

Maize Starch-g-poly(n-vinylimidazole) Synthesis and its Application in Sewage Water Treatment

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ABSTRACT

In this study, maize starch-g-poly(N-vinylimidazole) was prepared and characterized by determining its degree of substitution and elucidating its structure by FTIR. Graft copolymerization of vinyl imidazole onto starch was carried out in aqueous solution using potassium persulfate (KPS)/ sodium bisulfite (NaBS) initiator. Evidences of grafting were obtained via FTIR spectroscopy and X-ray diffraction before and after grafting. The effects of initiator concentration, monomer concentration, reaction time and temperature on the graft copolymerization were studied by determining the grafting percentage (% G), grafting efficiency (% GE) and the amount of homopolymer formed (%H). The optimum grafting conditions were determined as follows: [(KPS)/ (NaBS)]= 8×10^{-2} (mol.L⁻¹), [M]=3.5 (mol.L⁻¹), reaction temperature=55 °C and reaction time=2 h. The efficacy of the graft copolymers to uptake metal ions from their aqueous solutions was also studied. Results showed that the graft copolymers adsorbed more metal ions than starch itself. The graft copolymers were found to have antimicrobial activity against *Streptococcus pneumonia* and *Bacillus subtilis* as gram-positive and against *Escherichia coli* as gram-negative bacteria and against *Aspergillus fumigatus* and *Candida albicans* as fungi

INTRODUCTION

Starch or Amylum is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as an energy store. It is the most common carbohydrate in human diets and is present in large amounts in such staple foods as potatoes, wheat, maize (corn), rice and cassava. Pure starch is a white, tasteless, and odorless powder that is insoluble in cold water or alcohol. It consists of two types of molecules; the linear and helical amylose and the branched amylopectin. Depending on the plant, starch generally contains 20-25% amylose and 75 - 80% amylopectin by weight [1]. Products based on starch are potentially renewable, biodegradable, and ecofriendly [2]. Native starch has some limitation properties. Chemical modification of starch greatly improves its chemical and physical properties [3,4]. Azole compounds, which contain nitrogen atoms in different positions, can form complexes with various transition metals [5]. The coordination chemistry of imidazole and imidazole derivatives has attained great interest for several years [5]. Most of the copolymers are prepared through graft polymerization of vinyl or acryl monomers onto the biopolymer backbone [6]. Grafting of polyvinyl and polyacrylic synthetic materials onto the polysaccharides are mainly achieved by radical polymerization. The chemical and radiation initiating systems are employed to graft copolymerize these monomers onto polysaccharides. Thus, Poly (N-vinyl imidazole) (PVI) has been grafted onto carboxymethyl starch (CMS) in an aqueous solution using (KPS) as initiator and was examined for heavy metal ions removal from aqueous solutions. The grafted material was more sensitive to Cd(II) and Zn(II) ions than to Mn(II) [7]. Ceric ammonium nitrate-initiated graft polymerization of acrylonitrile (AN) onto spherocrystals formed in slowly-cooled solutions of jet cooked corn starch yielded graft copolymers containing higher percentages of grafted polyacrylonitrile (PAN) than comparable polymers prepared from granular corn starch. Molecular weights of PAN in grafted spherocrystals were higher by about a factor of six than the PAN molecular weight in grafted granular corn starch [8]. Cassava starch grafted with polystyrene (PS-g-starch) was synthesized via free-radical polymerization of styrene using suspension polymerization technique indicating the capability of polymerization of styrene monomer on the granular starch [9]. Polyacrylamide grafted starch (St-g-PAM) has been synthesized by microwave assisted method involving a synergism of microwave radiation and ceric ammonium nitrate (CAN) to initiate the grafting reaction. The higher the

percentage grafting of the graft copolymer, the higher is its intrinsic viscosity and consequently the higher the algal flocculation efficacy. The harvested algal biomass can be used for industrial applications (e.g., biodiesel production) or for food security (e.g., animal feed or as human food supplements) ^[140]. Environmental pollution, as a consequence of industrialization process, is one of the major problems that has to be solved and controlled ^[141]. Heavy metals are not degradable and have increasing significance owing to their harmful effect on human physiologies and other biological systems when they exceed the tolerance levels and become an ecotoxicological hazards of prime interests ^[142,143]. Adsorption is an effectively purification and separation technique used in water and waste water treatment. Starch has been modified by grafting to be used as metal adsorbant like Pregelled starch (PS) which was first crosslinked with epichlorohydrin (ECH) to obtain insoluble cross-linked pregelled starch (CPS). The latter was graft co-polymerized with different amounts of dimethylaminoethyl methacrylate (DMAEM) using potassium permanganate/sulphuric acid redox system. Pregelled starch could become an active and relatively insoluble metal scavenger for heavy toxic divalent cations such as Cu^{2+} , Pb^{2+} , Cd^{2+} and Hg^{2+} ions contaminated in water as well as efficient acid dyes removal ^[144]. The graft copolymer of crosslinked starch/acrylonitrile was prepared and used for the dynamic adsorption of the metal ions in water ^[145]. Ceric ions induced graft copolymerization of N,N dimethyl acrylamide (DMA) and acrylamide (AM) were carried out onto the hydroxyethyl starch (HES). These grafted copolymers were used for the removal of metal ions from their aqueous solutions. The metal ions absorption efficiency follows the order $\text{Hg(II)} > \text{Cu(II)} > \text{Zn(II)} > \text{Ni(II)} > \text{Pb(II)}$, pH 5.5 was found to be optimal for the removal of the studied metal ions ^[146]. A series of amphoteric starch-based grafting flocculants (3-chloro-2-hydroxypropyl trimethyl ammonium chloride modified starch-graft-poly(acrylamide-coacrylic acid)) denoted as SCPAMPAA were successfully synthesized with different grafting ratios and used for the removal of different charged contaminants from water ^[147] and synthesis of Starch-g-(Poly N, N-dimethylacrylamide-co-acrylic acid) was carried out by solution polymerization technique using potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) as the initiator. The synthetic graft copolymer was used for removal of hexavalent chromium ions [Cr (VI)] from its aqueous solution ^[148]. An antimicrobial is an agent that kills microorganisms or inhibits their growth. Imidazoles are one of the most promising and vigorously pursued areas of contemporary antifungal chemotherapy depicting broad spectrum and potent activity. They have relatively simple molecular nucleus, which is amenable to many structural modifications. These agents have several favorable properties such as excellent bioavailability, good tissue penetrability and permeability and a relatively low incidence of adverse and toxic effects. They have been found effective in the treatment of various infectious diseases ^[149]. CMS-g-PVI has bactericidal properties and can be used for seed treatment to control xanthomonads associated with bacterial leaf spot (BLS) ^[150]. In the present study, we prepared different poly (N-vinylimidazole)-grafted-starch copolymers with different graft percentages (%G) having metal ions uptake properties and antimicrobial activity.

