

Maize Starch-g-Poly (Itaconic Acid) and its Application in Sewage Water Treatment and as Antimicrobial Agent

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Abstract

Maize starch-g-poly(itaconic acid) was prepared and characterized by determining its degree of substitution and elucidating its structure by FTIR. Graft copolymerization of itaconic acid 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)]= 6×10^{-2} (mol.L⁻¹), [M]=1.1 (mol.L⁻¹), reaction temperature=55°C and reaction time=3.75h. The efficacy of the graft copolymers to uptake metal ions from their aqueous solutions was also studied. Results showed that the graft copolymers adsorb 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 *Candida albican* as fungi.

Keywords: Starch; Itaconic acid; Grafting; Metal uptake; Antimicrobial

Introduction

Polymers are natural components found in the human body, such as proteins and nucleic acids. Cellulose is the main structural component of plants. Condensation polymers are the biggest part of the natural polymers, monomers bind with each other forming water as a by-product. Starch is also produced from hundreds of glucose units through condensation polymerization splitting out water molecules as they chemically combine. Starch is one of the basic food group carbohydrates and is found in cereal grains and potatoes. It is also referred to as a polysaccharide [1]. Glucose as the monosaccharide unit is the main constituent of the common polysaccharides. Polysaccharides are carbohydrate polymers consisting of tens to hundreds to several thousand monosaccharide units. Plants, animals and humans synthesize and metabolize polysaccharides to be stored for food, structural support or energy [2]. Starch is a very important and abundant natural product, occurring in every part of green plants. It is the chemical storage form of the sun energy and it is the major source of energy for the

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organisms on Earth [3]. Up to now, corn (maize) is the largest source of starch with other known sources as rice, potato, tapioca and wheat. Amylopectin (amylase free) can be isolated from 'waxy' maize starch, whereas amylose (amylopectin free) is best isolated after specifically hydrolyzing the amylopectin with pullulanase [4]. Starch was isolated from corn grains following the method of Sandhu et al [5]. Starch is a known biodegradable polymer increasingly used in many sections of industry because of its respective physicochemical properties [6]. As a consequence of unfavorable properties of the native starch, like poor solubility and high hydrophilicity, it is being subjected to various modifications. Chemical grafting is one of the most popular methods for modifying structure and properties of biopolymers [7]. It's well known that heavy metals ions such as Pb (II) may cause severe health problems in animals and human because it can specifically bind to proteins, nucleic acid and small metabolites in living organisms inhibiting their functions. Heavy metals are detected in industrial waste water originating from metal plating, mining activities, smelting, battery manufacture [8], petroleum refining [9], printing and photographic industries. Different types of methods have been proposed for the treatment of water and industrial waste water containing heavy metals [10] such as biological treatments, procedures using a filtration membrane, advanced oxidation processes, electrochemical methods and adsorption processes have proven to be more viable alternative due to low cost of processing and instrumentation, ease of operation and no need for large facilities [11]. Starch has been modified by grafting to be used as metal adsorbant. An antimicrobial is an agent that kills microorganisms or inhibits their growth [12]. Due to available antimicrobials disability to treat infectious diseases, many researchers have focused on the investigation of natural products as source of new bioactive molecules [13,14]. For this purpose, a variety of methods are found and since not all of them are based on same standards, results obtained will also be deeply influenced not only by the method selected, but also by the microorganisms used to proceed the test, and by the degree of solubility of each test-compound [15,16]. The test systems should ideally be simple, inexpensive, reproducible, and rapid and maximize high sample throughput in order to cope with a varied number of extracts and fractions. The complexity of the bioassay must be determined by laboratory facilities and staff quality availability [14,17]. Itaconic acid (IA) is a gifted organic acid. It is used globally in the preparation of bioactive compounds in the agricultural, pharmaceutical and medicinal components and industrial synthesis of resins such as polyesters, plastics and artificial glass. The applications of IA have been extended to bio medical fields, such as dental, ophthalmic, wound dressing, contact lens, drug delivery fields. It can easily co-polymerize and provide polymer chains with carboxylic acid groups, which are highly hydrophilic and capable to form hydrogen bonds with corresponding groups [18]. It is an unsaturated dicarboxylic acid, structurally similar to petrochemical-derived acrylic and methacrylic acids. It ranks among the 12 most important building block chemicals from sugar according to the US-Department of Energy (DOE). The acid can be an interesting starting material for synthesis of polymers due to its dual polymerization mechanism i.e. by free-radical mechanism or formation of ester bonds [19]. It is one of the low cost monomers obtained from renewable resources by fermentation with *Aspergillus terreus* using carbohydrate materials as molasses and hydrolyzed starch [20]. A novel adsorbent, 2-mercaptobenzamide modified itaconic acid-grafted-magnetite nanocellulose composite [P(MB-IA)-g-MNCC] was synthesized for adsorbing mercury(II) [Hg(II)] ions selectively from aqueous solutions. The optimum pH for Hg(II) adsorption was found to be 8.0, and the adsorption attained equilibrium within 60 min. The maximum adsorption capacity was found to be 240.0 mg/g [21]. Itaconic acid produced by *Escherichia coli* expressing recombinant α -amylase, using soluble starch as its sole carbon source. The recombinant α -amylase from *S. bovis* (SBA) showed activity at 28° C, which is the optimal temperature for production of itaconic acid, while α -amylase from *B. amyloliquefaciens* displayed no noticeable activity. *E. coli* cells expressing SBA produced 0.15 g/L itaconic acid after 69 h cultivation under pH-stat conditions, using 1% starch as the sole carbon source [22]. A cellulose based adsorbent, poly (2-mercaptobenzamide modified itaconic acid)-

