

A Study of the Synthesis, Kinetics, and Characterization of Reactive Graft Copolymers of Poly(vinyl imidazole) and Cellulose for Use as Supports in Enzyme Immobilization and Metal Ion Uptake

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ABSTRACT: In continuation to our earlier work to use bioresource for developing alternate materials for use at the interface of biotechnology and polymer science, we have utilized pine needles as a renewable stock of cellulose to synthesize graft copolymers of vinyl imidazole. Kinetics of *N*-VIm by simultaneous γ -irradiation method has been investigated as a function of total dose, monomer concentration, and amount of water. Effect of water–methanol solvent composition on graft yields and polymerization kinetics has also been studied at the optimum grafting conditions of the total dose and monomer concentration. Effect of some additives such as ZnCl₂, Mohr salt, tetramethylethylene diamine, potassium persulfate, ammonium persulfate as grafting ac-

celerators and promoters has also been studied. Graft copolymers have been characterized by elemental analysis, FTIR, and swelling studies. The graft copolymers have been used as supports for metal ions sorption, enzyme immobilization, and as potential biomimicking catalysts. Sorption behavior of Fe²⁺ ions and Cu²⁺ ions and the immobilization of bovine serum albumin and protease as a function of graft yield has been reported. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 1522–1530, 2006

Key words: bovine serum albumin; grafting; metal ion uptake; protease; reactive polymers

INTRODUCTION

Modified cellulose has potential applications as supports in enzyme and metal ions remediation. Vinyl imidazole (VIm) is a versatile monomer that has catalytic activity on acidification of the imidazole ring. It can also interact with metal ions, and its complexes with copper exhibit enzyme-like activity. Proteins that contain metal ions were supposed to bind at the imidazole groups of histidine. Such propositions led to further studies of ion binding capacity of poly(VIm) with copper or iron ions. Tsuchida and Niside¹ discussed poly(VIm)-Cu²⁺ complex as a catalyst having activity equivalent to tyrosinase. This complex was reported to accelerate polymerization of active phenols to poly(ethers). The esterolytic activity of poly(*N*-VIm) and some of its copolymers have been reported in a number of works.^{2–5} More metal ion sorption studies have been subsequently reported on Poly(*N*-VIm) and its copolymers. Rivas et al.⁶ have reported effective pH-dependent metal ion sorption of bivalent

and other metal ions such as uranyl, vanadyl, Re, and Mo on the poly(*N*-VIm). The ability of poly(*N*-VIm) hydrogels to remove the metal ion from polluted water has been reported.⁷ Cellulose is widely available and environmentally safe and in functionalized forms is capable of lowering transition metal-ion concentration to parts per billion concentrations. Further, cellulose can be derivatized to possess a number of different functional groups, such as carboxymethyl and amines, to which metal ions can bind either by chemical or by physical adsorption.⁸ Tobacovic and co-workers⁹ grafted cellulose with nitrogen-containing monomers, including *N*-Vim, by radical polymerization in acetonitrile, and the resulting graft copolymers were used as supports in the sorption of some metal ions.

Tailored cellulose has also been used as supports for protein adsorption. Enzyme immobilization on porous cellulose beads shows better loading than that on ordinary cellulose.¹⁰ Cellulose derivatives modified with aliphatic diamine residues,¹¹ nitrilotriacetic acid residues,¹² and xylylene diamine¹³ were synthesized by a nucleophilic substitution reaction, starting from cellulose tosylates. These derivatives were cast into films from their solutions and were used as enzyme-compatible support matrices. Butler¹⁴ has carried out

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enzyme immobilization by adsorption on hydrophobic derivatives of cellulose and other hydrophilic materials. Varavinita et al.¹⁵ have reported immobilization of a thermostable α -amylase onto cellulosic fiber from bagasse.

We have recently reported well-defined and efficient cellulose-based reactive polymeric supports for metal ions sorption and enzyme immobilization.^{16–17} In these studies we have established that efficiency and selectivity of these supports can be enhanced by suitable modification of the cellulose itself or by modification of the graft copolymers. In the present study, we report grafting of *N*-VIm onto cellulose extracted from pine needles to obtain graft copolymers designed to have moderate hydrophilicity, high biocompatibility, and cost effectiveness. Kinetics of *N*-VIm by simultaneous irradiation method has been investigated as a function of total dose, monomer concentration and amount of water, water:methanol solvent composition along with the effect of $ZnCl_2$, Mohr salt (FAS), tetramethylethylene diamine (TEMED), potassium persulfate (KPS), ammonium persulfate (APS) as grafting accelerators and promoters. Graft copolymers have been characterized by elemental analysis, Scanning electron micrography (SEM), FTIR and water uptake studies. To explore the potential end-uses of these supports, metal ions and protein binding studies have been carried as a function of graft yields. Sorption of bivalent metal ions with tendency for variable oxidation states and thus having catalytic ability (Cu^{2+} and Fe^{2+}); and immobilization of bovine serum albumin (BSA) and protease as model protein and enzyme has been investigated.

EXPERIMENTAL

Materials

Cellulose was extracted from pine needles as described below. Vinyl imidazole (Fluka Chemicka, Buchs, Switzerland) was vacuum distilled and other reagents were used as received.