EXPERIMENTAL

Materials

Starch was purchased from Nasr Chemical Co.-Egypt. Vinyl imidazole, Potassium persulfate, Sodium bisulfite, Ethanol 99%, Methanol 99%, Acetic acid, DMF, 1,4-dioxane, THF and Benzene were purchased from Sigma-Aldrich Co.-Germany. Copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from Merck Co.-Germany. Cadmium chloride ($\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$) was purchased from Koch-light lab. Ltd-England. Cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) was purchased from May & Baker Ltd Dagenham-England. *Bacillus subtilis*, *Streptococcus pneumonia*, *Escherichia coli*, *Aspergillus flavus* and *Candida albicans* from culture collection of the regional center for mycology and biotechnology, El Azhar University, Cairo, Egypt.

Synthesis of Maize Starch-g-poly(N-vinylimidazole)

Pre-determined amount of starch (W_0) was dissolved in 20 ml distilled water, then a specified amount of vinyl imidazole (VI) was added and the mixture was heated at specific temperature for 15 min, then a determined amount of sodium bisulfite/potassium persulfate redox initiator was dissolved in 5 ml distilled water and added drop wise to the mixture. The mixture was left for 2 h at 60-70 °C then the graft was precipitated into cold methanol. The precipitate was filtered and left to dry in an air oven for 48 h, and its weight was determined (W_2). Ethanol was used for soxhlet extraction for 8 h to get rid of any formed homopolymer. The grafted samples after extraction were dried in an air oven at 40 °C until reaching a constant weight (W_1).

The grafting parameters were calculated according to the following equations (1-3) ^[21]:

$$\text{Graft Yield (G\%)} = [(W_1 - W_0) / W_0] \times 100 \dots\dots\dots \text{Equation 1}$$

$$\text{Homopolymer (H\%)} = [(W_2 - W_1) / W_3] \times 100 \dots\dots\dots \text{Equation 2}$$

$$\text{Grafting Efficiency (GE\%)} = [(W_1 - W_0) / (W_2 - W_0)] \times 100 \dots\dots\dots \text{Equation 3}$$

Where W_0 , W_1 are the weights of the initial matrix and grafted matrix (i.e., wt. of the grafted product before and after extraction), respectively; whereas W_2 is the crude product before extraction and W_3 is the weight of monomer.

Solubility Test

Solubility of different grafts (starch-g-PVI) in different organic solvents (1% acetic acid, 1% acetic acid: ethanol, ethanol, DMF, THF, benzene and 1,4-dioxane) in addition to cold and hot distilled water has been tested. The test was done by putting 0.02 g of

starch and each graft in 10 ml of each solvent for 24 h. Another test was done by putting 0.02 g of starch and each graft in 10 ml dist. water and heated with stirring for a definite time to dissolve.

Metal Ions Uptake

A definite weight (0.02 g) of tested samples were immersed in 10 ml of known concentration (2×10^{-4} mol.L⁻¹) of the chloride salt solutions of the investigated heavy metal ions (Co²⁺, Cu²⁺ and Cd²⁺) at room temperature (~25 °C) till equilibrium (for 24 h). After filtration, the concentration of the remaining metal ions was estimated by using atomic absorption technique and consequently, the concentration (ppm) of adsorbed metal ions can be calculated by difference [22,23]. Metal ions uptake was not tested on poly(N-vinylimidazole) (PVI) due to its water solubility. Starch, Starch-g-poly(N-vinylimidazole) being partially soluble, the test was performed on the insoluble fraction of them. Metal ions removal% was calculated using the following equation 4 [24].

Metal ions removal%=[Conc. of adsorbed metal ions in polymer (ppm)/Initial conc. of metal ions (ppm)] × 100
Equation 4

Antimicrobial Test

Briefly, the test bacteria/fungi 100 µl were grown in fresh media 10 ml until they reached a count of approximately 10⁸ cells.mL⁻¹ for bacteria or 10⁵ cells.mL⁻¹ for fungi [25]. Microbial suspension 100 µL was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method [26]. Of the many available media, NCCLS recommends Mueller-Hinton agar due to its good batch-to-batch reproducibility results. Disc diffusion method for filamentous fungi was tested by using approved standard method (M38-A) developed by researchers [27] for evaluating the susceptibilities of filamentous fungi to antifungal agents. Disc diffusion method for yeasts was developed by using approved standard method (M44-P) by NCCLS [28]. Plates were inoculated with filamentous fungi as *Aspergillus flavus* at 25 °C for 48 h; Gram-positive bacteria as *Bacillus subtilis* and *Streptococcus pneumoniae*; Gram-negative bacteria as *Escherichia coli*. They were incubated at 35–37 °C for 24–48 h and fungi as *Candida albicans* was incubated at 30 °C for 240–48 h, then the diameters of the inhibition zones were measured in millimeters.

Standard discs of Ampicillin as Gram-positive antibacterial agent, Gentamycin as Gram-negative antibacterial agent and amphotericin B as antifungal agent served as positive control tests for antimicrobial activity, but filter disc impregnated with 10 µL of solvent (distilled water, chloroform, DMSO) was used as a negative control. The agar used is Mueller-Hinton agar that is rigorously tested for composition and pH value. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and the standard zones of inhibition have been determined for susceptible and resistant values. Blank paper discs (Schleicher and Schuell, Barcelona-Spain) with a diameter of 8 mm were impregnated with 10 µL of tested concentration of the stock solutions. When a filter paper disc impregnated with a tested chemical is placed on agar, the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar, it will not grow on the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a “Zone of inhibition” or “Clear zone”.