grafted-magnetite nanocellulose (P(MB-IA)-g-MNCC) was synthesized by graft co-polymerization of itaconic acid onto magnetite nanocellulose (MNCC) using Ethylene glycol dimethacrylate (EGDMA) as crosslinking agent and $K_2S_2O_8$ as free radical initiator. Adsorbent dosage of 2.0 g/L was sufficient for the complete removal of Cd(II) ions from aqueous solutions [23]. In the present study, we prepared different poly (itaconic acid)-grafted-starch copolymers with different graft percentages (%G) having metal uptake properties and antimicrobial activity.

Experimental

Materials

Starch was purchased from Nasr Chemical Co.-Egypt. Itaconic acid, Potassium persulfate, Sodium bisulfite, Acetic acid, DMF, 1,4-dioxane, THF, Benzene and Acetone 99% were purchased from Sigma-Aldrich Co.-Germany. Copper chloride ($CuCl_2 \cdot 2H_2O$) was purchased from Merck Co.-Germany. Cadmium chloride ($CdCl_2 \cdot 2H_2O$) was purchased from Koch-light lab. Ltd. England. Cobalt chloride ($CoCl_2 \cdot 6H_2O$) was purchased from May & Baker Ltd Dagenham-England and *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-Egypt.

Synthesis of maize starch-g-poly(itaconic acid)

Pre-determined amount of starch was dissolved in 20 ml dist. water, then a specified amount of itaconic acid (IA) 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 dropwisely to the mixture. The mixture was left for 2 h at 60-70 °C then the graft was precipitated into cold acetone. The precipitate was filtered and left to dry in an air oven for 48 h, and its weight was determined (W_2). Acetone was used for soxhlet extraction for 8h to get rid of any formed homopolymer [24]. 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 mentioned equations.

The grafting parameters were calculated according to the following equations (1, 2 and 3) [25]:

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

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

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

Where W_0 is the weight of the initial matrix and W_1 is the weight of 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-PIA) in different organic solvents (1 % acetic acid, 1 % acetic acid: ethanol, ethanol, DMF, THF, benzene and 1,4-dioxane). The test was done by putting 0.02 g of starch and each graft in 10 ml of each solvent for 24 h.

Metal ions uptake

A definite weight (0.02 g) of tested samples were immersed in 10 ml of known concentration ($2 \times 10^{-4} \text{ mol.L}^{-1}$) of the chloride salt solutions of the investigated heavy metal ions (Co^{2+} , Cu^{2+} and Cd^{2+}) at room temperature ($\sim 25^\circ\text{C}$) till equilibrium (for 24h). 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 [26,27]. Metal ions uptake was not tested on poly (itaconic acid) (PIA) due to its water solubility. Starch and Starch-g-poly (itaconic acid) being partially soluble, the test was performed on the insoluble fraction of them. Metal ions removal % was calculated using the following equation (4) [28].

Metal ions removal % = $[\text{Conc. of adsorbed metal ions in polymer (ppm)} / \text{Initial conc. of metal ions (ppm)}] \times 100$ 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 $10^8 \text{ cells.mL}^{-1}$ for bacteria or $10^5 \text{ cells.mL}^{-1}$ for fungi [29]. 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 [30]. 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 [31] 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 [32]. 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 $35\text{--}37^\circ\text{C}$ for 24–48 h and fungi as *Candida albicans* was incubated at 30°C for 24–48 h and then the diameters of the inhibition zones were measured in millimeters.

Standard discs of Ampicillin as *Gram-positive* antibacterial agent, Getamycin 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 & 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 [33]. Agar-based methods such as Etest and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods [34].

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 ($^1\text{H-NMR}$) was recorded on a Varian Mercury VX-300 NMR Spectrometer. $^1\text{H-NMR}$ spectra was run at 300 MHz in dimethyl sulfoxide (DMSO) for starch and the different grafts. Chemical shifts are quoted in δ 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 $\text{Cu-K}\alpha$ radiation ($\lambda=0.154$ nm) at an accelerating voltage/current of 50 kV/40mA. 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 1000000 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. 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(itaconic acid) grafts

Effect of various reaction parameters on the grafting process

The effect of the initiator concentration on the extent of grafting is represented in FIG. 1, where the reaction temperature, the reaction time and the monomer concentration were kept constant at 60°C for 2h and $[\text{M}]=0.7$ mol.L^{-1} , respectively with 1:25 liquor ratio. The results indicated that both percent grafting (%G) and percent efficiency (%GE) values increased with increasing initiator concentration until it reached a maximum value at around 6×10^{-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, the possible recombination of the initiator radicals (cage effect) and due to the possible chain transfer of the growing chains to the initiator molecules which come 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 6×10^{-2} mol.L^{-1} . These results were in good accordance with the results obtained for the %G and %GE. Increasing the initiator concentration, (12×10^{-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 was 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.