Extraction of cellulose

Pine needles were dried in oven at 150°C and were crushed in a mixer to a fine powder. Cellulose was

separated by improved ammonia digestion method, as reported earlier.¹⁸ Pine needles were dried at 150°C in an oven. These were crushed to a fine powder and digested in 1-L round bottom flask in ammonia for 8 h at low pressures. The digested powder was repeatedly washed with water to ensure the removal of lignin and other water-soluble organic components to get clear supernatant. The resulting mass was treated with calcium hypochlorite to remove any colored impurities. The excess of bleaching powder was removed by treatment with dilute mineral acids. Cellulose thus obtained was stirred with solvents of varied polarities to ensure extraction of any remaining low molecular weight organic impurities. Cellulose powder was then dried in an oven at 40°C.

Graft copolymerization of *n*-vim onto cellulose

Cellulose (1 g) and definite amount of water and monomer were irradiated for specified time periods in a γ -chamber. The irradiated samples containing both homopolymer and graft copolymers were filtered, dried, and weighed. Dissolved homopolymer was precipitated by acetone and weighed. The dried samples were extracted with water by stirring for 2 h and the process was repeated with methanol. The process was repeated to ensure complete removal of homopolymer till constant weight was obtained. Initially the optimum total dose of radiation was evaluated by variation of radiation dose at constant monomer concentration, which was followed by variation of monomer concentration at the optimum total dose of radiation. Other reaction conditions were optimized by changing monomer concentrations as water contents were varied at optimum total dose of radiation and monomer concentration.

Graft copolymerization in the presence of additives

Effect of water–methanol solvent system was studied using $[N\text{-Vim}] = 165.5 \times 10^{-2}$ mol/L and at a dose rate of 0.24 KGy, keeping 10 mL as total solvent composition. Effect of the accelerators was also studied at these conditions by the addition of 43.8×10^{-5} mol each of APS, KPS, Mohr salt, and TEMED. Expressions used to evaluate kinetics and grafting values were same as reported earlier.¹⁸

$$\text{Percent total conversion (\% } C_t) = \frac{\text{Weight of polymer grafted} + \text{Weight of homopolymer formed}}{\text{Weight of monomer charged}} \times 100$$

$$\text{Percentage of grafting (} P_g) = \frac{\text{Weight of polymer grafted}}{\text{Weight of cellulose}} \times 100$$

Percent grafting efficiency (% GE)

$$= \frac{\text{Weight of polymer grafted}}{\text{Weight of polymer grafted} + \text{Weight of homopolymer formed}} \times 100$$

Rate of polymerization (R_p) (moles L⁻¹sec⁻¹)

$$= \frac{\text{Weight of polymer grafted} + \text{Weight of homopolymer formed}}{\text{Mol. wt. of monomer} \times \text{time of reaction (s)} \times \text{vol. of reaction mixture (mL)}} \times 1000$$

Rate of grafting (R_g) (moles L⁻¹Sec⁻¹)

$$= \frac{\text{Weight of homopolymer formed grafted}}{\text{Mol. wt. of monomer} \times \text{time of reaction (s)} \times \text{vol. of reaction mixture (mL)}} \times 1000$$

Rate of homopolymerization (R_h) (moles L⁻¹Sec⁻¹)

$$= \frac{\text{Weight of polymer grafted}}{\text{Mol. Wt. of monomer} \times \text{time of reaction (s)} \times \text{vol. of reaction mixture (mL)}} \times 1000$$

$$\text{Percent uptake } (P_u) = \frac{(\text{Total amount of ions in feed} - \text{amount of ion rejected})}{\text{Total amount of ions in feed}} \times 100$$

$$\text{Percent swelling } (P_s) = \frac{\text{Weight of swollen polymer} - \text{Weight of dry polymer}}{\text{Weight of dry polymer}} \times 100$$

Characterization of polymers

SEMs of graft copolymers were studied on Cambridge Stereoscan 150. Elemental analysis (N) and FTIR spectra of cellulose-g-poly(*N*-Im) were recorded, respectively, on Carlo Erba-1150 instrument and Nicolet Magha IR 750 Spectrometer Series II in KBr pellets. Swelling studies were performed in distilled water at room temperature, as reported earlier.¹⁸

Metal ion sorption study by graft copolymers

Copper sulfate and ferrous sulfate (CDH) were used as received. All weights were measured using Denver TR-203 balance. The graft copolymers of known weights were immersed in 50-mL solutions each of Cu²⁺ and Fe²⁺ salts for 24 h. Filtrate of the solutions was analyzed for the concentration of rejected ions on DR 2010 Spectrophotometer (Hach Co.) by using specialty pillow reagents. Various calculations were made from the following equations¹⁶:

Protease immobilization

Cellulose and cell-g-poly(*N*-VIm) with varying graft yield were used as supports for protease immobilization. Protease (isolated and characterized at the Department of Biotechnology, Himachal Pradesh University, Shimla, India) assay was performed by Manachini method.¹⁹ Casein (Hammartein) solution (0.5% w/v) was dissolved in 50 mM Tris-HCl buffer (pH = 8) and 5% (w/v) trischloroacetic acid using tyrosine 10–100 μg/mL as standard. Known weights of cellulose and cellulose graft copolymers (50 mg) were immersed in 1 mL of protease enzyme (50 μL) for 24 h. After 24 h polymers were filtered and washed with Tris-HCl and followed by dipping in 4 mL of casein

solution. The reaction mixture was incubated at 55°C for 10 min and the reaction was terminated by addition of 5 mL of 5% TCA, vortexed again, and allowed to stand for 20 min. The contents were filtered through Whatman No. 1 filter paper and the absorbance of the supernatant was measured at 275 nm on UV 160Å Shimadzu Spectrophotometer.

