Homogeneous and Heterogeneous Grafting of 4-Vinylpyridine onto Chitosan

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ABSTRACT: Modification of chitosan by grafting with 4-vinylpyridine (VP) was carried out both in homogeneous and heterogeneous phases, using potassium persulfate ($K_2S_2O_8$) and sodium bisulfite ($NaHSO_3$) as redox initiators. The effect of monomer concentration, initiator concentration and redox ratio, time and temperature on the extent of grafting (*G*%), homopolymer formation, and the efficiency of grafting were studied. Values of grafting percentages up to 96% were reached in heterogeneous conditions and up to 130% in homogeneous conditions (in 5% acetic acid). The grafting was confirmed by FTIR and ^{1}H NMR spectroscopy. The grafted samples were characterized by scanning electron microscopy, X-ray diffraction, and thermogravimetric analysis. The crystallinity of the used chitosan was not affected by grafting, it even increased slightly. Dye uptake of the grafted samples towards the different types of dyes (acidic and basic) was investigated and was found to improve profoundly over the native chitosan with a higher uptake for the acidic dye. The grafted samples showed an increased swelling in water, which increased further upon quaternization of the graft copolymers. The extent of swelling is higher in acidic and basic media more than in neutral pH. The grafted copolymers are soluble with difficulty in warm acetic acid solution. The quaternized graft copolymer was found to be soluble in water. The biological activity of the quaternized graft copolymers (*G* = 130 and 80%) was investigated and was found to have an inhibition effect on both the *Azotobacter* fungus and the bacterium *Fusarium oxysporium*. The effect on the micro organisms is proportional to the amount of VP in the graft copolymer. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 99: 3308–3317, 2006

Key words: chitosan; 4-vinylpyridine; graft copolymer; swelling behavior; dye uptake; quaternization

INTRODUCTION

The modification of natural polymers, by grafting technique, is a promising method for the preparation of new materials. This method enables one to introduce special properties and enlarge the field of the potential applications, especially, for biopolymers present in abundance. Chitosan is the product obtained from N-deacetylation of chitin with strong alkali.[1,2] Chitin is an intractable and abundant (second or third to cellulose) naturally occurring polysaccharide forming part of the shell of crustaceans and insects. Whereas chitin contains an acetamide group situated in the C-2 of the anhydroglucose ring, chitosan is a random copolymer of β-(1–4)-2-acetamido-2-deoxy-D-glucose and β-(1–4)-2-amino-2-deoxy-D-glucose units.[3,4] The presence of free amino groups in chitosan enhances the solubility of this polysaccharide in dilute acids as compared with chitin as well as imparting a positive charge to the polymer. Many uses of chitosan are based on its positive charge, which is attracted to negatively charged materials as for example most living tissues, polyanions, bacteria, fungi, enzymes, and microbial cells. Many attempts have been taken to further modify the natural polymer chitosan by graft copolymerization with several vinyl monomers. Representative examples of grafting of chitosan are acrylonitrile,[5,6] vinyl acetate[5,7,8] methyl acrylate[5,9–11] methyl methacrylate[5,11,12] hydroxyethyl methacrylate,[13–16] methoxy-poly(ethylene glycol),[17] acrylamide,[18–20] acrylic acid,[18–22] 4-vinylpyridine (VP),[23,24] styrene,[25–27] vinylpyrrolidone,[28] and isopropylacrylamide[29,30] and have been grafted onto chitosan using several initiators. In a comprehensive review Jenkins and Hudson[31] have discussed the recent advances toward controlling radical-based reactions of grafting chitin and chitosan with many acrylic and vinyl monomers under different experimental conditions. Another review dealing with the metal complexation of chitosan and its derivatives[32] threw more light on the importance of chitosan and its value. The antibacterial activities of quaternary ammonium salt of chitosan have been also discussed.[33]

The present work deals with the grafting copolymerization of chitosan with VP to investigate eventual changes in its behavior. The combined effect of the principal reaction variables of the grafting process was studied systematically. The capacity of the grafted chitosan samples for acidic or basic dyes was investi-

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gated. Since chitosan is nontoxic, bio-absorbable,[34,35] and swells to different extents in different pH media, it is an excellent candidate for drug release formulations.[36] Therefore, the swelling behavior of the graft/chitosan copolymers is investigated at different pH values. Another interesting modification was made by quaternarization of the grafted chitosan, which could be used as antibacterial material [33] The biological activity of the quaternized grafts towards two kinds of organisms: a fungus and a bacterium have been investigated. The fungus (*Fusarium oxysporium*) is considered to be one of the most important fungi that cause *Fusarium*-wilt and rot diseases for many economic crops i.e., wilt of cotton, wilt of tomato, rot of gladiolus, dry rot of potato, and ear rot of maize. On the other hand, the bacterium (*Azotobacter*) has the properties of fixing atmospheric nitrogen and secreting certain growth hormones necessary for improving plant growth and increasing soil fertility.[37–39]

EXPERIMENTAL

Samples

Chitosan was kindly supplied by Professor Dr. Furuhata of Tokyo Institute of Technology (T.I.T). 4-Vinylpyridine (VP), from ACROS organics, was distilled just before use (bp 73°C). Initiators (potassium persulfate and sodium bisulfite) were analytical grade reagents from Merck chemicals and were used as received. All solvents were from Aldrich and were purified by distillation, according to the conventional methods. Eriochrome Black T and Methylene Blue dyes were purchased from Aldrich and were used as received.

Grafting reactions

Heterogeneous grafting reaction

An exact amount (0.5 g) of dry chitosan was mixed with water in 50-mL stopper flask, with 1:50 liquor ratio; followed by the addition of monomer and initiator in this order. The flask was placed in a thermostated bath and the reaction mixture was shacked occasionally. After a suitable time, the product was filtered, washed with water to remove the unreacted monomer, and dried at 60°C until a constant weight was reached. Exhaustive extraction (using a soxhlet for 24 h) of the product with methanol allowed the separation of poly 4-vinylpyridine (PVP) homopolymer formed during the grafting reaction. The graft copolymer was thoroughly washed with water, followed by drying to constant weight. Separated by methanol extraction, the PVP homopolymer was precipitated in water and its structure was confirmed by Fourier transform infrared (FTIR) spectroscopy.

