J Polym Res (2011) 18:459–467
DOI 10.1007/s10965-010-9437-x

ORIGINAL PAPER

Grafting of acryloyl cyanoacetohydrazide onto chitosan

Said S. Elkholy · Khalid D. Khalil · Maher Zaki Elsabee

Received: 13 October 2009 / Accepted: 5 April 2010 / Published online: 13 April 2010

Abstract Chitosan was grafted with a novel monomer namely Acryloyl cyanoacetohydrazide (ACAH) which contains carbonyl and cyano groups. The graft copolymerization was conducted in heterogeneous phase using potassium persulfate ($K_2S_2O_8$) and sodium bisulfite ($NaHSO_3$) as redox initiators. The effect of monomer concentration, initiator concentration and ratio, time and temperature on the extent of grafting (G%) and the efficiency of grafting were studied. Homopolymer formation has not been observed under all the investigated conditions. The grafted samples were characterized by FTIR spectroscopy, X-ray diffraction and thermogravimetric analysis. The crystallinity of the used chitosan was reduced by grafting. 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 quaternized graft copolymer was found to be soluble in water. The fungicidal activity of the quaternized graft copolymers towards three soil-borne sugar beets pathogens was investigated in vitro. The effect on the micro organisms is proportional to the amount of ACAH in the graft copolymer.

Keywords Chitosan · Acryloyl cyanoacetohydrazide · Graft copolymer · Swelling behavior · Dye uptake · Quaternization · Biological activity

S. S. Elkholy · K. D. Khalil · M. Z. Elsabee (✉)
Department of Chemistry, Faculty of Science, Cairo University, Giza, Cairo 12613, Egypt
e-mail: mzelsabee@yahoo.com

Introduction

Chitin is an intractable and abundant (second to cellulose) naturally occurring polysaccharide forming part of the shell of crustaceans, fungi and insects. Chitosan is the product obtained from N deacetylation of chitin with strong alkali [1–3]. 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 [4, 5]. 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.

Chitosan can be easily modified by a variety of techniques among it is grafting. Grafting has been used to prepare new materials based on chitosan which however have improved properties for example as a flocculent agent [6] by grafting chitosan with acrylamide using gamma radiation, for dye absorption [7] where chitosan grafted with a series of methacrylate monomers, for metal uptake [8] and waste water purification especially removal of heavy metals as Hg [9]. Of interest also grafting of chitosan could enhance its already known antimicrobial capacity [10, 11]. Many reviews dealing with chitosan modifications including grafting were published [12–15] with a comprehensive list of the monomers type, the initiators and grafting conditions as well as the polymers characterization.

The present work deals with the grafting copolymerization of chitosan with a new monomer: acryloyl cyanoacetohydrazide, ACAH, which contains two active moieties: a carbonyl and a cyano group. The reaction variables of the

grafting process were studied systematically. The capacity of the grafted chitosan samples for acidic or basic dyes was investigated. Since the graft copolymers are based on a biodegradable and biocompatible material (chitosan) it could have biomedical applications which necessitate measuring its swelling behavior. The grafted chitosan was further modified by quaternization to increase its antimicrobial capacity [16, 17].

Sugar beet (*Beta vulgaris L., Chenopodiaceae*) is one of the most important cash crops grown mainly in the areas of temperate climatic conditions for sugar production. It has great economic importance for Egypt [18] since it is the second crop plant for the sugar production after sugar cane. Sugar beet is attacked by several root-rot diseases the most serious of which are those caused by *R. solani* and *S. rolfsii* and also a wilt disease caused by *Fusarium* species [19]. Taking this economic importance into consideration the present work was designed to investigate the in vitro effect of chitosan and its graft copolymers on the growth activities of the sugar beet pathogens: *R. solani, S. rolfsii* and *F. solani*.