Bovine serum albumin immobilization

Bovine serum albumin (Merck) was used as a protein standard and it was immobilized by Lowry Method.²⁰ 1% CuSO₄ (i), 2% sodium potassium tartarate (ii), 2% sodium carbonate in 0.1 N NaOH (iii), Lowry alkaline reagent [mixture of 1 mL of (i) and 1 mL of (ii) + 98 mL of (iii)], Folin–Ciocalteu's phenol reagent (1:1 with distilled water), and BSA (10–100 μg/mL) were taken. Candidate polymers (50 mg) were dipped in 1 mL of BSA (100 μg/mL) for 24 h. The samples were filtered after 24 h and washed with 5 mL of distilled water, and to the filtered polymers, 3 mL of Lowry alkaline reagent was added. After mixing thoroughly on vortex mixer it was allowed to stand for 15 min at room temperature. Folin–Ciocalteu's phenol reagent (0.3 mL) was added to it and the contents were vortexed and allowed to stand for 30 min for maximum color development. Optical density was measured at 670 nm against a reagent blank, and the concentration of protein in the samples was calculated from a standard curve prepared by the same procedure using BSA from varying concentrations, 10–100 μg/mL.

RESULTS AND DISCUSSION

N-VIm is sluggish to homopolymerize as placement of *N* and vinyl group in the molecule, coupled by reso-

nance factors, does not favor/facilitate high reactivity of *N*-VIm as compared with that of the symmetric 4-vinyl pyridine. Different accelerators were used to affect its reactivity, as out of two nitrogen atoms present in the imidazole ring, metal ions present in the reaction system are expected to link at the unhindered site (that is nitrogen not attached to the vinyl group) and affect the electronic environment of the monomer. Analysis of different kinetic parameters as function of various reaction conditions is discussed.

Effect of total dose and monomer concentration on grafting parameters

Total monomer conversion (% C_t) and grafting parameters are dependent on the extent of total radiation dose and monomer concentration and as a function of monomer concentration at different doses and vice versa. It has been observed that % C_t increases with the increase in total dose of radiation (from 0.216 to 0.24 KGy), and then tends to be constant at 87.4 at total dose rate of 0.24. On further increase of total radiation dose to 0.288 and 0.312 KGy, % C_t decreases. Percent grafting (P_g) increases initially and then decreases for whole range of the total radiation dose studied [Fig. 1(a)]. Percent grafting efficiency (% GE) varies in a different manner, as it decreases first, and then increases finally at a total dose rate of 0.312 KGy. These values indicate that more homopolymer is formed at the cost of grafting. Similar trends as that observed for % C_t has also been observed for the rate of total polymerization (R_p) and rate of grafting (R_g), as these also increase initially and then decrease with the increase of total radiation dose. On the other hand, the rate of homopolymerization (R_h) shows steady increase with the increase of total radiation dose till 0.264 KGy before a sharp decrease is observed at the higher radiation doses. It can be concluded from the trend observed for R_h that though the overall polymerization increases initially with the total dose of radiation, at the higher dose of radiation more homopolymerization is favored at the cost of grafting due to more mutual annihilation of the growing monomer macroradicals [Fig. 1(b)].

Effect of variation of [VIm] on grafting parameters was studied in the range 55.2×10^{-2} to 276.0×10^{-2} mol/L at 0.24 KGy and it has been observed that % C_t increases slowly or rather tends to be constant over the range of [VIm] studied [Fig. 2(a)]. P_g on the other hand increases steadily with the increase in [VIm] and reaches the highest value of 91.9 at [VIm] = 276.0×10^{-2} mol/L. It increases rather sharply when [VIm] was increased from 220.8×10^{-2} to 276.0×10^{-2} mol/L. Similar trends for % GE have been observed as it also increases with [VIm] increase, but tends to remain constant at the higher concentrations. R_p , R_g , and R_h also increase steadily with [VIm], and as ob-

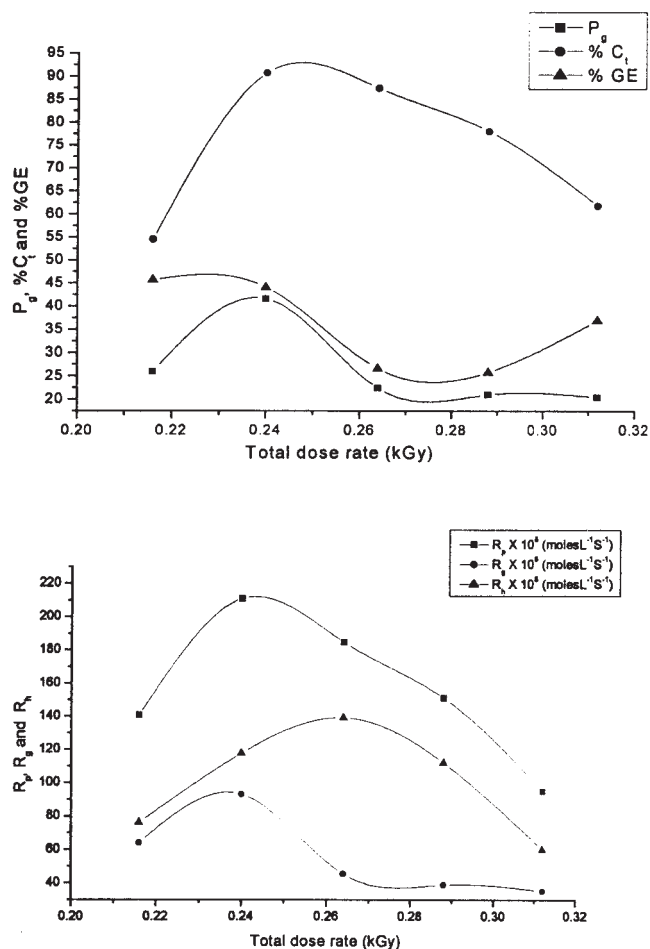


Figure 1 Effect of total dose rate on (a) P_g , % C_t , and % GE and (b) R_p , R_g , and R_h (cellulose = 1 g [VIm] = 110.40×10^{-2} mol/L and water = 10 mL).

served in case of P_g , maximum values for the different rates have been observed at 276.0×10^{-2} mol/L [Fig. 2(b)]. It also follows from the foregone discussion that homopolymerization processes are more dominant than that of the grafting reactions.