Grafting parameters such as grafting percentage (%*G*), homopolymer percentage (%*H*), and grafting efficiency (%*E*) were determined as follows:

$$\%G = \frac{W_2 - W_1}{W_1} \times 100$$

$$\%H = \frac{W_4 - W_2}{W_3} \times 100$$

$$\%E = \frac{W_2 - W_1}{W_3} \times 100$$

where, W_1, W_2, W_3, and W_4 denote the weight of initial chitosan, grafted chitosan after extraction with methanol, vinylpyridine monomer charged, and grafted chitosan before extraction with methanol, respectively.

Homogeneous grafting reaction

The same above procedure was repeated except that the chitosan sample was dissolved in 5% diluted acetic acid and mixed with the monomer and the initiators in this order. At the end of the grafting reaction, the medium was neutralized with enough ammonia to precipitate the graft copolymer. The flask was filtered and the precipitate was extracted with methanol as described earlier. The graft copolymer was dried and subjected to elemental analysis (C, H, and N) and IR-spectroscopy. The elemental analyses were conducted in the analytical unit in the University of Halle, Halle, Germany.

FTIR spectroscopy

FTIR spectra were taken using FTIR spectrometer Bruker Vector 22 Germany in the range of 400–4000 cm^{-1}.

^{1}H NMR spectroscopy

The spectra were recorded on a Varian Mercury 400 spectrometer. The solvent used was 95% D_2O:5% CD_3COOD (unless otherwise noted). The spectra were measured at 70°C.

Thermal analysis

Thermogravimetric analysis (TGA) was carried out using Shimadzu TGA-50H at a heating rate of 10°C/min under nitrogen atmosphere.

Scanning electron microscopy

The surface morphology was investigated with a JEOL JEM-100S M microscope. The sample surface was coated with gold and fixed on a plate holder with a silver paste, then investigated using back scattered electron to detect the image of the surface with high resolution accelerated voltage 60 kV and 50-μA beam current.

X-ray diffraction analysis

X-ray diffraction measurements were carried out using Scintag/USA XGEN-4000 at 45 kV and 40 mA using Ni-filtered Cu Kα radiation. A measure of the crystallinity was obtained by comparing the area of the crystallinity peaks to the whole area.

Dye uptake and fastness

In 100-mL round bottom flask, 0.5 g of the grafted sample was charged together with 25 mL of 1% dye solution (molar ratio 1:50). The flask was placed in a water thermostat and the temperature was raised during 30 min to 95°C. The mixture was refluxed for 25 min at this temperature. The solution was left to cool to room temperature, and then filtered. The filtrate was transferred to a 25-mL measuring flask and completed to the mark. The UV-visible spectra of the samples were then measured. A calibration curve for each dye was constructed and the amount of the dye absorbed onto each grafted sample could be then determined from the difference in absorption before and after the reaction with the grafted samples. To investigate the dye stability onto the grafted copolymers, the dyed samples were boiled in water for 30 min, left to cool, and the liquor solution was transferred to a measuring flask and completed to the mark and finally the concentration of the leached dye was determined by UV spectroscopy, as described earlier, using the calibration curve of each dye.

Swelling measurements

Swelling measurements were carried out in distilled water, pH 6.5, acidic buffer pH = 3.4, and in basic buffer at pH = 10, at room temperature. The well-known tea-bag method was used.[21] A known weight of the dry sample was placed into a tea-bag and immersed in the aqueous medium. After certain time, the bag was taken out and hung for 5–10 min to eliminate excess unabsorbed water and then weighed. The degree of swelling was calculated using the relation $(W - W_0)/W_0 \times 100$, where W and W_0 are the weight of the swollen and dry samples, respectively.

Quaternization

Quaternization of chitosan and its grafted copolymers was achieved by heating the samples with dimethyl sulfate in the presence of NaOH. When the quaternization was performed using excess dimethyl sulfate per mole of chitosan, the obtained material was found to be soluble in water.

Preparation of the media for the fungus and the bacterium

The medium for Azotobacter was prepared using the following reagents: mannitol, 5 g; sucrose, 5 g; K_2HPO_4, 0.25 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; NaCl, 0.1 g; $CaSO_4$, 0.05 g; $CaCO_3$, 0.25 g; $MnSO_4{\cdot}4H_2O$, $FeCl_3{\cdot}6H_2O$, $Na_2MoO_4{\cdot}2H_2O$, traces; and agar, 15–20 g, and were dissolved in 1 L of distilled water and the pH was adjusted to 7 $\times$ 0.1*M* NaOH solution. The medium of the fungus were prepared by adding the following reagents, sucrose, 30 g; $NaNO_3$, 2 g; KH_2PO_4, 1 g; $MgSO_4{\cdot}7H_2O$, 0.5 g; KCl, 5 g; $FeSO_4$, 0.01 g; and 20 g agar in 1 L of distilled H_2O.[38,39] The antibacterial ability was examined by measuring the inhibition zone caused by the graft copolymer.

RESULTS AND DISCUSSION

The used chitosan was characterized by elemental analysis, IR, NMR, and acid–base potentiometric titration to determine the degree of deacetylation. The elemental analysis was found to be C = 41.94, H = 7.62, N = 7.60% and using the ratio of C/N of the original chitosan one gets a degree of deacetylation (DDA) from the following formula[40]:

$$DDA = \left(1 - \frac{C/N - 5.145}{6.816 - 5.145}\right) \times 100$$

A 77.8% DDA value was obtained. The NMR spectrum of the pure chitosan sample is shown in Figure 1. The band at 2.3 ppm could not be used for the determination of the DDA because of the presence of a high contamination of acetic acid, thus, rendering the height of the peak exaggerated. The two bands at 4.8 and 5.3 ppm corresponding to C-2 proton in the acetylated and deacetylated parts, respectively, were used for the DDA determination.[41] This section of the spectrum was enlarged and when the area of the two bands were compared, a value of DDA of 86% was obtained.