Series	Initiator Conc. $K_2S_2O_8/NaHSO_3$ (mol.L ⁻¹)	%G	%GE	%H
I	3×10^{-2}	88.7	37.74	64.25
II	4×10^{-2}	128.63	56.16	44.09
III	6×10^{-2}	203.23	86.8	13.6
IV	12×10^{-2}	141.1	60.3	40.8
V	14×10^{-2}	128.5	54.91	46.33

TABLE. 1. Effect of initiator concentration on the %G, %GE and %H for starch-g-PIA, where the reaction temperature, reaction time and the monomer concentration were kept constant at 60 °C for 2h and [M]=0.7 mol.L⁻¹, respectively with 1:25 liquor ratio.

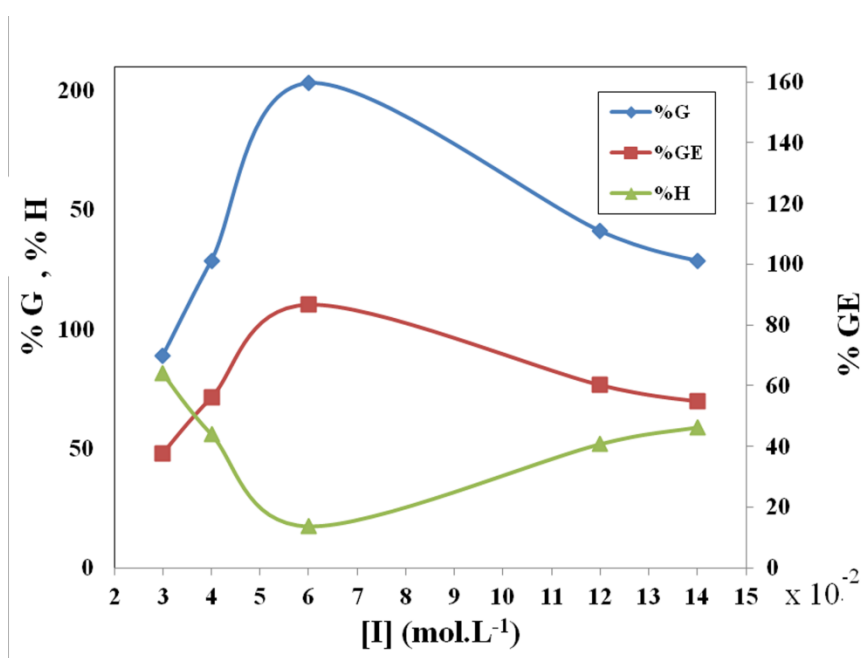


FIG. 1. Effect of initiator concentration on graft of itaconic acid onto starch keeping the temperature, the reaction time and the monomer concentration constant at 60 °C for 2 h and [M]=0.7 mol.L⁻¹ respectively with 1:25 liquor ratio.

FIG. 2 illustrates the effect of IA concentration [M] on the grafting process, while the reaction temperature, the reaction time and the initiator concentration were kept constant at 60 °C for 2 h and [I]=6X10⁻² 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]=1.1 mol.L⁻¹ as seen in TABLE 2. A further increase in the monomer concentration (1.2 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 observations was reported for the grafting of acrylamide onto kappa-carrageenan [35], ethyl acrylate onto cellulose [36], methyl acrylate onto starch [37] and methyl methacrylate onto sodium alginate [38].

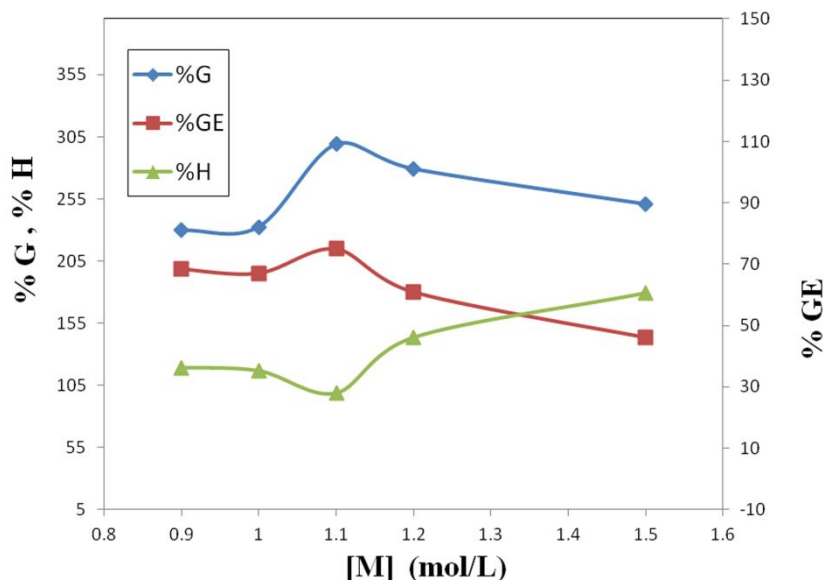


FIG. 2. Effect of monomer concentration on graft of itaconic acid onto starch keeping the temperature, the reaction time and the initiator concentration constant at 60°C for 2 h and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ respectively with 1:25 liquor ratio