Effect of amount of water and water-methanol composition

Amount of water was varied from no water to 20 mL at constant total dose rate and 165.0×10^{-8} mol/L of the monomer. In the absence of water, no P_g was observed, though some homopolymerization has been observed. All the kinetic parameters have been observed to increase with the increase of the amount of water up to 10 mL, which afford a maximum P_g of 91.9. Thereafter all the parameters decrease but for R_h , which increases regularly with the increase in the amount of water. The higher volume of water though contributes to more % C_t , but it also accounts for more in homopolymer formation rather than increasing the grafting reactions. Such results are expected as larger

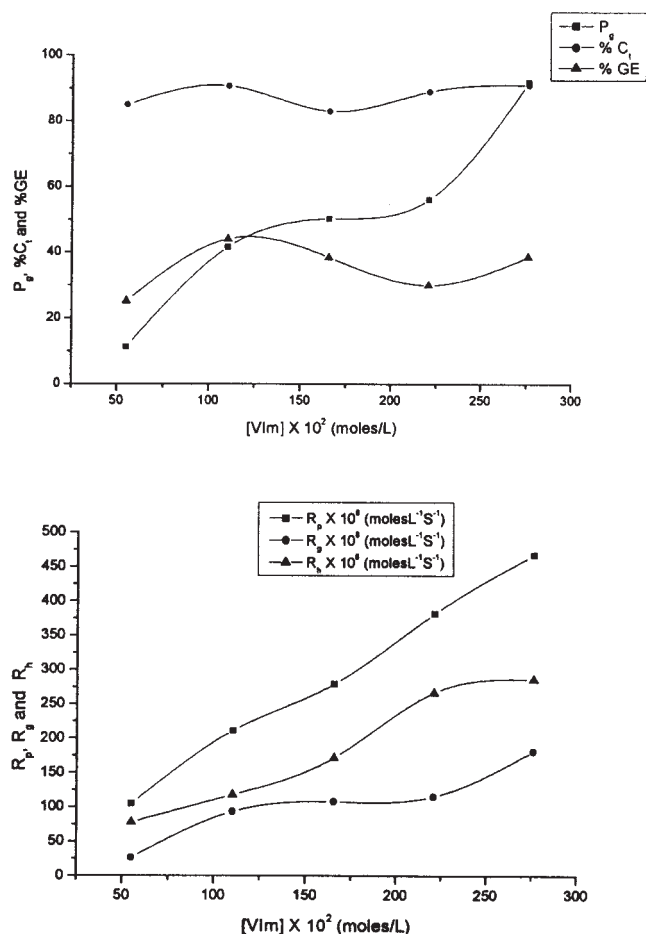
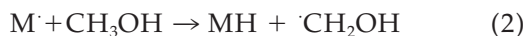
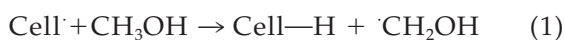


Figure 2 Effect of [VIm] on (a) P_g , $\%C_i$, and $\%GE$ and (b) R_p , R_g , and R_h (cellulose = 1 g, total dose = 0.240 kGy, and water = 10 mL).

volumes of water decrease the accessibility of growing monomer macroradicals to the backbone [Figs. 3(a) and 3(b)]. The effect of methanol as additive with water was also studied as methanol is a good solvent for poly(*N*-VIm). However, it has been observed that grafting reactions are adversely affected in the presence of methanol and no graft yield was obtained despite very high R_h [Fig. 4(a,b)]. It is thus obvious that monomer chains are propagated but chain transfer reactions are more predominant. Methanol scavenges active radical sites created on the cellulose backbone [eq. (1)] and also inactivates monomer radical species [eq. (2)]²¹:



Effect of ZnCl_2 and other additives on graft yield and polymerization parameters

ZnCl_2 is a decrystallizing agent for cellulose. Its presence decreases various polymerization parameters

and the decrease in all the polymerization parameters is steady. At the [monomer: ZnCl_2] molar ratio higher than 1 : 1, no graft yield was obtained, though homopolymer formation has been observed [Figs. 5(a) and 5(b)]. There was also constant decrease in $\%C_i$, which decreases to almost half at 1 : 1 *M* ratio of [ZnCl_2 :VIm] as compared with its value in the absence of ZnCl_2 . Grafting reactions are more adversely affected than that of homopolymer formation. It appears that Zn^{2+} ions form complex with VIm at the N: of the monomer. Formation of such a complex lowers monomer reactivity because of the electronic effects. Such an inference is further supported by the fact that experimental values of all the parameters studied decrease with the increase in [ZnCl_2].²²

It is also evident from Table I that various grafting parameters decrease in the presence of additives that are otherwise known promoters or accelerators of grafting. P_g , $\%GE$, and R_g decrease in the presence of these additives than that observed in their absence. $\%C_i$ and overall polymerization processes (R_p) are maximum in FAS and minimum and almost same in both the persulfates studied. R_h decreases but it is more so