IR spectroscopy was used to determine the DDA using the ratio $AI655/A3450 \times 100/1.33$,[42] which lead to a value of $DD = 77\%$. Finally, the degree of deacetylation was determined by a potentiometric acid–base titration. The chitosan was dissolved in a known amount of acid (in excess). From the titration of this

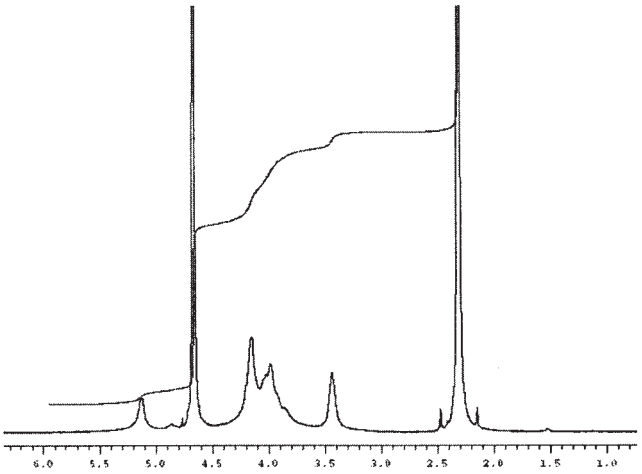


Figure 1 ^{1}H NMR spectrum of chitosan.

solution with a 0.1*N* NaOH, a curve with two inflection points was obtained. The difference of NaOH solution volumes corresponding to these points is equivalent to the amino groups and allows the determination of the DA of the chitosan using the following formula[43]:

$$DA = (1 - 161Q)/(1 + 42Q)$$

Where $Q = N\Delta v/m$, Δv is the volume of NaOH between the two inflection points (in L), N is the normality of the sodium hydroxide, and m is the weight of the dry sample.

A value of 83.9% was obtained by the latter method. It seems that the last two methods are the most reliable for the determination of DD in chitosan.

The existence of the grafting was evidenced by the weight increase and by observing the difference in the IR- spectra of the pure chitosan and the graft copolymer as shown in Figure 2.

The O—H stretching absorption vibration for chitosan forms a broad band around 3500–3200 cm^{-1}, which is due to the intermolecular hydrogen bonding, overlapped with the N—H stretching band that is supposed to occur in the same range. Primary amines have two bands in this region. The N—H bending vibration appears in the range from 1641 to 1602 cm^{-1} as two sharp bands.[44] The spectrum of the grafted copolymer shows difference corresponding to the appearance of a band at 2197 cm^{-1}, which is attributed to the poly 4-vinylpyridine chains. Two characteristic bands for CH bending of the aromatic ring of the vinylpyridine moiety that appear around 750 and 823 cm^{-1} are very indicative of the presence of the polyvinylpyridine polymer in the graft. The band at 3191 cm^{-1} in chitosan disappears almost after grafting while that at 3400 cm^{-1} increases in intensity. This seems to be due to the increased hydrogen bonding by the pyridine moiety. Quaternization reduces the intensity of the N—H bending at 1602 cm^{-1}. ^{1}H NMR spectroscopy also indicates the occurrence of grafting as evidenced by the spectrum of one grafted sample with 80% VP, shown in Figure 3.

The presence of pyridine ring is confirmed by the presence of the bands at 7.1 and 8.5 ppm because of the pyridine ring[44] and a band at 1.9 ppm for the methylene protons of the polyvinyl pyridine.

Elemental analysis of the soxhlet-extracted samples was used as a further evidence for the process of grafting through the noticeable increase in the nitrogen content with grafting (the 80% *G* sample was found to have C = 56.01 and N = 9.54%).

The surface morphology of two grafted samples was compared to that of the un-grafted chitosan and is shown in Figure 4. The chitosan surface shows a fibrous structure while the grafted samples seems to be covered by the polyvinylpyridine layer and the extent of coverage increases with the increase of *G*% (as shown in Fig. 4(b) 80% *G* and Fig. 4(c) 105% *G*). The photos indicate that grafting seems to occur mainly on the surface, this could be due to the high crystallinity of chitosan,[45] which render the penetration of VP in the chitosan bulk rather difficult.

Influence of the reaction conditions on the grafting extent

The reaction conditions of grafting, the effect of time, monomer concentration, initiator concentration, the oxidizing:reducing agent ratio and the temperature on the %*G*, %*H*, and %*E* have been investigated under homogeneous and heterogeneous conditions. The heterogeneous condition produces powder like graft,

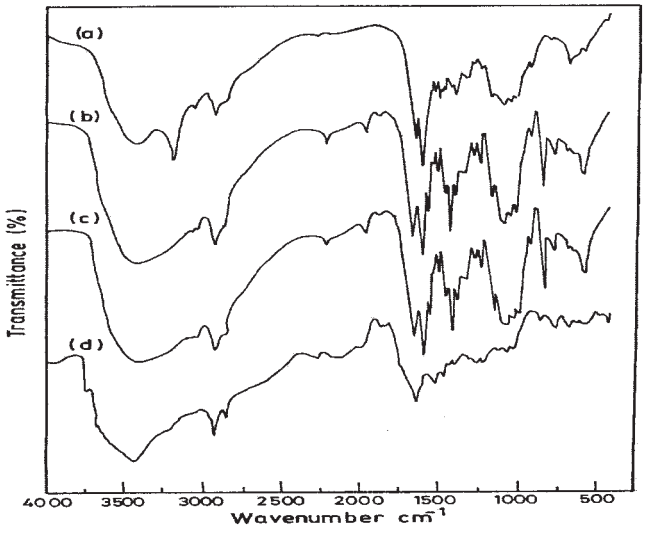


Figure 2 IR spectra of (a) chitosan, (b) VP/chitosan copolymer 26% *G*, (c) 120% *G*, and (d) quaternized VP/chitosan graft copolymer with 98% *G*.