Series	Monomer conc. Itaconic acid (mol.L^{-1})	%G	%GE	%H
VI	0.9	229.74	68.42	36.19
VII	1	232.52	67.01	35.19
VIII	1.1	299.5	74.97	27.93
IX	1.2	279.5	60.87	46.03
X	1.5	251	45.97	60.49

TABLE. 2. Effect of monomer concentration on the %G, %GE and %H for starch-g-PIA keeping the temperature, the reaction time and the initiator concentration constant at 60°C for 2 h and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ respectively with 1:25 liquor ratio

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 $[M]=1.1 \text{ mol.L}^{-1}$, $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ for 2h, respectively with 1:25 liquor ratio as illustrated in FIG. 3 and tabulated in TABLE 3. The obtained results indicated that the %G and %GE increased with increasing temperature up to 55°C then they started to decrease. This is probably attributed to increasing rate of termination and chain transfer reactions and decomposition of KPS to give O_2 (a radical scavenger), which reacts with primary free radicals resulting in decreased graft copolymerization reaction at higher temperatures [39]. 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.

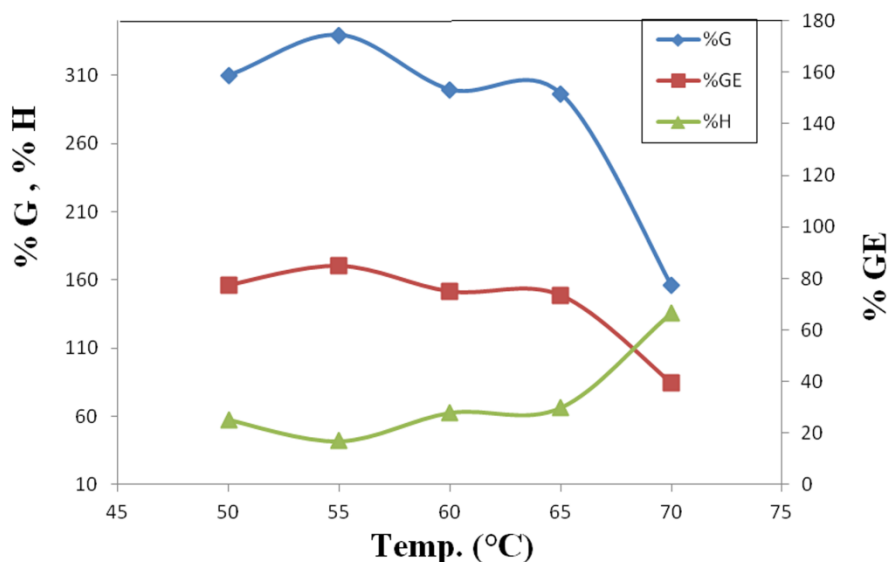


FIG. 3. Effect of Temp on graft of itaconic acid onto starch keeping the monomer and the initiator concentrations and the reaction time constant with $[M]=1.1 \text{ mol.L}^{-1}$ and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ for 2h respectively with 1:25 liquor ratio.

Series	Temperature/ °C	%G	%GE	%H
XI	50	309.85	77.44	25.21
XII	55	339.45	84.82	16.97
XIII	60	299.5	74.97	27.93
XIV	65	296.57	73.48	29.9
XV	70	156.19	39.65	66.4

TABLE. 3. The effect of the reaction temperature on the %G, %GE and %H for starch-g-PIA keeping the monomer and the initiator concentrations and the reaction time constant being $[M]=1.1 \text{ mol.L}^{-1}$ and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ for 2h respectively with 1:25 liquor ratio

The effect of reaction time on %G, %GE and %H is illustrated in FIG. 4 and TABLE 4. The monomer and the initiator concentrations and the reaction temperature were kept constant being $[M]=1.1 \text{ mol.L}^{-1}$, $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ at 55 °C, respectively with 1:25 liquor ratio. The results indicated that both the %G and %GE increased with increasing time till reached its maximum at 3.75 hours then decreased. The %H, on the other hand, was found to decrease with increasing time then increased. The grafting loss may be attributed to decrease 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. So the optimum time to reach the highest G% and the highest GE% was 3.75 hours.