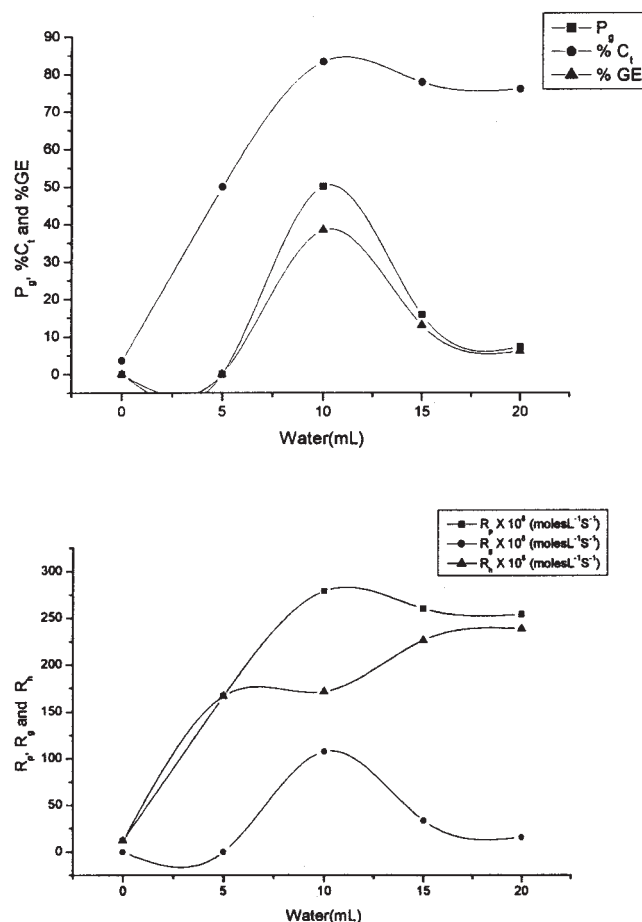


Figure 3 Effect of solvent on (a) P_g , $\%C_i$, and $\%GE$ and (b) R_p , R_g , and R_h (cellulose = 1 g, total dose rate = 0.240 kGy, [VIm] = 276.00×10^{-2} mol/L).

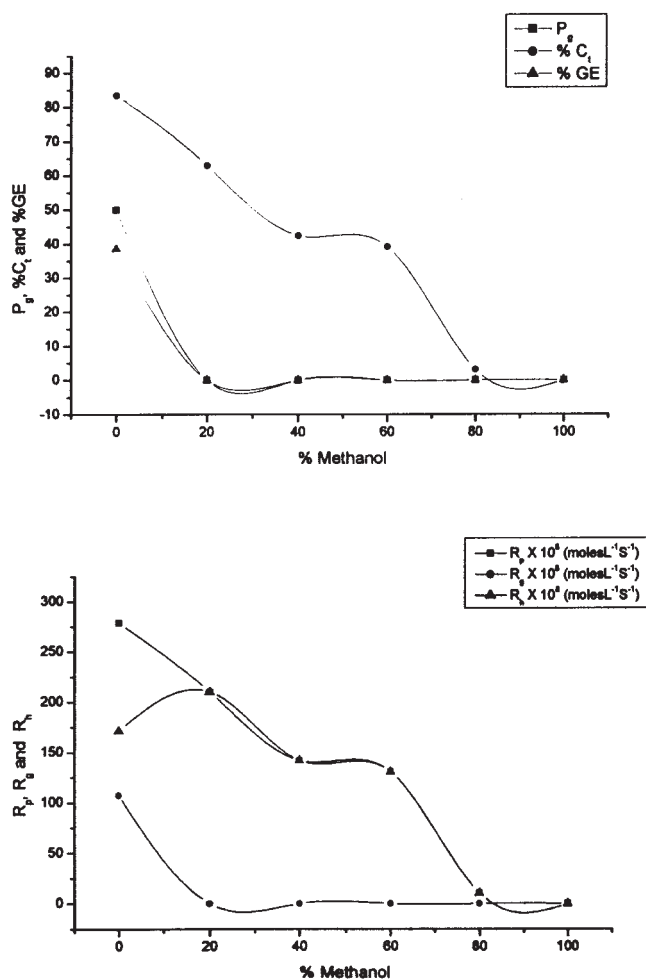


Figure 4 Effect of % methanol in methanol–water composition on (a) P_g , % C_t , and % GE and (b) R_p , R_g , and R_h (cellulose = 1 g, total dose rate = 0.240 kGy, $[Vim] = 276.00 \times 10^{-2}$ mol/L).

in case of $K_2S_2O_8$. The ratio of grafting processes to homopolymerization processes is positive (more than 1) in $K_2S_2O_8$ and minimum in case of FAS. It appears that grafting is augmented more by the persulfates because of the formation of more free radicals on dissociation.

Characterization of graft copolymers

SEMs of the cellulose graft poly(VIm) with different P_g were examined. Fibrils of grafted poly(VIm) chains are visible in the micrographs of graft copolymer [Fig. 6(a,b)]. Elemental analysis of cellulose has been reported in an earlier communication.¹⁸ Graft copolymer ($P_g = 33$) containing carbon (48.8%), hydrogen (9.4%), and nitrogen (11.8%) provide evidence of monomer incorporation onto backbone. Percent of nitrogen corresponds to the gravimetric value of the P_g . FTIR spectra of cellulose have a sharp peak at 1645.69 cm^{-1} observed because of carboxylate ion formed in