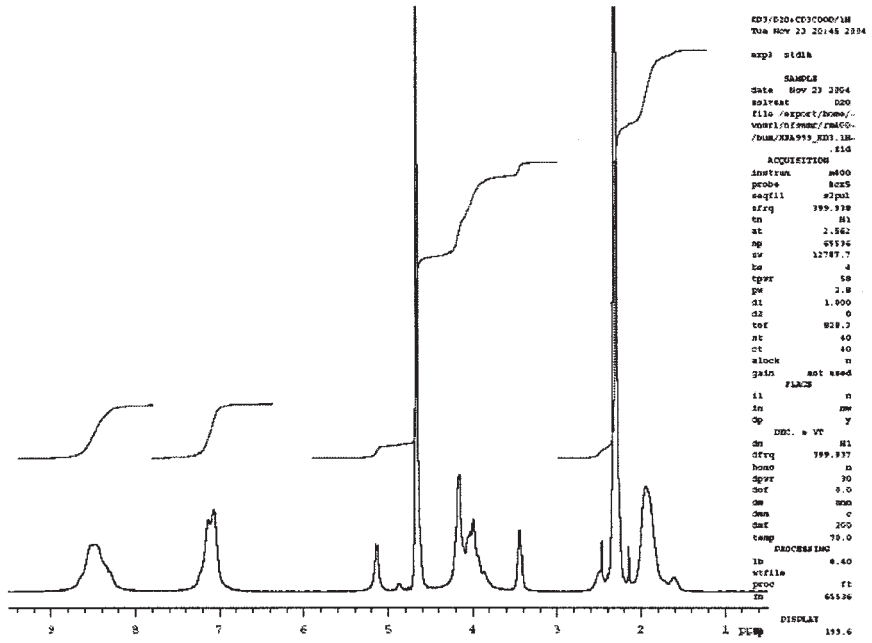


Figure 3 ^{1}H NMR spectrum of 80% VP grafted chitosan.

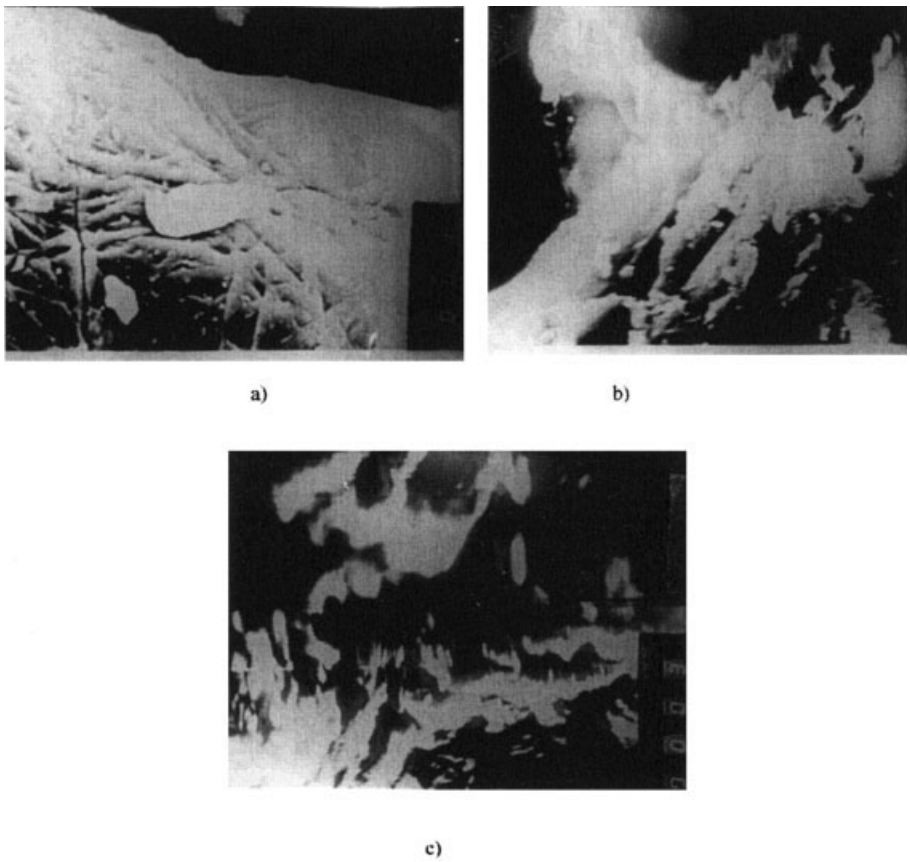


Figure 4 SEM images of (a) chitosan, (b) chitosan graft with PVP 80% *G*, (c) chitosan grafted with PVP 105% *G*.

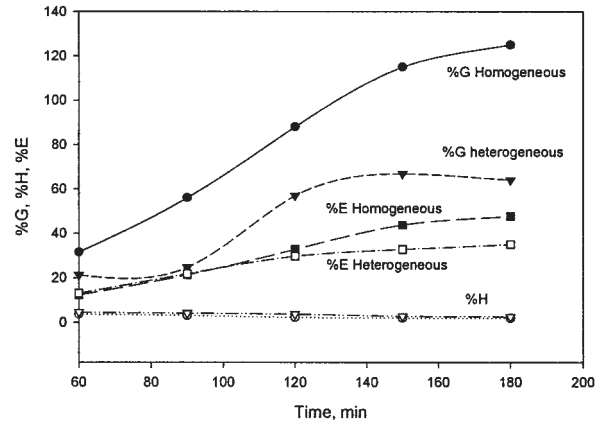


Figure 5 Effect of time on the extent of %*G*, %*H*, and %*E* for the homogeneous and heterogeneous grafting of VP onto chitosan. [VP] = 1.0*M*, Initiator [I] = $3 \times 10^{-4}M$, Temperature, T = 55°C.