For the disc diffusion, the zone diameters were measured with slipping calipers of National Committee for Clinical Laboratory Standards [29]. Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [30].

Instrumentation

Fourier transform infrared (FTIR) spectra were recorded in the frequency range of 600–4000 cm⁻¹ using Jasco FTIR 4100 spectrophotometer (Japan). Proton nuclear magnetic resonance (¹H NMR) was recorded on a Varian Mercury VX-300 NMR Spectrometer. ¹H-NMR spectra were run at 300 MHz in dimethyl sulfoxide (DMSO) for starch and the different grafts. Chemical shifts are quoted in and were related to that of the solvent. X-ray diffraction (XRD) pattern of samples were obtained using an X-ray powder diffractometer (a Philips Xpert MPD Pro) with Ni-filtered Cu-Kα radiation (λ=0.154 nm) at an accelerating voltage/current of 50 kV/40 mA. The relative intensity was recorded in the scattering range 2θ, varying from 4° to 60° at a scan speed of 1 step s⁻¹. Scanning electron microscopy (SEM) images were obtained using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification 14x up to 1000 and resolution for Gun.1n, FEI company, Netherlands. Samples were prepared by placing a small part of film on a carbon tape on a stub, which was coated with a thin layer of gold using K550X sputter coater England. Proton Nuclear Magnetic Resonance (¹H-NMR) was recorded on a Varian Mercury (VX-300) NMR Spectrometer. ¹H-NMR spectra were run at 300 MHz in dimethyl sulfoxide (DMSO) as solvent for starch and its copolymers. Atomic absorption was done on an Analyst 100 Winlab-Perkin Elmer to determine the amount of metal ions remaining in the sample liquor.

RESULTS AND DISCUSSION

Effect of Various Reaction Parameters, Characterization, and Applications of Maize Starch-g-poly(N-vinylimidazole) Grafts

Table 1. Effect of initiator concentration [I] on the %G, %GE and %H for starch-g-PVI.

Series	Initiator Conc. $K_2S_2O_8/NaHSO_3$ (mol.L ⁻¹)	%G	%GE	%H
A	3×10^{-2}	52	44	56
B	4×10^{-2}	75.5	64.3	35.7
C	6×10^{-2}	77.7	66.16	33.8
D	8×10^{-2}	81.4	68.8	31.44
E	10×10^{-2}	53	45	55.6

The reaction temperature, reaction time and the monomer concentration were kept constant at 55 °C for 2h and [M]=0.5 mol.L⁻¹, while the liquor ratio was kept constant at 1:25.

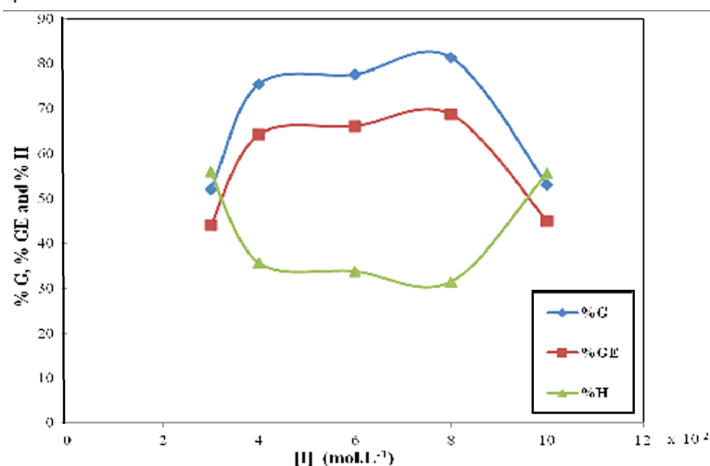


Figure 1. Effect of initiator concentration on graft of vinyl imidazole onto starch keeping the temperature, the reaction time and the monomer concentration constant at 55 °C for 2 h and [M]=0.5 mol.L⁻¹, respectively with 1:25 liquor ratio.

Effect of various reaction parameters on the grafting process:

The effect of initiator concentration on the extent of grafting is represented in **Figure 1** and tabulated in **Table 1**, where the reaction temperature, the reaction time and the monomer concentration were kept constant at 55 °C for 2 h and [M]=0.5 mol.L⁻¹, respectively with 1:25 liquor ratio. The results indicated that both percent grafting (%G) and percent grafting efficiency (%GE) values increased with increasing initiator concentration until it reached a maximum value at around 8×10^{-2} mol.L⁻¹ as seen in **Table 1**. These results seem to be reasonable as increasing initiator concentration will increase the amount of primary radicals on the polymer matrix and consequently more monomers will be consumed in the grafting process. A further increase in initiator concentration is accompanied by a considerable decrease in the grafting yields. This is probably attributed to the competition between initiation and termination reactions and due to the possible chain transfer of the growing chains to the residual initiator molecules which comes on the expense of the graft formation. On the other hand, the H% was found to decrease with the increase in initiator concentration till reached 8×10^{-2} mol.L⁻¹. These results were in good accordance with the results obtained for the %G and %GE. Increasing the initiator concentration, (10×10^{-2} mol.L⁻¹), led to an increase in the %H as the diffusion of the initiator radicals towards the starch matrix became difficult due to the increase in the medium viscosity and consequently, homopolymerization is most likely to occur. The obtained result coincides well with the decrease in both the %G and %GE with the increase of initiator concentration over the optimum concentration.