the bleaching process. FTIR spectra of cellulose¹⁸ and its graft copolymers prepared at different radiation doses were studied to obtain evidence of incorporation of poly(*N*-VIm) [Fig. 7]. Prominent peak at $3405\text{--}3432 \text{ cm}^{-1}$ is due to the stretching of —OH (polymeric association), which sharpens on grafting. Sharpening of the peak due to —OH stretching is more so in graft copolymers with higher P_g , thus grafting opens up cellulose matrix. Apart from these bands, high absorption bands were also observed in the range $1621\text{--}1638 \text{ cm}^{-1}$ due to the stretching of various multiple bonds as —C=N— and —C=C— of the imidazole moieties of poly(*N*-VIm). A sharp doublet appear at $2340\text{--}2370 \text{ cm}^{-1}$ because of the overtones and combination bands of tertiary ammine salts, which in the present case may appear because of the interaction of nitrogen of imidazole ring and metal ions present in the reaction system. Another peak in the range $1050\text{--}1160 \text{ cm}^{-1}$ is attributed to the stretching of —C—O—C— of anhydroglucose units. The ratio of absorbances of

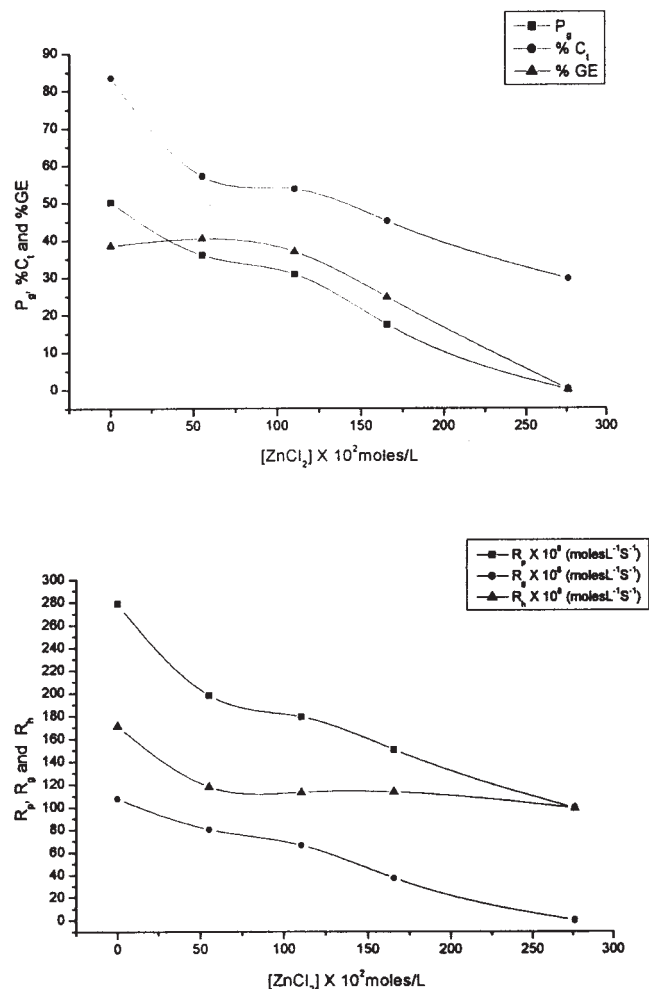


Figure 5 Effect of $[ZnCl_2]$ on (a) P_g , % C_t , % GE and (b) R_p , R_g , and R_h (cellulose = 1 g, total dose rate = 0.240 kGy, $[Vim] = 276.00 \times 10^{-2}$ mol/L).

TABLE I
Effect of Additives on Grafting Parameters

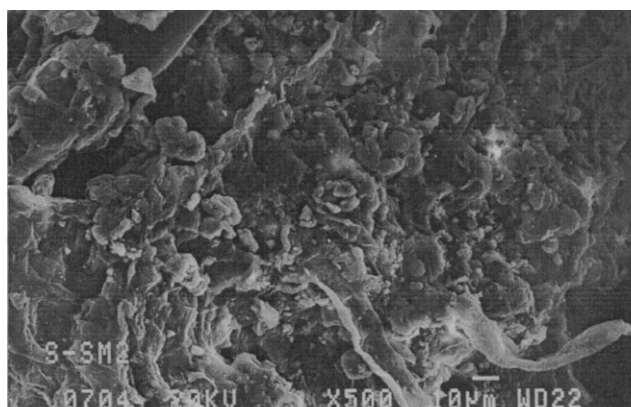
Additive	P_g	% C_t	% GE	$R_p \times 10^8$ (m/L S)	$R_g \times 10^8$ (m/L S)	$R_h \times 10^8$ (m/L S)
—	50.2	83.0	38.5	278.8	107.4	171.3
$K_2S_2O_8$	33.6	35.9	60.0	119.9	71.9	47.9
$(NH_4)_2S_2O_8$	20.5	35.4	37.1	118.2	43.8	74.3
Mohr salt	22.7	50.8	28.6	169.3	48.6	120.7
TEMED	25.9	35.7	45.7	121.2	55.4	65.7

Amount of cellulose = 1.0 g, [additive] $\times 10^5 = 43.8$ moles, [VIm] $\times 10^2 = 165.6$ moles/L, and total dose of radiation = 0.240 KGy.