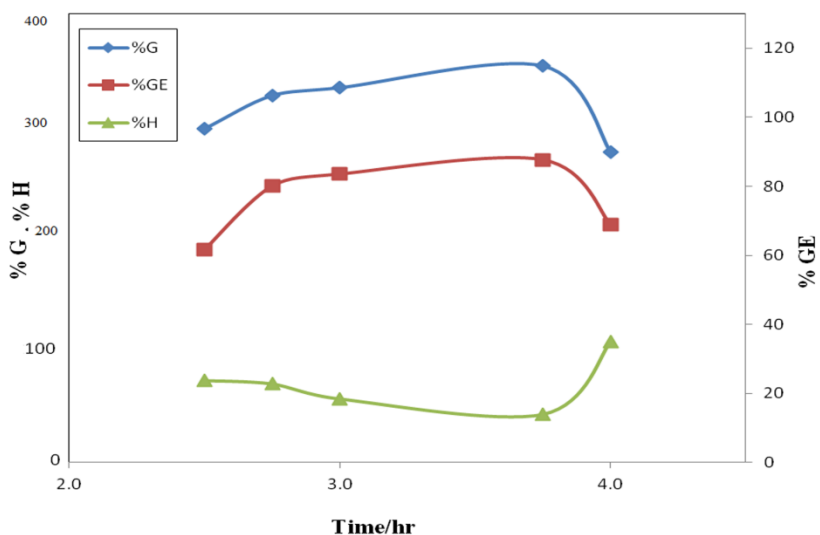


FIG. 4. Effect of reaction time on graft of itaconic acid onto starch keeping the monomer and the initiator concentrations and the temperature constant with $[M]=1.1 \text{ mol.L}^{-1}$ and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ at 55°C respectively with 1:25 liquor ratio.

Series	Time/h	G%	GE%	H%
XVI	2.5	297.5	61.66	23.74
XVII	2.75	327.04	80.05	22.77
XVIII	3	334	83.5	18.43

TABLE. 4. The effect of reaction time on the %G, %GE and %H for st-g-PIA. The monomer and the initiator concentrations and the reaction temperature were kept constant being $[M]=1.1 \text{ mol.L}^{-1}$ and $[I]=6 \times 10^{-2} \text{ mol.L}^{-1}$ at 55°C , respectively with 1:25 liquor ratio.

Characterization of maize starch-g-poly(itaconic acid) grafts

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

Fourier transform infrared (FTIR) analysis

FTIR spectra of starch, PIA and starch-g-PIA of different %graft are illustrated in FIG. 5. The data revealed a peak at 3434 cm^{-1} corresponding to the O-H stretch related to the starch and the starch-g-PIA. Peak observed at 2934 cm^{-1} corresponded to C-H alkane stretching, while peaks at 1422 cm^{-1} corresponded to -C-H bending related to starch, PIA and starch-g-PIA. Also a peak observed at 1154 cm^{-1} corresponded to C-O stretching and at 1725 cm^{-1} corresponding to Carboxylic acid C=O stretch related to the starch-g-PIA itself [40]. The intensity of all of these peaks increased with increasing the graft yield.

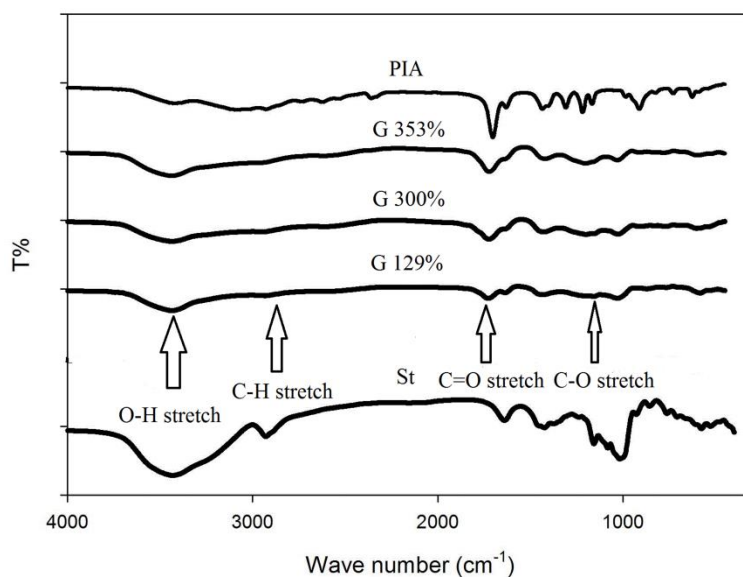


FIG. 5. FTIR spectra of (St) Starch; st-g-PIA (G 129%); st-g-PIA (G 300%); st-g-PIA (G 353%) and PIA

A proposed chemical structure of the grafted copolymer is illustrated as follows (FIG 6):

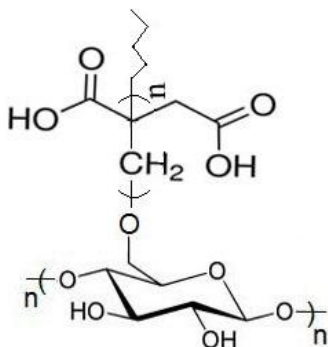


FIG. 6. Proposed chemical structure of Starch-g-PIA

NMR

Synthesis of starch-g-PIA has been proved by comparing its ¹H-NMR spectrum with that of native starch FIG. 7. 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) [41]. On the other hand, the ¹H-NMR spectrum of starch-g-PIA showed in addition to the above mentioned signals of starch, a quartet signal at $\delta=2.4$ ppm attributed to the hydrogen atoms of methylene groups in the main chain of itaconic acid and a broad signal at $\delta=13.00$ – 11.00 ppm attributed to the hydrogen atom bonded to oxygen atom of the carboxylic group of itaconic acid [42].