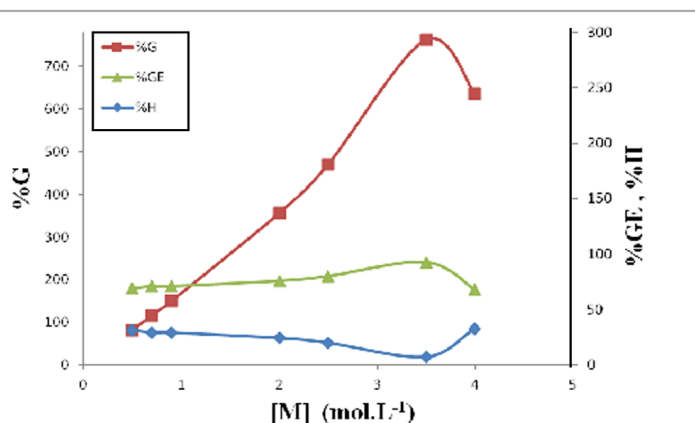


Figure 2. Effect of monomer concentration on graft of vinyl imidazole onto starch keeping the temperature, the reaction time and the initiator concentration constant at 55 °C for 2 h and [I]= 8×10^{-2} mol.L⁻¹, respectively with 1:25 liquor ratio.

Table 2. Effect of monomer concentration on the %G, %GE and %H for starch-g-PVI keeping the temperature, the reaction time and the initiator concentration constant at 55 °C for 2 h and $[I]=8 \times 10^{-2}$ mol.L⁻¹, respectively with liquor ratio kept constant at 1:25.

Series	Monomer conc. (mol.L ⁻¹)	%G	%GE	%H
F	0.5	81.4	68.8	31.44
G	0.7	116.6	70.88	29.12
H	0.9	150.1	70.97	29.03
I	2	356	75.74	24.26
J	2.5	470	80	20
K	3.5	762.5	92.7	7.3
L	4	636.3	67.7	32.31

Figure 2 illustrates the effect of VI concentration [M] on the grafting process, while the reaction temperature, the reaction time and the initiator concentration were kept constant at 55 °C for 2 h and $[I]=8 \times 10^{-2}$ mol.L⁻¹, respectively with 1:25 liquor ratio. The obtained results indicated that both %G and %GE increased, while the %H decreased as a result of increasing the monomer concentration till reached its maximum at $[H]=3.5$ mol.L⁻¹ as seen in **Table 2**. A further increase in the monomer concentration (4 mol.L⁻¹) resulted in a remarkable decrease in both the %G and %GE probably due to the limitation of the number of active sites created on the starch molecules as a result of the increase in the medium viscosity which hindered the diffusion of the monomer towards the polymeric chains, and consequently, under this condition, the %H increased. Similar observation was reported for the grafting of acrylamide onto kappa carrageenan [31], ethyl acrylate onto cellulose [32], methyl acrylate onto starch [33] and methyl methacrylate onto sodium alginate [34].

The effect of variation of the reaction temperature on %G, %GE and %H, while keeping the monomer and the initiator concentrations and the reaction time constant being $[M]=3.5$ mol.L⁻¹, $[I]=8 \times 10^{-2}$ mol.L⁻¹ for 2 h, respectively with 1:25 liquor ratio as illustrated in **Figure 3** and tabulated in **Table 3**. The obtained results indicated that both the %G and %GE increased with increasing the reaction temperature up to 55 °C then they started to decrease then level off. This is probably attributed to increasing rate of termination and chain transfer reactions and decomposition of KPS to give O₂ (a radical scavenger), which reacts with primary free radicals [35]. Also inefficient activity of Potassium persulfate/Sodium bisulfite at elevated temperatures would give rise to less efficient initiation resulting in decreased graft copolymerization reaction at higher temperatures. On the other hand, the %H had the opposite trend of the graft yields. The optimum temperature to reach the highest %G and %GE was noticed at 55 °C.

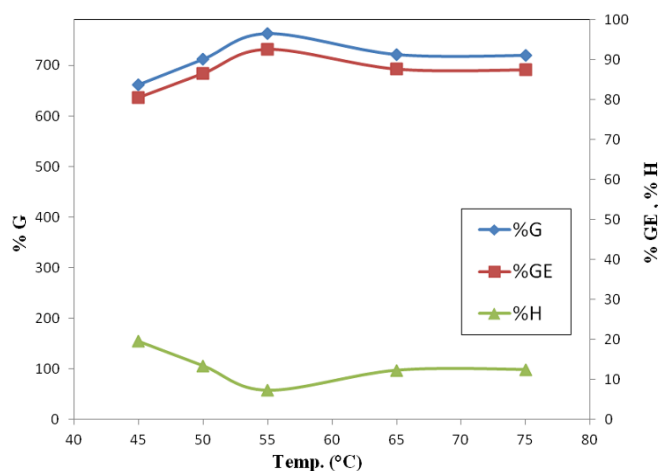


Figure 3. Effect of Temp on graft of vinyl imidazole onto starch keeping the monomer and the initiator concentrations and the reaction time constant with $[M]=3.5$ mol.L⁻¹ and $[I]=8 \times 10^{-2}$ mol.L⁻¹ for 2 h respectively with 1:25 liquor ratio.

Table 3. Effect of the reaction temperature on the %G, %GE and %H for starch-g-PVI keeping the monomer and the initiator concentrations and the reaction time constant at $[M]=3.5$ mol.L⁻¹ and $[I]=8 \times 10^{-2}$ mol.L⁻¹ for 2 h respectively with keeping the liquor ratio constant at 1:25.

Series	Temperature/ °C	%G	%GE	%H
M	45	662.3	80.52	19.5
N	50	712	86.6	13.43
O	55	762.5	92.7	7.3
P	65	721.5	87.72	12.3
Q	75	719.7	87.5	12.5

The effect of reaction time on %G, %GE and %H is illustrated in both **Figure 4** and **Table 4**. The monomer and the initiator concentrations and the reaction temperature were kept constant with $[M]=3.5$ mol.L⁻¹, $[I]=8 \times 10^{-2}$ mol.L⁻¹ at 55 °C, respectively

with 1:25 liquor ratio. The results indicated that both the %G and %GE increased with increasing time till it reached its maximum at 2 hours then slightly decreased and then leveled off.