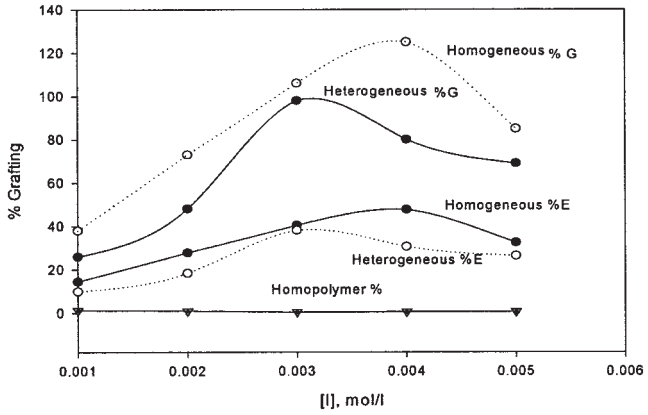


Figure 7 Effect of initiator concentration on %*G*, %*H*, and %*E* [VP] = 1*M*, T = 55°C.

which could be handled easily while the homogeneous could lead some time to paste like mixture. However, it has been found that the homogeneous method produces higher grafting yield with higher grafting efficiency (%*E*) in most cases. Figure 5 illustrates the effect of time on the three parameters in both conditions. %*G* and %*E* are higher for the homogeneous condition.

The effect of monomer concentration [VP], in Figure 6, was studied by using a set of predetermined reaction conditions based on preliminary tests. As can be seen from Figure 6, the percentage of grafting increases with increasing the vinyl pyridine concentration reaching 122 and 96% at 1.5*M* VP for homo and hetero conditions, respectively. The effect of initiator concentration and the ratio of its components (Figs. 7 and 8) on the %*G*, %*H*, and %*E* were studied at 55°C with 1*M* VP for 3 h. Here, the maximum %*G* was obtained at $\sim 3 \times 10^{-4}M$ of redox initiators with ratio 1:0.75 potassium persulfate:sodium bisulfite. From Figures 7 and 8, the maximum %*G* was obtained at 55°C and after 3 h. The effect of temperature is illustrated in Figure 9 and shows that the heterogeneous grafting has a maximum in %*G* beyond which the grafting percentage decreases and %*E* decreases consequently, while under homogeneous conditions the %*G* increases with temperature till 55°C then decline slightly.

The grafted VP/chitosan copolymers were found to be insoluble in water but soluble in acetic acid solution after warming. The low %*H* formation indicates that the oxidizing agent apparently attacks the chitosan backbone and free radicals are created onto the glucoside ring directly, followed by the monomer attacking this center leading to the formation of the PVP side chain. A possible mechanism for the grafting reaction could be illustrated in the following Scheme 1:

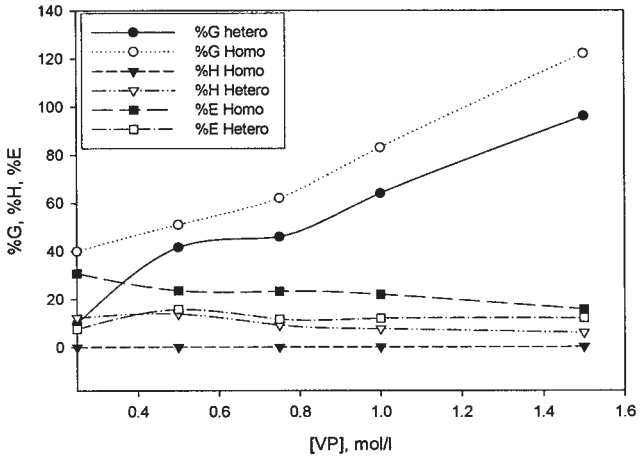


Figure 6 Effect of 4-vinylpyridine concentration on the %*G*, %*H*, and %*E* under homogeneous and heterogeneous conditions. [I] = $3 \times 10^{-4}M$, T = 55°C.

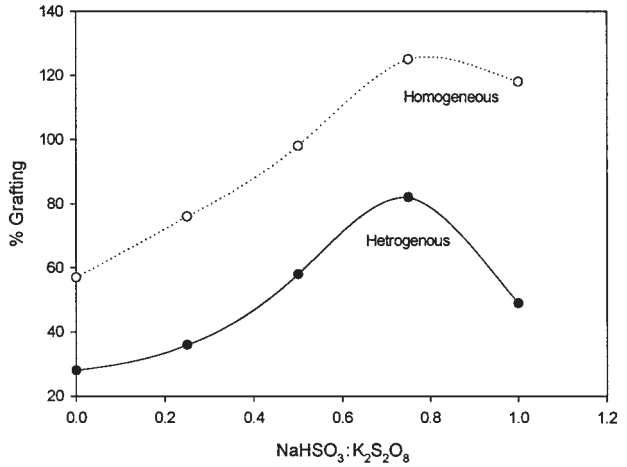


Figure 8 Effect of the initiator ratio on the extent of grafting [VP] = 1*M*, T = 55°C.

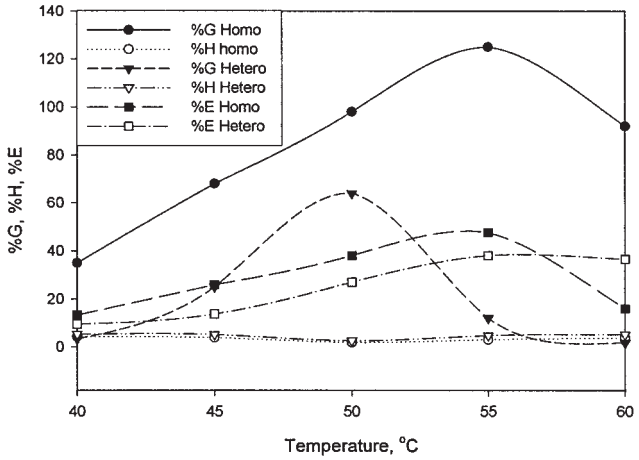


Figure 9 Effect of temperature on the %*G*, %*H*, and %*E*. [VP] = 1*M*, [I] = $3 \times 10^{-4}M$.