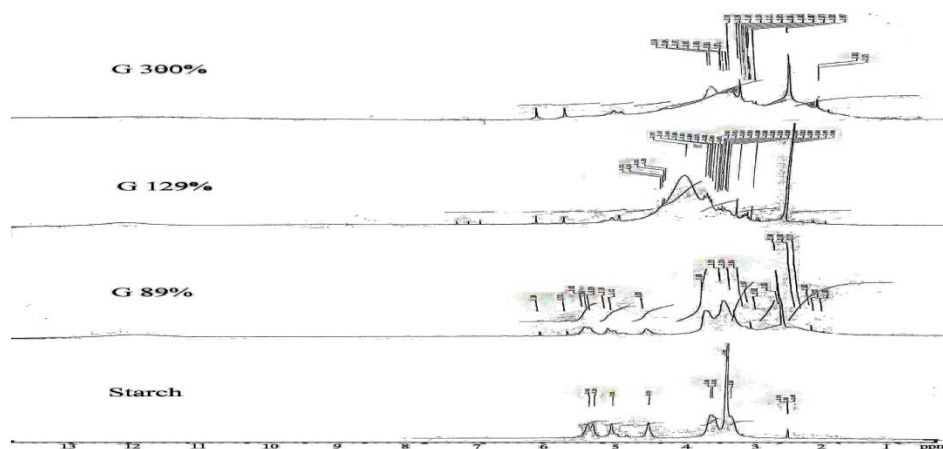


FIG. 7. NMR spectra of Starch, (G 89%), (G129%) and (G 300%).

X-ray diffraction (XRD)

XRD analysis for starch, PIA and starch-g-PIA with different % grafts were illustrated in FIG. 8. The results showed that starch had peaks at $2\theta=17^\circ$, 19° , 22° and 24° degree [43] while PIA had peaks at $2\theta=18^\circ$, 22° , 24° , 30° , 31° , 36° , 39° , 44° , 55° and 56° degree. The St-g-PIA was amorphous thus X-rays would be scattered in many directions leading to a large bump distributed in a wide range (2 Theta) instead of high intensity narrower peaks.

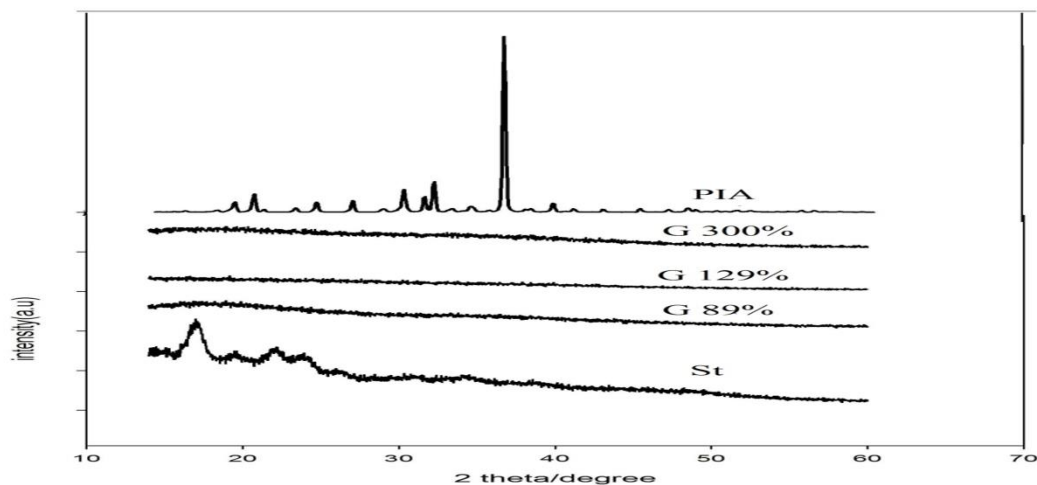


FIG. 8. XRD for (St) Starch; st-g-PIA (G 89%); st-g-PIA (G 129%); st-g-PIA (G 300%) and PIA.

Scanning electron microscopy (SEM) (Magnification x 1000)

Scanning electron microscopy (SEM) images of A (St), B (G 89%), C (G 129%) and D (G 300%) grafts were illustrated in FIG. 8. The images showed that the granular nature of starch (St) surface was completely changed into a rock-like nature of grafts surfaces due to the extensive itaconic acid (IA) monomers introduced and this rock-like nature increased with increasing G%. Also, more G% showed more pores (hollow zones) helping in capturing metal ions in water treatment.