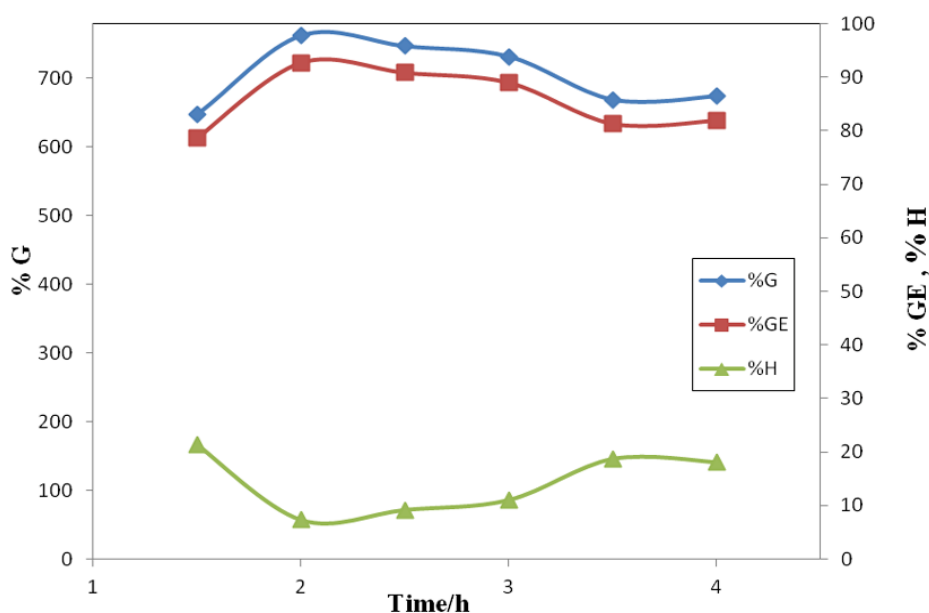


Figure 4. Effect of reaction time on graft of vinyl imidazole onto starch keeping the monomer and the initiator concentrations and the temperature constant with $[M]=3.5 \text{ mol.L}^{-1}$ and $[I]=8 \times 10^{-2} \text{ mol.L}^{-1}$ at 55°C respectively with 1:25 liquor ratio.

Table 4. Effect of reaction time on the %G, %GE and %H for starch-g-PVI keeping the monomer and the initiator concentrations and the temperature constant; $[M]=3.5 \text{ mol.L}^{-1}$ and $[I]=8 \times 10^{-2} \text{ mol.L}^{-1}$ at 55°C respectively and the liquor ratio was kept constant at 1:25.

Series	Time/h	%G	%GE	%H
R	1.5	647.3	78.7	21.3
S	2	762.5	92.7	7.3
T	2.5	747	90.82	9.18
U	3	731.8	88.97	11.03
V	3.5	668.9	81.33	18.67

The %H, on the other hand, was found to decrease with increasing the reaction time then slightly increased and then leveled off. The grafting loss may be attributed to depletion in all the consuming reactants. In addition, the decrease in number of available active sites for grafting and the retardation in diffusion of reactants, also due to the long-grafted chains at the starch surface.

All these factors may be possible reasons for the diminished grafting at longer reaction times. Other investigators reported similar time dependency of grafting parameters ^[36,37]. So, the optimum time to reach the highest %G and the highest %GE was 2 hours.

Characterization of Maize Starch-g-poly(N-vinylimidazole) Grafts

Characterization of the synthesized St-g-PVI had been performed using different analysis tools including:

Fourier Transform Infrared (FTIR) analysis:

FTIR spectra of starch, PVI and starch-g-PVI of different% graft is illustrated in **Figure 5**. The data revealed a peak at 3435 cm^{-1} corresponding to the O-H stretch related to the starch and the starch-g-PVI. Peak observed at 2928 cm^{-1} corresponded to C-H alkane stretching, while peaks at 1649 cm^{-1} corresponded to C=N stretching ^[38], at 1502 cm^{-1} corresponded to C=C aromatic stretching ^[38] and at 659 cm^{-1} corresponded to =C-H bending related to PVI and starchg-PVI. A peak observed at 1422 cm^{-1} corresponded to -C-H bending related to starch, PVI and starch-g-PVI. Also a peak observed at 1154 cm^{-1} corresponded to C-O stretching related to the starch-g-PVI itself ^[39]. The intensity of these peaks increased with increasing the graft yield.

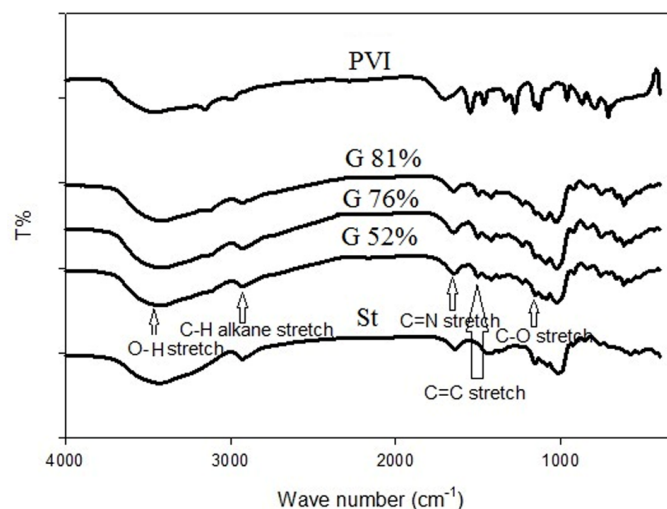
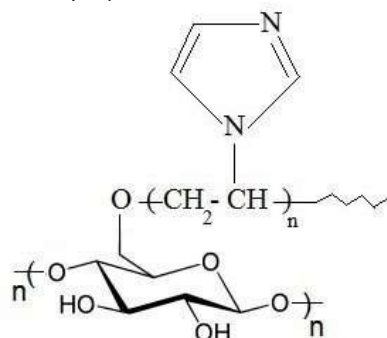


Figure 5. FTIR spectra of (St) Starch; starch-g-PVI (G 52%); starch-g-PVI (G 76%); starch-g-PVI (G 81%) and PVI.