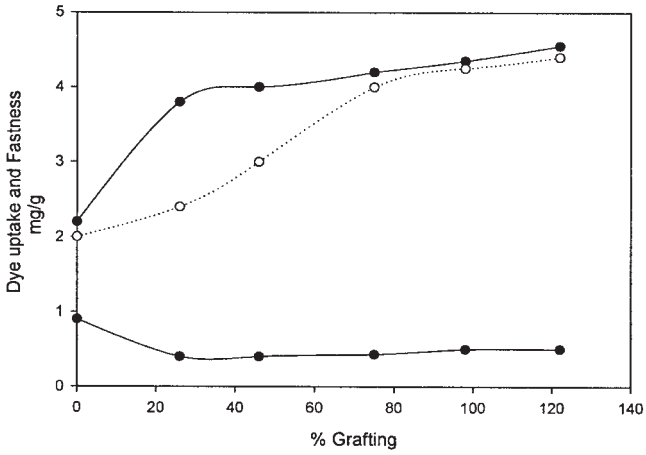
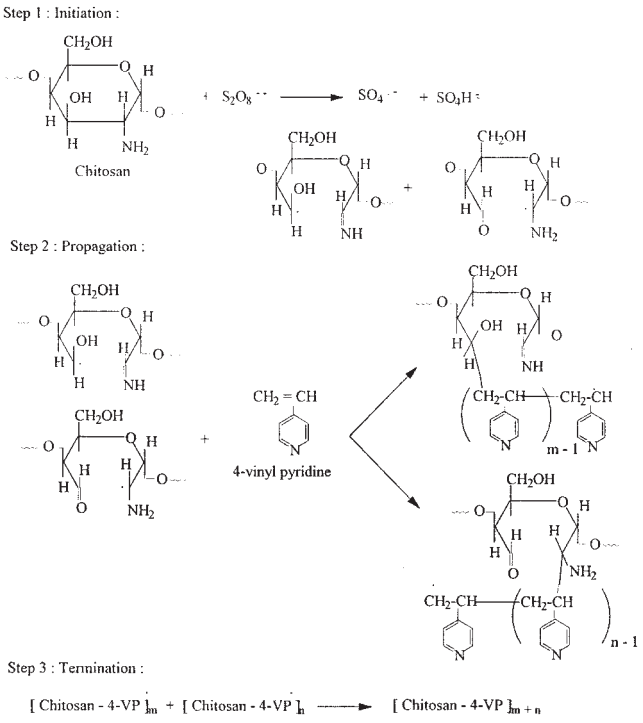


Figure 10 Dye uptake and washing fastness of chitosan and its grafted copolymers, $T = 37°C$.

Dyeing of polyvinylpyridine/chitosan copolymer

The results of the dye uptake by chitosan and its grafted copolymers are illustrated in Figure 10 (the dye uptake is represented as mg dye/g polymer). Two types of dyes have been used: an acidic dye Erochrome Black T (EBT) and a basic one Methylene Blue (MB). The figure contains also the results of washing fastness. The behavior of the two dyes, as shown in the figure, indicates that the grafted samples absorb higher amount of both the types of dyes when compared with that of the original chitosan polymer. However, the acid dye has higher absorption and shows very high fastness since when the samples treated with the acidic dye are boiled in water for 20 min, no dye was released. This could indicate a chemisorption interaction with the basic polymer, while the basic dye is apparently physically adsorbed onto the polymer surface and is washed to greater extent when the dyed samples are boiled in water, as indicated by the lower curve in Figure 10.

X-Ray curves for copolymer samples prepared by heterogeneous grafting are shown in Figure 11. By



Scheme 1 Illustration of possible mechanism of grafting of VP onto chitosan.

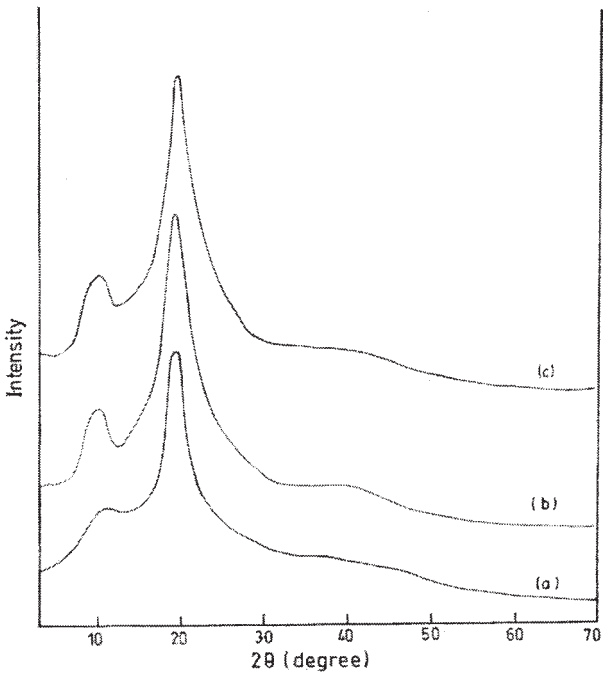


Figure 11 X-ray curves for (a) chitosan, (b) chitosan 80% *G*, (c) chitosan 105% *G*. The intensity of scattering is plotted as a function of 2θ.