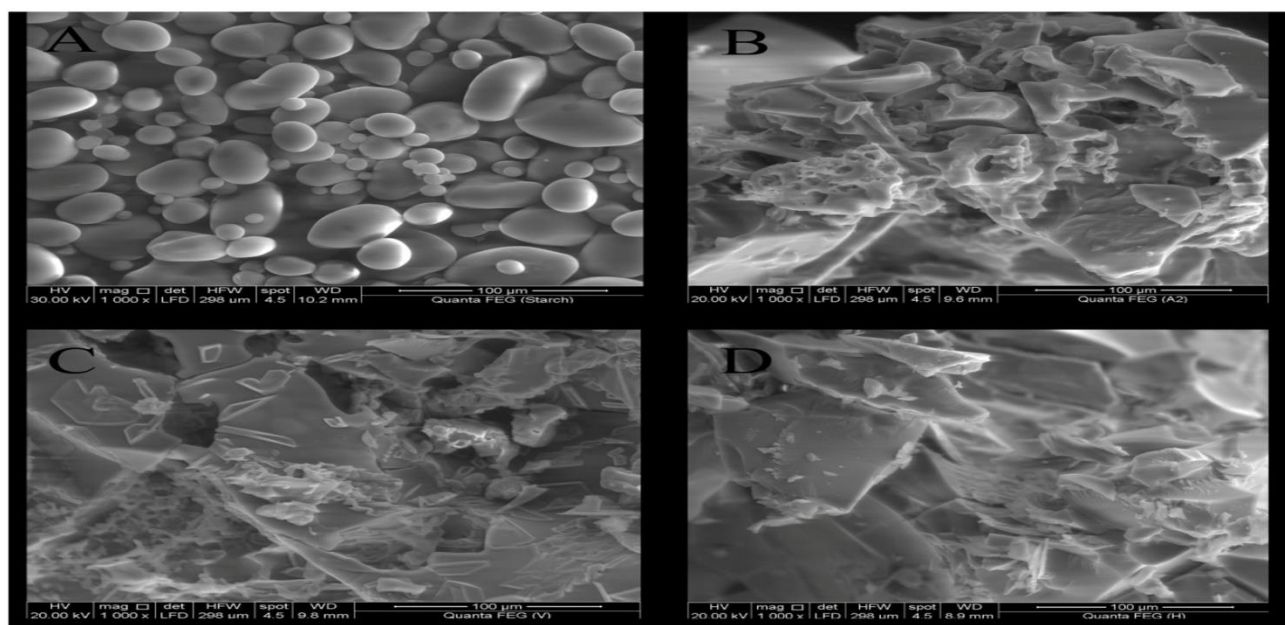


FIG. 8. SEM images of Starch, (G 89%), (G 129%) and (G 300%).

Solubility test

Solubility of different grafts, as example, (G 89%, G 129% and G 300%) in different organic solvents in addition to cold and hot distilled water, and in acetic acid is summarized in TABLE 5. Starch was partially soluble in dist.water and hot water and insoluble in 1% acetic acid, 1% acetic acid: ethanol, ethanol, hot ethanol, 1,4-dioxane, THF and benzene, while PIA was soluble in distilled water, hot water, 1% acetic acid, 1% Acetic acid:Ethanol, ethanol, hot ethanol, 1,4-dioxane and THF and insoluble in benzene. The starch-g-PIA was found to be partially soluble in dist. water and hot water, 1% acetic acid, 1% acetic acid: ethanol, THF and 1,4-dioxane, soluble in hot ethanol and insoluble in ethanol and benzene. As the G% increased, the solubility% increased due to the hydrophilic nature of PIA.

Solvent	Starch	PIA	(G 89 %)	(G 129%)	(G 300 %)
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	PS	PS	S
Ethanol	IS	S	IS	IS	PS
Hot ethanol	IS	S	S	S	S
1,4-dioxane	IS	S	PS	PS	S
THF	IS	S	PS	PS	S
Benzene	IS	IS	IS	IS	IS

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

TABLE. 5. Solubility results of the grafts (G 89%, G 129% and G 300%), Starch and PIA

Applications of maize starch-g-poly(itaconic acid) grafts

Metal ions uptake

As shown in TABLE 6, St-g-PIA (89%) and (353%) adsorbed Cu^{2+} ions the most, it reached 54% and 72.3% respectively, followed by Co^{2+} ions, its adsorption reached 46.1% and 71.8 %, respectively, while the least adsorbed ions were Cd^{2+} ions, their adsorption values were 29.6% and 70.4 %, respectively at pH 7. The metal ion uptake of starch is due to OH groups donating protons to aqueous solutions. As itaconic acid is being grafted onto starch, more COOH groups increasing metal ion uptake by donating more protons to the metal ions as seen in FIG. 9. Thus starch had the lowest metal ion uptake ability then St-g-PIA (89%) then St-g-PIA (353%). The highest metal adsorption was observed with Cu^{2+} ions followed by Co^{2+} ions then Cd^{2+} ions. There were factors affecting the metal ions adsorption such as ionic sizes, electropositivity 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] 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 $\text{Cu}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+}$ which confirmed the results obtained. Thus as the graft percent increased, the metal ions uptake percent increased and the highest metal adsorption was observed with Cu^{2+} ions followed by Co^{2+} ions then Cd^{2+} ions.