Experimental

Samples

Chitosan was kindly supplied by Dr. Furuhata of Tokyo Institute of Technology (T.I.T). Acryloyl cyanoacetohydrazide (ACAH) was synthesized in our laboratory and its structure was confirmed and characterized as mentioned below. The 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 from Aldrich and were used as received.

Preparation of acryloyl cyanoacetohydrazide (ACAH)

N-acryloyl-*N*′-cyanoacetohydrazide (ACAH) was prepared according to reference [18].

ACAH has a m.p. of 195–197 °C. The structure of the prepared monomer was confirmed by ^{1}H–NMR, FTIR spectroscopy and elemental analysis and is represented as:

$$CH_2 = CH - CO - NH - NH - CO - CH_2 - CN$$

Elemental analysis was carried out in the Central Microanalytical Unit, Cairo University with the following data: Found (calculated): C% 47.20 (47.05), H% 4.9 (4.57) and N% 26.85 (27.45).

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 thermostat 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 for the purification of the graft copolymer. The structure of the obtained graft copolymer was investigated by Fourier transform infrared (FTIR) spectroscopy.

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

$$\%G = [(W_2 - W_1)/W_1] \times 100$$
$$\%E = [(W_2 - W_1)/W_3] \times 100$$

Where, W_1, W_2 and W_3 denote the weight of initial chitosan, grafted chitosan after extraction with methanol, and ACAH monomer charged, respectively.

FTIR spectroscopy

FTIR spectra were taken using FTIR spectrometer Bruker Vector 22 Germany in the range of 400 to 4,000 cm^{-1}.

Thermal analysis

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

X-ray diffraction analysis

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

Dye up take 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 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 above using the calibration curve of each dye.

Swelling measurements

Swelling measurements were carried out in distilled water, pH=6.5, in acidic buffer pH=3.4 and in basic buffer at pH=10, at room temperature. The well-known teabag method was used [18]. 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, hung for 5–10 min in order to eliminate excess unabsorbed water, and then weighed. The degree of swelling was calculated using the relation $[(W–W_o)/W_o] \times 100$, where W and W_o are the weight of the swollen and dry sample, 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 according to [10]. The antibacterial ability was examined by measuring the inhibition zone caused by the graft copolymer.

Bioassay of the graft copolymers

Sources and culture of fungi The fungi used in this work were isolated from diseased sugar beet roots. *(Aspergillus niger, Caldosporium herbarum and Fusarium moniliformae*) [10].

Germination of macroconidia and sclerotia

The procedure for the germination has been described in details in [10]. Five plates were prepared for each treatment and the means were compared.

Production of Sclerotia PDA (potato dextrose agar) was used for *R. solani* and Czapek-Dox agar for *S. rolfsii*. Dried polymer powder was mixed with the medium to produce the required concentration and poured into Petri dishes. The fungi were transferred to the dishes and incubated at 27°C for 9 days. For *R. solani*, 1 ml of the hyphal suspension was added to each dish. This was prepared by transferring two 6 mm diameter PDA disks bearing hyphae into potato dextrose broth (PDB) in 250 ml Erlenmeyer flasks, each containing 50 ml of the medium.

The flasks were incubated at 27 °C for 3 days and filtered; the mycelial mats were washed with sterile distilled water. This mycelium was homogenized with 100 ml sterile water in a sterile micro blender for 3 min to form a heavy suspension. For *S. rolfsii*, one 6 mm diameter agar disk bearing hyphae of the fungus was transferred to each dish. The number of sclerotia produced per plate in each treatment was visually counted. Five plates were prepared for each treatment and the means were compared.

Production of macroconidia The details are given in reference [10].

Statistics The experiments were conducted in 3–5 replicates and the results obtained were treated statistically with an analysis of variance and the significance was expressed at LSD 5% and 1%.