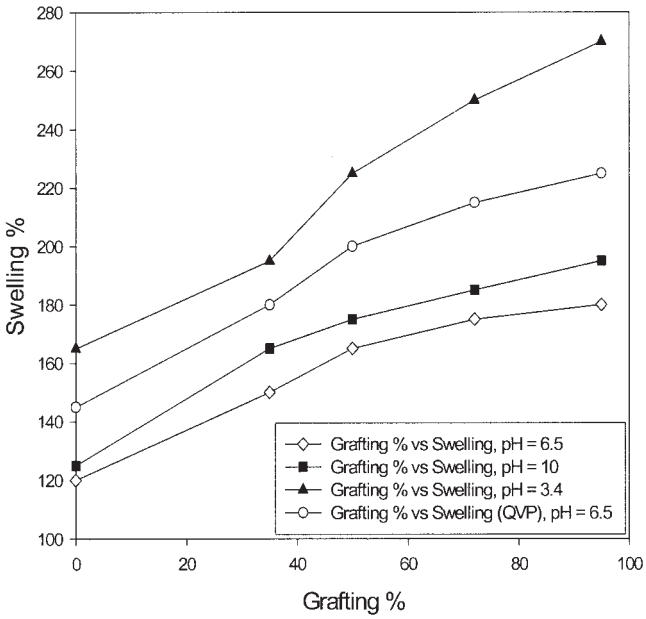


Figure 12 Effect of %*G* and pH on the extent of swelling of the chitosan/VP copolymers at room temperature, time of swelling = 6 h. Quaternized samples at pH 6.5.

comparing the area of he crystalline peaks to the whole area under the curve, an estimate of the percent crystallinity was obtained. It is interesting to notice that using heterogeneous method of grafting, the crystallinity of the original chitosan (49.3%) increased to 54.3% for the 80% *G* and to 55.4% for the 105% *G* samples. This may be due to the fact that grafting occurred mainly on the surface of the samples and did not disrupt the internal structure drastically. This comes in accordance with the SEM graphs given previously.

Swelling behavior of chitosan and its grafted copolymer

It has been found that the water uptake increases with time and reaches saturation after ~6 h. The swelling % after 6 h is plotted versus *G*% as shown in Figure 12. The swelling increases with the increase in the extent of grafting percent, in this way the hydrophilicity of the graft copolymer is higher than the original chitosan, which could have important consequence in biomedical applications. The pH has a strong effect on the extent of swelling increasing in the following order: pH 3 > pH 10 > pH 6.5. This behavior could be used in controlled release of drugs.[36] The swelling of the quaternized samples was measured only at pH 6.5.

Quaternization of polyvinylpyridine/chitosan copolymer

Quaternization of chitosan and its grafted copolymer can be achieved by reaction with dimethyl sulfate in basic medium (NaOH solution). It has been found that the degree of swelling increases upon quaternization for all the treated samples reaching 45% water uptake, as shown in Figure 12. At high degree of the reaction with dimethyl sulfate, the samples were found to be soluble in water, a property which could have important consequence for the antibacterial application of these copolymers.[44] Figure 13 illustrates the ^{1}H NMR spectrum of a quaternized sample in D_2O. The two signals at 7.6 and 8.7 ppm correspond to C (2, 6) and C (3, 5) of the pyridine group, respectively, both shifted to lower field due to deshielding of the methyl group on the N^+ atom, the bands show also some broadness due to the incomplete quaternization of all the pyridine groups. The signal for the methyl groups at 3.0 ppm correspond to the residual —$COCH_3$ of the un-hydrolyzed chitin (one can notice the reduced intensity of the band in D_2O compared with that in deutrated acetic acid/D_2O mixture. The (N—CH_3) groups at 3.4 ppm are shown in the complex spectrum in addition to signal at 2.2 ppm, which could be due to the —CH_2— of the polyvinylpyridine side chain and at 3.9 ppm for the CH_2—O of the chitosan.[46,47] The strong signal of the solvent (water) at 4.7 ppm obscures the OH of the chitosan. The spectrum offers additional proof of quaternization, in addition to the water solubility of the quaternized samples.

Biological activity of the quaternized graft copolymers

Preliminary results of the biological activity measurements show that grafting with VP and quaternization of the copolymer produces inhibiting effect on both micro organisms. The inhibition of growth of the fungus will be useful for the plant, decreasing the diseases it causes. However, a negative inhibition influ-

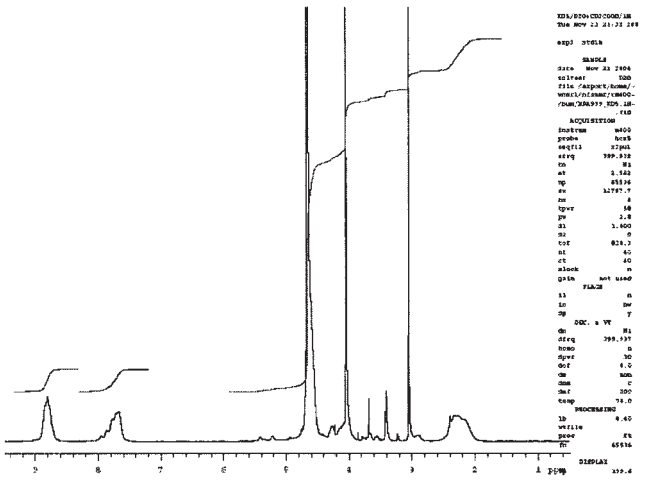


Figure 13 ^{1}H NMR spectrum of quaternized grafted chitosan copolymer, 130% *G*.

TABLE I
Values of the Inhibition Zone of the Quaternized Graft Copolymers for *Azotobacter* and *Fusarium oxysporium*

Graft copolymer (G%)	Azotobacter (cm)	Fusarium oxysporium (cm)
VP (130)	6.2	4.5
VP (80)	5.3	3.6

ence on the growth function of the bacteria will be harmful for the plant, decreasing the fixation of useful atmospheric nitrogen. Table I shows the inhibition zones for both micro organisms; the inhibition zones increase with increasing the grafting percentage. More detailed investigations are being performed.