A proposed chemical structure of the grafted copolymer is illustrated as follows:



¹H NMR

Synthesis of starch-g-PVI has been proved by comparing its ¹H-NMR spectrum with that of native starch **Figure 6**. The ¹H-NMR spectrum of St showed a singlet signal at $\delta=5.10$ ppm corresponded to the hydrogen atom bonded to the anomeric carbon 1 of glucose unit [H_1]. A multiplet signal appeared at $\delta=3.64-3.34$ ppm corresponded to the hydrogen atoms bonded to carbon atoms 3, 4, 5 and 6 of glucose unit [H_3, H_4, H_5 and H_6] and a singlet signal at $\delta=3.20$ ppm attributed to the hydrogen atom bonded to carbon atom 2 of glucose unit [H_2]. Sharp signal at $\delta=2.50$ ppm related to solvent dimethyl sulphoxide (DMSO) ^[40]. On the other hand, the ¹H NMR spectrum of starch-g-PVI showed in addition to the above mentioned signals of starch, a signal at $\delta=2$ ppm attributed to the hydrogen atoms of methylene groups in the main chain of vinyl imidazole and a multiplet signal appeared at $\delta=7.21-6.86$ ppm corresponded to the hydrogen atoms bonded to carbon atoms 2, 4 and 5 of imidazole ring [H_2, H_4 and H_5] ^[41].

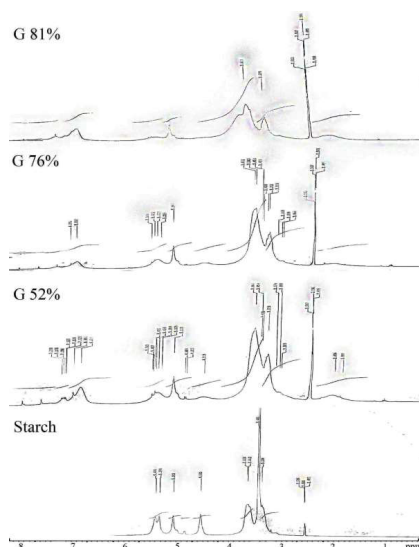


Figure 6. NMR spectra of Starch, G 52%, G 76% and G 81% Grafts.

X-ray Diffraction (XRD)

XRD analysis for starch, PVI and starch-g-PVI with different% grafts were illustrated in **Figure 7**. The results showed that starch had peaks at $2\theta=17^\circ$, 19° , 22° and 24° degree [42], while PVI had peaks at $2\theta=18^\circ$, 22° , 24° , 30° , 31° , 36° , 39° , 44° , 55° and 56° degree. The XRD analysis of St-g-PVI showed the combined signals of both starch and PVI side chains indicating the successful grafting of PVI onto starch. The intensity of the signals corresponding to PVI side chains increased with the increase in the graft yield, while those signals corresponding to the starch molecules decreased in intensity as a result of grafting. These findings gave an additional proof for the obtained results.

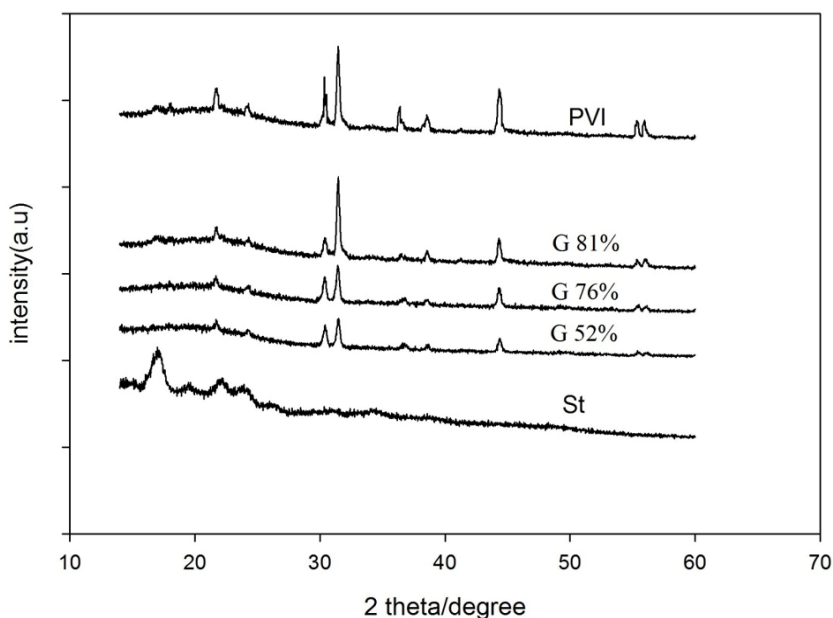


Figure 7. XRD for (St) Starch; starch-g-PVI (G 52%); starch-g-PVI (G 76%); starch g-PVI (G 81%) and PVI.

Scanning Electron Microscopy (SEM) (Magnification 1000X)

Scanning electron microscopy (SEM) images of A (St), B (G 52%), C (G 76%) and D (G 81%) grafts are illustrated in **Figure 8**. The images showed that the granular nature of starch (St) surface was completely changed into a network-like surface due to the introduction of extensive amounts of vinyl imidazole (VI) monomer and this network-like nature increased with increasing %G. Also, more% G showed more porous nature which helped in capturing more metal ions from their aqueous solutions.