Metal ions	Starch/ads.%	(G 89%)/ads.%	(G 353%)/ads.%
Cu^{2+}	34.1	54	72.3
Co^{2+}	40.7	46.1	71.8
Cd^{2+}	29.3	29.6	70.4

TABLE. 6. Metal ions uptake of different starch-g-PIA grafts at pH 7

Antimicrobial activity

Starch had no antimicrobial activity [47], starch-g-PIA had good antimicrobial activity as shown in TABLE 7. Mean zone of inhibition in mm \pm 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 and the grafts compared to Amphotericin B. As for *Candida albicans*, (G 89%) had inhibition activity 63.8%, (G 129%) had inhibition activity 76% and (G 353%) had inhibition activity 76% compared to Amphotericin B. As for *Streptococcus pneumonia*, (G 89%) had inhibition activity 60.1%, (G 129%) had inhibition activity 68.1% and (G 353%) had inhibition activity 89.5% compared to Ampicillin. As for *Bacillus subtilis*, (G 89%) had inhibition activity 47.5%, (G 129%) had inhibition activity 52.2% and (G 353%) had inhibition activity 69.1% compared to Ampicillin. As for *Escherichia coli*, (G 89%) had inhibition activity 66.8 %, (G 129%) had inhibition activity 77.4% and (G 353%) had inhibition activity 94% compared to Getamycin. Where (G 353%) had the best antibacterial and antifungal activity as the graft % increased, antimicrobial activity increased.

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

- Itaconic acid disrupts the electron transport chain located in the inner membrane of *Gram-positive* and *Gram-negative* bacteria responsible for energy production by binding to the carriers of the electron transport chain directly or inserting into the inner membrane causing the electron carriers to move apart or be displaced from the membrane entirely.
- Itaconic acid inhibits enzyme activity affecting the composition and permeability of the bacterial cell membrane leading to cell death.

And the antifungal activity of starch-g-PIA (in aqueous media) can be possibly illustrated that carboxylic group of itaconic acid Binds physicochemically to ergosterol in fungal membrane resulting in the production of aqueous pores leading to altered permeability, leakage of vital cytoplasmic components, and death of the organism [49].

Sample	Inhibition zone diameter (mm)				
	<i>Bacillus subtilis</i> (G^+)	<i>Streptococcus pneumonia</i> (G^+)	<i>Escherichia coli</i> (G^-)	<i>Aspergillus flavus</i> (fungus)	<i>Candida albicans</i> (fungus)
Amphotericin B (antifungal agent)	--	--	--	23.7 ± 0.1	25.4 ± 0.1
Ampicillin (antibacterial agent)	32.4 ± 0.3	23.8 ± 0.2	--	--	--
Getamycin (antibacterial agent)	--	--	19.9 ± 0.3	--	--
Starch	NA	NA	NA	NA	NA
(G 89%)	15.4 ± 0.63	14.3 ± 1.2	13.3 ± 1.2	NA	16.2 ± 0.58
(G 129%)	16.9 ± 1.2	16.2 ± 1.5	15.4 ± 0.63	NA	19.3 ± 1.2
(G 353%)	22.4 ± 0.63	21.3 ± 1.2	18.7 ± 0.58	NA	19.3 ± 0.63

TABLE. 7. Antimicrobial activity of Starch and starch-g-PIA with different G%

Conclusion

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

[(KPS)/ (NaBS)]= 6×10^{-2} mol.L⁻¹, [M]=1.1 mol.L⁻¹, reaction temperature=55°C and reaction time=3.75h.

Synthesis of Starch-g-poly(itaconic acid) (St-g-PIA) had been done and its structure was proven by FTIR, NMR, XRD and SEM analyses.

FTIR spectra of starch, PIA and starch-g-PIA of different Graft% were illustrated revealing different peaks corresponding to starch, PIA and starch-g-PIA. The intensity of all of these peaks increased with increasing the graft yield. Synthesis of starch-g-PIA has been proved by comparing its 1H-NMR spectrum with that of native starch.

The St-g-PIA is amorphous thus X-rays would be scattered in many directions leading to a large bump distributed in a wide range (2 Theta) instead of high intensity narrower peaks.

Scanning electron microscopy (SEM) images showed that as G% increased, rock-like nature of the grafts increased with increasing IA content. Also, more G% showed more pores (hollow zones) helping in capturing metal ions in water treatment. Results for the solubility test showed that as the G% increased, the solubility% increased due to the hydrophilic nature of PIA.

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 $\text{Cu(II)} > \text{Co(II)} > \text{Cd(II)}$ at pH 7.

Chemical modification of starch by grafting with PIA has special use in antimicrobial activity against *Candida albicans*, *Streptococcus pneumonia*, *Bacillus subtilis* and *Escherichia coli* as fungi, Gram-positive and Gram-negative bacteria, respectively. 353 % G had the best antimicrobial activity due to the high content of grafted PIA.

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