Thermal behavior

Figure 14 shows the thermogravimetric curves of chitosan [Fig. 14(a)], PVP homopolymer [Fig. 14(b)], and two grafted copolymer samples with 65% *G* [Fig. 14(c)] and 98% *G* [Fig. 14(d)]. The first endotherm of pure chitosan is due to elimination of water starting around 52°C followed by one step degradation at about 314°C. The thermal degradation of PVP follows several steps. The first is elimination of water (2.5%) followed by small weight decrease 8.7% then a dramatic weight loss (54%) starting at 280°C with T_{max} at 302°C. The final stage occurs at 320–380°C. The TGA of the grafted sample [Fig. 14(c)] shows the same first endotherm; however, there are two steps for the decomposition, a small endotherm probably due to the decomposition of the polyvinylpyridine branches, followed by a major endotherm at around 388°C. At the same time, one can notice a slight improvement in the thermal resistance for the graft copolymer since the major decomposition of chitosan occurs at 314°C while that of the graft copolymer occurs at 379°C and 388°C for the 65 and 98% *G* respectively. This means that there is synergetic effect when PVP is grafted to chitosan, the H-bonding between the N of the pyridine ring and the O—H of chitosan slightly enhances the thermal stability of the final material.

CONCLUSIONS

Poly 4-vinylpyridine could be grafted onto chitosan up to 130% under homogeneous conditions (in 5% acetic acid) and up to 98% in the heterogeneous conditions (as suspension in water). The grafting efficiency in homogeneous conditions is higher than that in heterogeneous conditions for all the investigated parameters. It was possible to control the extent of grafting by varying the reaction parameters, namely, monomer concentration, initiator concentration, temperature, and time. The grafted samples are insoluble in common solvents, however, they are soluble in hot diluted acids, therefore, one can exclude crosslinking reaction between the chains but an increased hydro-

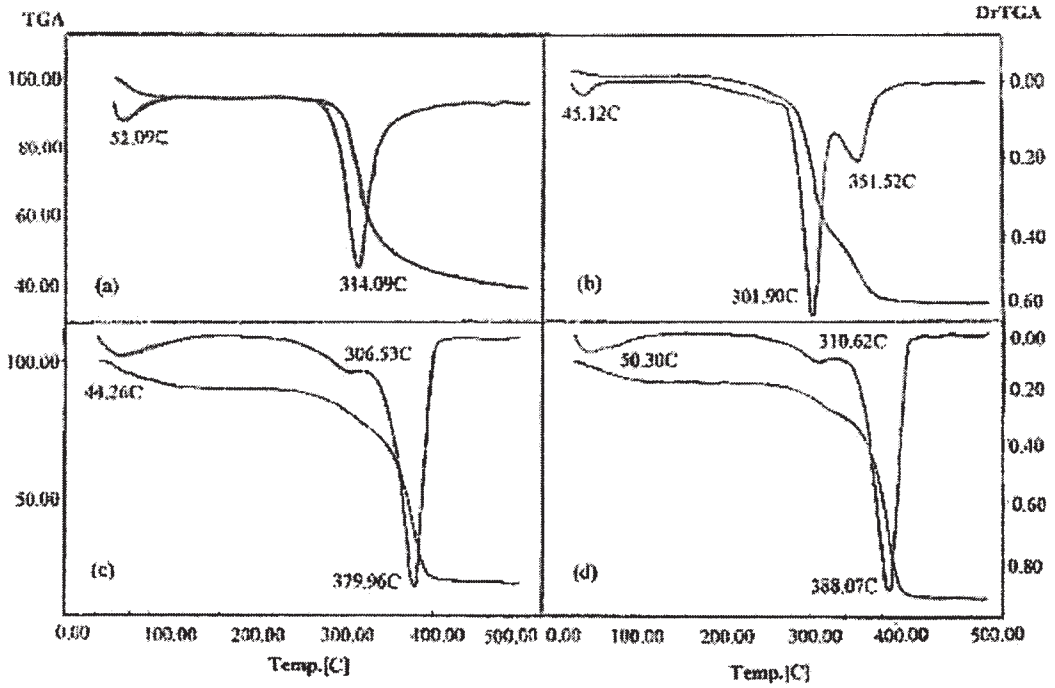


Figure 14 Thermogravimetric and differential curves for (a) chitosan, (b) PVP homopolymer, (c) PVP/chitosan graft copolymer 65%*G*, (d) PVP/chitosan 98% *G*.

gen bonding makes the solubility more difficult. Dye uptake of the grafted samples was studied using acidic and basic dyes. It has been shown that the acid dye uptake is much higher than that for the basic dye, which is due to the basic character of chitosan and the presence of 4-vinyl pyridine in the graft copolymer. The dye fastness is also much higher for the acidic than that of the basic dye and this is due to the chemisorption of the acidic dye onto the basic chitosan graft copolymer. SEM microscopy showed that grafting occurs probably on the surface, this could be a consequence of the high crystallinity of chitosan. X-ray analysis of samples prepared by heterogeneous grafting showed higher percent crystallinity with increasing grafting. The swelling behavior of chitosan and its grafted copolymers showed that water uptake increases with increasing the grafting content. Moreover, the swelling in acidic medium is higher than in basic medium and the swelling in the latter medium is higher than that in neutral medium. This property could be useful for further practical biomedical applications, particularly for drug release applications. Further increase in the swelling capacity was achieved upon quaternization of chitosan/polyvinylpyridine copolymer with dimethyl sulfate in basic medium. Quaternization with 3 mol of dimethyl sulfate led to the preparation of soluble graft copolymer with a film forming ability, the antibacterial properties of this system shows that increasing *G*% leads to higher inhibition values for bacteria and fungi. The thermal stability of the graft copolymer did not show deterioration compared with that of the original chitosan, it increases slightly with percent grafting.

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