The susceptibilities of the test fungal spore *(Aspergillus niger, Caldosporium herbarum and Fusarium moniliformae*) as seeded in Dox's medium on filter paper discs (6 mm) soaked with 5 mg ml^{-1} of each compound, were determined. The soaked and completely dried filter paper discs were placed on the surface of the seeded Dox medium in triplicate tests for each compound. Plates were allowed to stand for 2 h to allow for diffusion. Later on, the plates were incubated for 48 h, after which the susceptibility of each organism to each compound was estimated by measuring the diameter of the zones of inhibition.

Determination of the minimum inhibitory concentration (MIC) of the fungicidal compounds

The MICs of the isolated compounds on *Aspergillus niger, Caldosporium herbarum* and *Fusarium moniliformae* were determined by the dilution method [10].

Results and discussions

The grafting percentage increases with time initially and reaches almost a limiting value after 2 h as can be seen in Fig. 1.

The effect of initiator concentration is shown in Fig. 2. The%G increases reaching a maximum then declines slightly; this initiator concentration (ca 0.004 mol/l) was taken in all other experiments. The ratio of redox system was also investigated to find the optimal ratio was found to be $NaHSO_3/K_2S_2O_8$=0.8. The temperature effect is shown in Fig. 3 where a maximum grafting is attained at around 55 °C then declines slightly; in the meantime the grafting efficiency is almost constant.

The%G increases with increasing [M], as is expected, as seen in Fig. 4. The remarkable feature of this system is that no homopolymer formation was observed under all these investigated conditions. Therefore no homopolymer formation information was given.

The grafting was evidenced by weight increase, IR spectroscopy and elemental analysis. As an example, it has been found by gravimetric weight increase that G% is 95% and the grafted sample has N% of 17.09 which corresponds to a G% of 102.8%.

The FTIR of chitosan and two grafted samples (Fig. 5) shows the O–H stretching absorption vibration for chitosan as a broad band around 3,500 to 3,200 cm^{-1}, which is due to the intermolecular hydrogen bonding, overlapped with the N–H stretching bands of amino groups that are 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 1,641 to 1,602 cm^{-1} as two sharp bands [19]. The spectrum of the grafted copolymer shows a band at 2,250 cm^{-1} which is attributed to the presence of CN group of ACAH, its intensity increases with increasing the grafting extent. Also, CH_2 bending vibration of ACAH appears at 1,450 cm^{-1}. The vibrational frequency of the carbonyl group decreases upon grafting due to the increase in ACAH concentration which has more weaker C=O bond due to resonance. In addition to the presence of the above bands, the change in the finger-print region confirms also the grafting process.

Figure 5 has proved that the initiation step for grafting was on the amino group at C_2. This is well illustrated by the disappearance of the doublet band at 3,445 and 3,422 cm^{-1} corresponding to the $–NH_2$ group and the appearance of a single band at 3,400 cm^{-1} for –NH group which indicates the abstraction of H atom by the KSO4* radical derived

