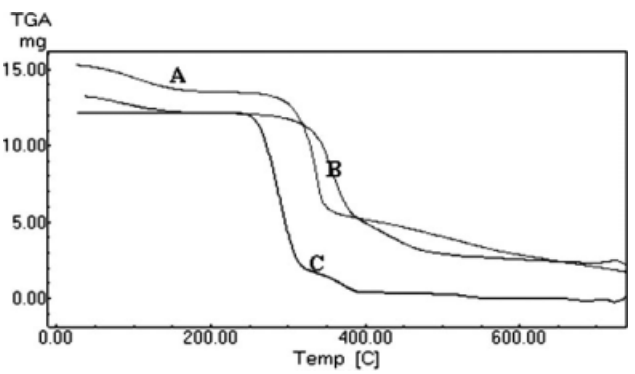
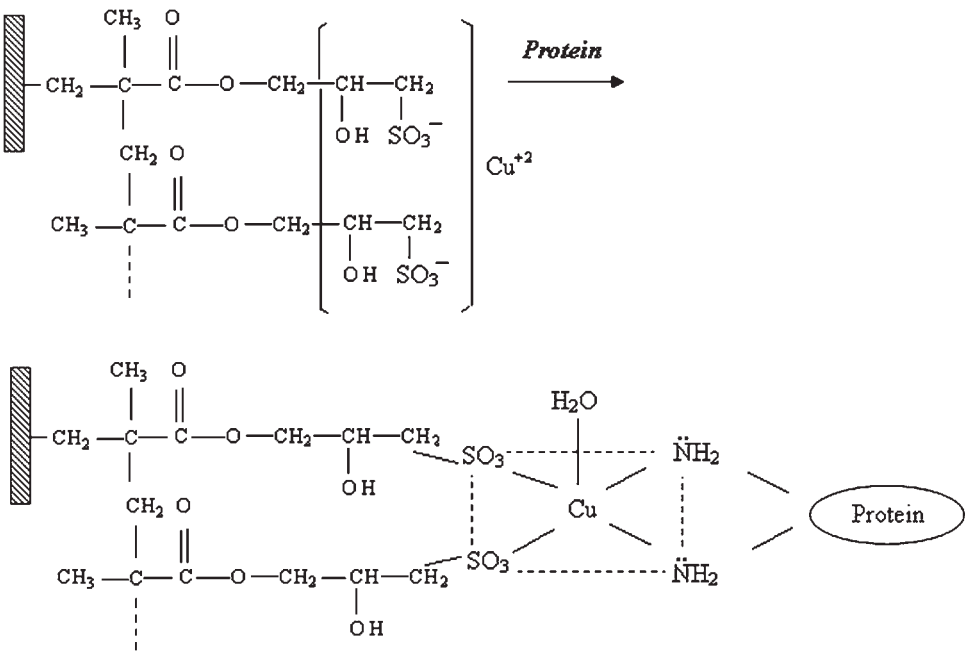


Figure 9 TGA thermographs of (A) ungrafted cellophane membrane, (B) 40.28% grafted cellophane membrane, and (C) homopolymer.



Scheme 3 Mechanism of proteins adsorption on the immobilized Cu^{2+} ions on the sulfonated grafted cellophane membranes.

identical; PI = 4.7 for BSA and 4.61 for β-Gal. This explains the reason of getting the higher percentage of proteins adsorption near this pH on which histidine, cystein, and tryptophan residues become neutral and consequently play an important role as a ligand, which can coordinate to the immobilization Cu^{2+} ions.[17]

In conclusion, the effect of pH on the protonation and hence the conformation changes of proteins has its reflection on the availability and number of coordinated histidine residues. It is known that BSA has only one to three exposed histidine,[10,33] and hence, it acquires less adsorption when compared with β-Gal, which has more histidine residues. Also, the molecular size plays a significant role in reaching the proteins to binding sites inside the pores. The decrease in protein binding capacity with increase in molecular size may be explained by the possibility that the binding of a large protein molecule blocks the access of multiple metal ions sites to reach the immobilized metal affinity membrane separation (IMAMS).[34–36]

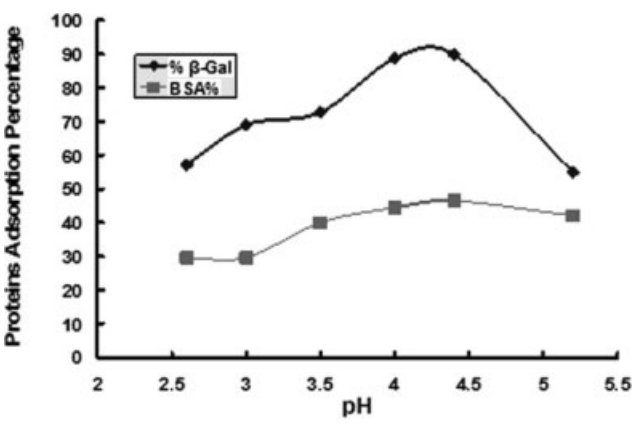


Figure 10 Effect of protein's solution pH on its adsorption percentage.

CONCLUSIONS

Immobilized Cu^{2+} ions cellophane–PGMA-grafted membranes have been prepared, characterized, and evaluated in the separation of β-Gal enzyme from its mixture with BSA. In the part of membrane preparation, the functionalization process through grafting of cellophane membranes with PGMA using persulphate initiation system has been studied by exploring the effect of different factors, such as monomer, initiator concentration, time, temperature, and solvent ratio. Different GPs have been obtained and controlled as a result of the variation of the grafting conditions. The occurrence of grafting has been proved by FTIR, thermogravimetric (TGA), and EDAX analysis. The introduced epoxy groups of PGMA are subsequently converted into sulfonic groups by sulfonation process using SS. In general, increasing the grafting degree has a negative impact on the sulfonation process and immobilization of Cu^{2+} ions. Indeed, grafted membranes prepared under different conditions produced different degrees of sulfonation, which revealed the importance of selecting the grafting conditions. The optimum sulfonation conditions are 0.25% SS,

sulfonation time 30 min, and sulfonation temperature 80°C and 30% ethanol. The sulfonation reaction has been verified through FTIR and EDAX analysis. Finally, the IMAC membranes showed high affinity toward the separation of β-Gal from its mixture with BSA, although the concentration of BSA, 0.5%, is 20 times higher than that of β-Gal concentration, 0.025%. Almost 90% of β-Gal has been adsorbed in comparison with 45% of BSA. This result implies the success of our technique in preparation of IMAC membranes for the separation of β-Gal enzyme from protein mixture.

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