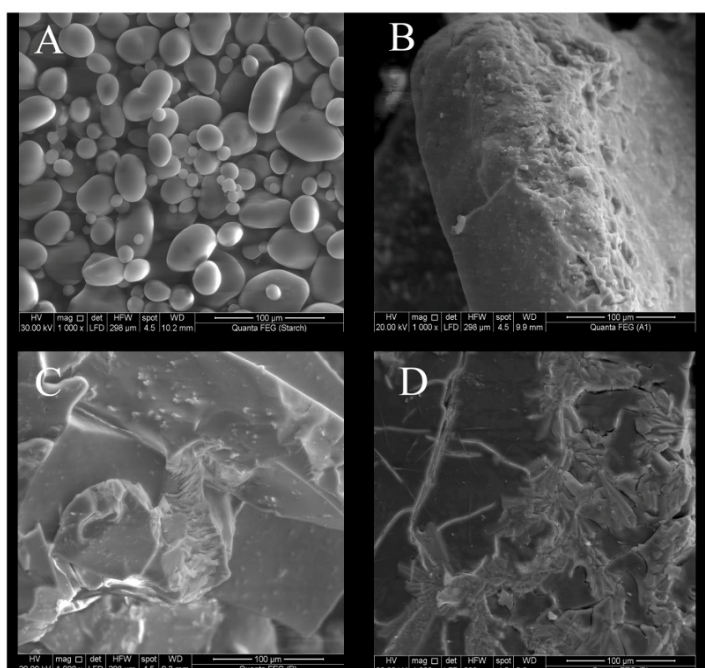


Figure 8. SEM images of Starch, G 52%, G 76% and G 81% Grafts.

Solubility Test

Solubility of different investigated St-g-PVI, as example, (G 52%, G 81% and G 347%) in different organic solvents in addition to cold and hot distilled water, and in acetic acid was tested qualitatively as showed in **Table 5**. Starch was partially soluble in dist. water and hot water and insoluble in 1% acetic acid, 1% acetic acid: ethanol, ethanol, DMF, 1,4-dioxane, THF and benzene, while PVI was soluble in dist. water, hot water, 1% acetic acid, 1% Acetic acid:Ethanol and ethanol and insoluble in DMF, 1,4-dioxane, THF and benzene. The starch-g-PVI was found to be partially soluble in dist. water and hot water and insoluble in 1% acetic acid, 1% acetic acid: ethanol, ethanol, DMF, THF, benzene and 1,4-dioxane, while the solubility of higher %G increased in case of 1% acetic acid and 1% acetic acid: ethanol. The solubility was tested quantitatively as shown in **Table 6**, as the G% increased, the solubility% increased due to the hydrophilic nature of PVI. The test was done by adding 0.02 g of starch or the grafts to 10 ml dist. water and left for 24 h. The results indicated that the graft with G 52% had solubility 50%, G 81% had solubility 73% and G 763% had solubility 100% in dist. water, as an example, while starch had solubility 17.5%. Another test was done by adding 0.02 g of starch or the grafts to 10 ml dist. water and heated with stirring for a definite time to dissolve. The results indicated that the graft with G 52% had solubility 55%, G 81.4% had solubility 76% and G 763% had solubility 100% in hot dist. water, while starch had solubility 70% as shown in **Table 6**.

Table 5. Solubility results of the grafts (G 52%, G 81% and G 347%), Starch and PVI.

S Solvent	Starch	PVI	G 52%	G 81%	G 347%
Dist. Water	PS	S	PS	PS	PS
Hot Water	PS	S	PS	PS	PS
1% Acetic acid	IS	S	PS	PS	S
1% Acetic acid: Ethanol	IS	S	IS	IS	PS
Ethanol	IS	S	IS	IS	IS
DMF	IS	IS	IS	IS	IS
1,4-dioxane	IS	IS	IS	IS	IS
THF	IS	IS	IS	IS	IS
Benzene	IS	IS	IS	IS	IS

Where S: soluble, PS: partially soluble and IS: insoluble

Table 6. Solubility% of starch and the grafts (G 52%, G 81% and G 763%) in both distilled and hot water at 60 °C for 60 min.

Sample	% Solubility	
	Dist. water	Hot water
Starch	17.50%	70%
G (52%)	50%	55%
G (81%)	73%	76%
G (763%)	100%	100%

Applications of Maize Starch-g-poly(N-vinylimidazole) Grafts

Metal ions uptake:

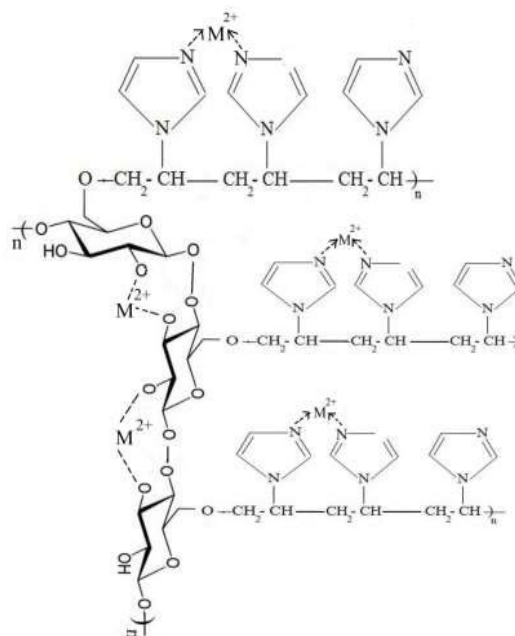
As shown in **Table 7**, starch, St-g-PVI (G 53%) and (G 76%) adsorbed Co^{2+} ions the most, it reached 40.7%, 42.7% and 68.8% respectively, followed by Cu^{2+} ions, its adsorption reached 34.1%, 37.5% and 41.2%, respectively, while the least adsorbed ions were Cd^{2+} ions, their adsorption values were 29.3%, 31.6% and 32.3%, respectively at pH 7. The metal ion uptake of starch is due to -OH groups donating protons to aqueous solutions as seen in **Scheme 1**. As the graft increases, more vinyl imidazole units were added with lone pair of electrons on nitrogen atoms are coordinative bounded to metal ions increasing metal ion uptake. Therefore, the chelation of metal ions can occur mainly on the surface of the polymer and in the pores that the hydrated ions can diffuse^[43]. Thus, starch had the lowest metal ion uptake ability then St-g-PVI (G 53%) then St-g-PVI (G 76%). Using cations with small ionic radii can be bound tightly by specific adsorption, and as the graft percent increased, the metal ions uptake percent increased.