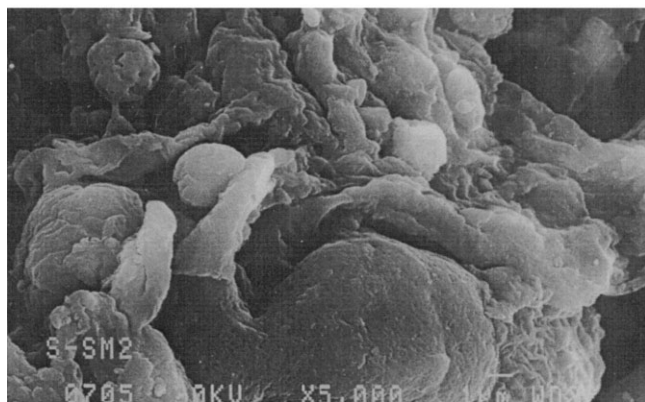
—C=N— stretching of VIm moiety to the ether linkage of anhydroglucose in different graft copolymer show a linear relationship with an increase in the P_g [Fig. 7].

Water uptake behavior of graft polymers

Grafted poly(VIm), being water-soluble polymer, increases hydrophilicity and enhances water uptake of the graft copolymers [Fig. 8]. It is evident from the plot of P_u vs. P_g that there is a linear relationship between the graft level and water uptake behavior of the graft



(a)



(b)

Figure 6 SEM of cell-g-p(VIm). (a) $P_g = 41.6$, (b) $P_g = 91.9$.

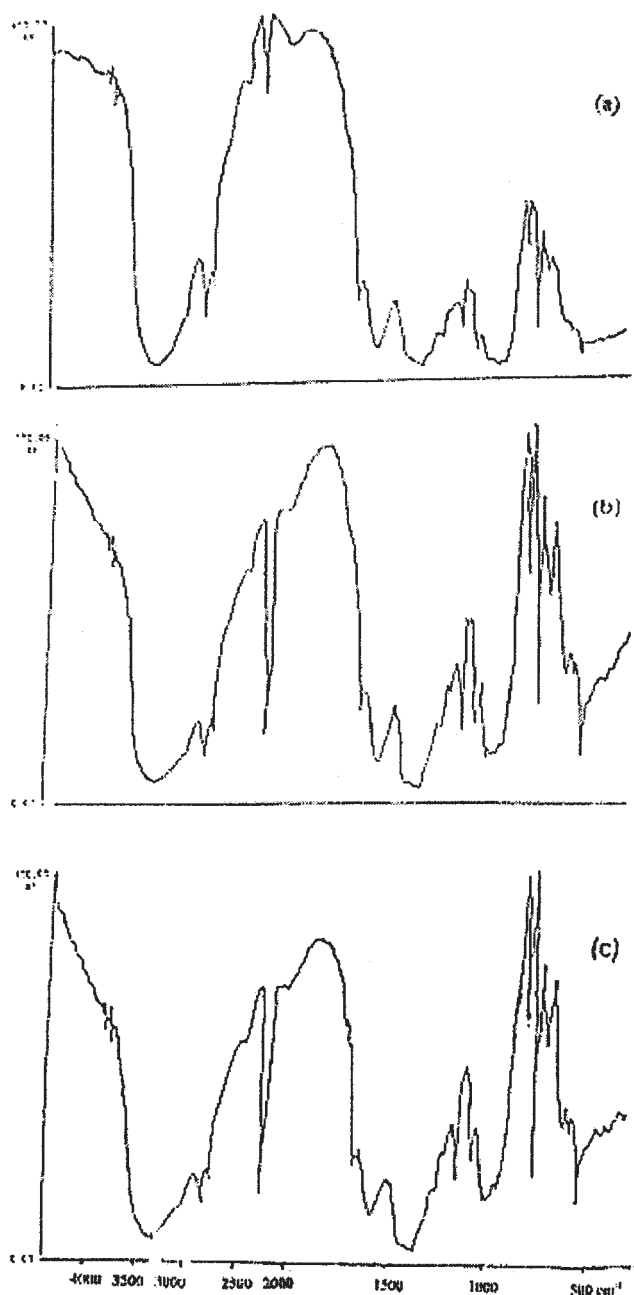


Figure 7 FTIR spectra of cellulose-g-poly(N-VIm) at different total radiation dose: (a) 0.240 kGy, (b) 0.284 kGy, and (c) 0.312 kGy on KBr pellets.

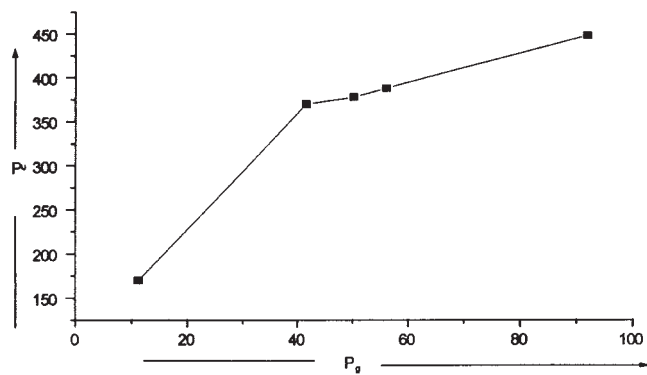


Figure 8 Variation of water uptake with P_g .

copolymers. It also reveals that grafting leads to opening of the cellulose matrix, thus increasing its accessibility to water. The direct relationship between P_g and P_u is explained by the pendent moieties of VIm that are water interacting, hence water uptake increases with the graft yield.