The highest metal adsorption was observed with Co^{2+} ions followed by Cu^{2+} ions then Cd^{2+} ions. There were factors affecting the metal ions adsorption such as ionic sizes, electro positivity and reactivity of metal ions^[44]. Ionic radii of Co^{2+} ions are 70 pm, Cu^{2+} ions are 73 pm and Cd^{2+} ions are 95 pm for six-coordination^[45] which confirmed the results obtained, where electropositivity and reactivity of the metals themselves to lose electrons easily had the order $\text{Cd} > \text{Co} > \text{Cu}$ ^[46]. Thus, as expected the reactivity of their metal ions to react with ligands would have the opposite order $\text{Cu}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$.

Table 7. Metal ions uptake of different starch-g-PVI grafts at pH 7.

Metal ions	Starch/ads.%	(G 53%)/ads.%	(G 76%)/ads.%
Co ²⁺	40.7	42.7	68.8
Cu ²⁺	34.1	37.5	41.2
Cd ²⁺	29.3	31.6	32.3

Thus, as the graft percent increased, the metal ions uptake percent increased and the highest metal adsorption was observed with Co²⁺ ions followed by Cu²⁺ ions then Cd²⁺ ions.



Scheme 1. Proposed Structure for the heavy metal ions chelation by starch-g-PVI.

Antimicrobial activity:

Starch had no antimicrobial activity [47], starch-g-PVI had good antimicrobial activity as shown in Table 8. Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (5 mg.ml⁻¹) concentration of tested samples. Where for *Aspergillus flavus*, there was no activity for starch, (G 52%) had inhibition activity 65.82% and (G 81.4%) has inhibition activity 78.5% compared to Amphotericin B. As for *Candida albicans*, (G 52%) had inhibition activity 67.7% and (G 81%) had inhibition activity 76% compared to Amphotericin B. As for *Streptococcus pneumonia*, (G 52%) had inhibition activity 77.3% and (G 81%) had inhibition activity 83.6% compared to Ampicillin. As for *Bacillus subtilis*, (G 52%) had inhibition activity 62.3% and (G 81%) had inhibition activity 64.5% compared to Ampicillin. As for *Escherichia coli*, (G 52%) had inhibition activity 81% and (G 81%) had inhibition activity 92% compared to Getamycin. Where (G 81%) had the best antibacterial and antifungal activity as the %graft increased, antimicrobial activity increased.

Table 8. Antimicrobial activity of Starch and starch-g-PVI with different G%.

Sample	Bacillus subtilis (G+)	Streptococcus pneumonia (G+)	Escherichia coli (G)	Aspergillus flavus (fungus)	Candida albicans (fungus)
Amphotericin B (antifungal agent)	-	-	-	23.7 ± 0.1	25.4 ± 0.1
Ampicillin (antibacterial agent)	32.4 ± 0.3	23.8 ± 0.2	-	-	-
Gentamycin (antibacterial agent)	-	-	19.9 ± 0.3	-	-
Starch	NA	NA	NA	NA	NA
(G 52%)	20.2 ± 0.67	18.4 ± 0.44	16.1 ± 0.46	15.6 ± 0.25	17.2 ± 0.34
(G 81%)	20.9 ± 0.32	19.9 ± 0.63	18.3 ± 0.46	18.6 ± 0.36	19.3 ± 0.44

- The test was done using the diffusion agar technique, well diameter: 6.0 mm (100 µl was tested),

RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms

- *NA: No activity, data are expressed in the form of mean ± SD.

Mechanically, the antibacterial activity of starch-g-PVI (in aqueous media) can be possibly illustrated with [48]:

- The electrostatic interactions between positive charge of VI and negative charge of Gram-positive and Gram negative bacterial membranes.

- Hydrophobic interactions between the alkyl chain of vinylimidazole and the cytoplasmic membrane of bacteria responsible for the conglomeration of starch-g-PVI on bacterial membranes resulting in the disruption of the membranes and cell death.
- The imidazole groups penetrate the cell membrane and bind to DNA leading to destabilization due to mutation altering the protein sequences resulting in the damage of DNA.

Also, the antifungal activity mechanism of starch-g-PVI can be explained similar to antibacterial activity, resulting in the disruption of the plasma membrane to alter its structure and function [49].

CONCLUSION

The effect of various reaction parameters on the graft yield, graft efficiency and amount of homopolymer of St-g-PVI system has been determined and the obtained results indicated that the optimum grafting conditions were as follows:

$[(KPS)/(NaBS)]=8 \times 10^{-2} \text{ mol.L}^{-1}$, $[M]=3.5 \text{ mol.L}^{-1}$, reaction temperature= 55°C and reaction time=2 h.

Synthesis of Starch-g-poly(N-vinylimidazole) (St-g-PVI) had been done and its structure was proven by FTIR, NMR, XRD and SEM analyses. FTIR spectra of starch, PVI and starch-g-PVI of different Graft% were illustrated revealing different peaks corresponding to starch, PVI and starch-g-PVI. The intensity of all of these peaks increased with increasing the graft yield. Synthesis of starch-g-PVI has been proved by comparing its $^1\text{H-NMR}$ spectrum with that of native starch. The St-g-PVI had sharp X-ray peaks at $2\theta=22^{\circ}$, 24° , 30° , 31° , 36° , 39° , 44° , 55° and 56° , degree indicating their starch and PVI content. Scanning electron microscopy (SEM) images showed that as G% increased, the network-like structure of the grafts increased with increasing VI content. Also, more G% showed more porous nature helping in capturing metal ions for water treatment. Results for the solubility test showed that as the G% increased, the solubility% increased due to the hydrophilic nature of PVI. Results of the adsorption of heavy metal ions (Co^{2+} , Cu^{2+} and Cd^{2+}) from their chloride salt solutions compared to the parent starch, showed that the metal ions uptake followed the following order $\text{Co(II)}>\text{Cu(II)}>\text{Cd(II)}$ at pH 7. Chemical modification of starch by grafting with PVI has special use in antimicrobial activity against *Aspergillus flavus*, *Candida albicans*, *Streptococcus pneumonia*, *Bacillus subtilis* and *Escherichia coli* as fungi, Gram-positive and Gram-negative bacteria, respectively. 81% G had the best antimicrobial activity due to the high content of PVI.

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