Metal ion sorption

Cellulose-based ion exchangers are better than the conventional ion exchangers in respect to higher chemical resistance, radiation stability, and low cost of preparation, and provide sufficient hydrophilic surface area for the partitioning of metal ions from their solutions. Interaction of cellulose with small molecules and metal ions arise from the capacity of its glycolic groups to do so, and substitutions by attaching acidic or basic groups on the backbone by grafting leads to the increased workability and diversification of the backbone properties. Since the graft copolymers prepared in the present study are not ion exchangers, these remove metal ions both by adsorption on N: and also by sorption in the bulk as the graft copolymers act a hydrogels. Hence, the general term sorption is used because of the expected multiplicity of metal ion uptake by the graft copolymers. Cellulose also binds metal ions at the glycolic position, but modified-cellulose, obtained by grafting with poly (4-VIm), enhances this property of cellulose, and metal ion uptake increases as a function of P_g . The uptake of Fe^{2+} ions is appreciably high and P_u , even up to 100%, has been observed [Fig. 9]. Cu^{2+} ion uptake is also considerable and it increases with the P_g . The binding of the Cu^{2+} ions by poly(VIm) moieties is effective and the complex thus formed has been reported by Tsuchida and Niside.¹ In both these cases the relationship of P_g and P_u is linear and well defined. The sorption of Cu^{2+} ions is comparatively lower than that of the Fe^{2+} ions and it is manifestation of the effective charge density of these ions.

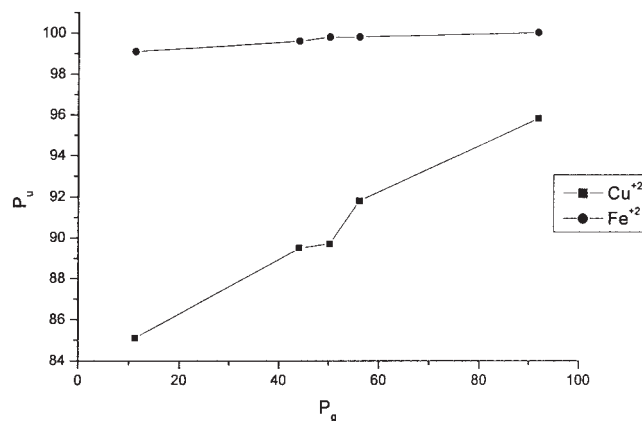


Figure 9 Variation of P_u vs. P_g of cell-g-p(VIm).

Immobilization of protease and BSA

Results on enzyme immobilization have been plotted in Figure 10. The enzyme used in the present study is metalloprotease isolated from *Bacillus* species and has a molecular weight of 16.9 KDa. Metalloproteases involve metal ions at its active sites, and given the affinity that N-VIm has for metal ions as discussed before, higher protease uptake has been expected. It has been observed that grafting onto cellulose effectively increases immobilization on polymeric support as compared with the native cellulose and the activity of enzyme increases with the increase in P_g and reaches nearly 100% (99.8%) in case of cell-g-poly(VIm) with a moderate P_g of 56.1 and tends to be almost constant (97.2) at higher P_g of 91.9. In other words, graft yield as low as 50% P_g is sufficient to immobilize almost all the available enzyme. Thus, immobilization efficiency observed is very high.

The adsorption of BSA (molecular weight, 66 KDa) onto different polymeric supports again reveals that

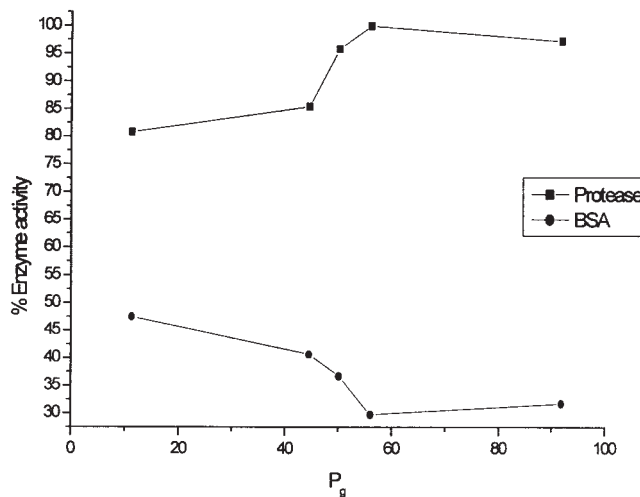


Figure 10 Immobilization of protease and BSA by cell-g-p(VIm) copolymer as a function of P_g .

native cellulose is not again good support for BSA adsorption. In contrast to the protease immobilization, it has been observed that adsorption of BSA has inverse relationship with P_{gr} , and overall grafting does not improve its uptake on these cellulosic supports. From these results it appears that apart from the interaction of the active sites of the protease with the imidazole moieties of the grafted VIm, the size effect plays important role in determining the selectivity of graft copolymers toward these two proteins. It is also explained given the comb-shaped structure of cellulose graft copolymer, which makes smaller molecules more accessible to the inner core of the graft copolymers chains. Thus, the BSA macromolecule is excluded because of its larger size as compared with that of the protease.

CONCLUSIONS

From the foregone discussion it can be concluded that the grafting of poly(VIm) onto cellulose results in effective supports both for the metal ion sorption and for the immobilization of enzyme (protease). Trends in results manifest that the effect of structural aspects of the supports are the main determinant that affect the quantum of immobilization. It has also been observed from these studies that the sorption of Fe^{2+} and Cu^{2+} ions is appreciable. The high efficiency combined with the marked selectivity for metal ions. The nitrogen containing moieties of the grafted polymers act as anchor to the enzyme. Further, compatibility of VIm to enzyme is also an important factor that determines the efficiency of these supports. On the other hand, lower BSA adsorption, especially at the higher graft yields, is due to the lack of accessibility of this protein to the inner core of the graft chains because of the size exclusion effect as expected in a comb-shaped graft copolymers with high graft levels. Since the capacity of

these supports is excellent both for the sorption of two metal ions with variable oxidation states and also for enzyme, these two factors can be combined to use these supports as bio-analogous catalysts and also for the development of protein separation processes.

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