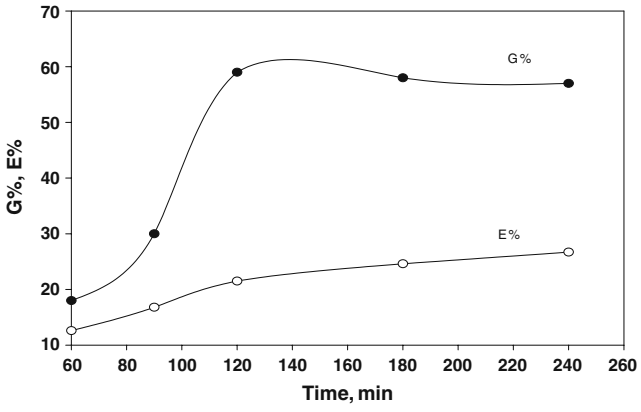

Fig. 1 Effect of time on the% G of ACAH onto chitosan, T=55 °C

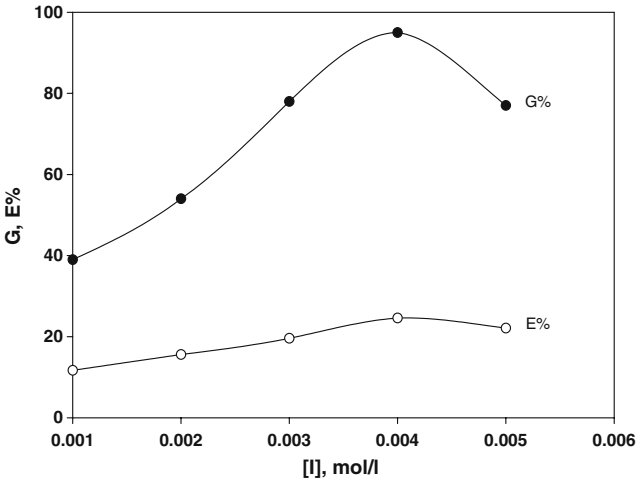

Fig. 2 Effect of initiator concentration on the%G, T=55 °C, [ACAH] = 1 M

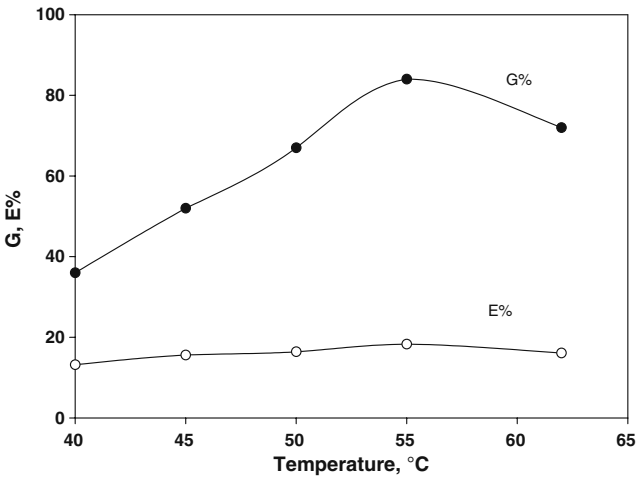

Fig. 3 Temperature effect on the%G, the same grafting conditions as in the previous system

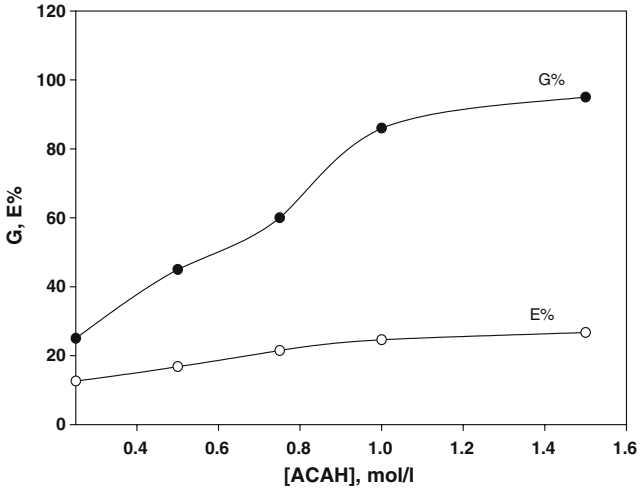

Fig. 4 Effect of monomer concentration [M] on the grafting extent

from the decomposition of the potassium [20] persulphate initiator.

$$Ch - NH_2 + KSO_4^* \rightarrow Ch - NH^* + KHSO_4$$

Swelling measurements of the graft copolymers

Since the grafted chitosan copolymers are considered biomaterials then swelling measurement is important for any biomedical and biological applications later on.

From Fig. 6 it is clear that the extent of swelling increases with increasing the% G. The introduction of polar groups of ACAH (–CN and –CONH groups) in the graft copolymer leads to an increase in the extent of swelling. Furthermore, quaternization of the PACAH/chitosan copolymers led to an improvement in the swelling degree. The pH has a strong effect on the extent of swelling increasing in the following order: pH 3.4 > pH 10 > pH 6.5.

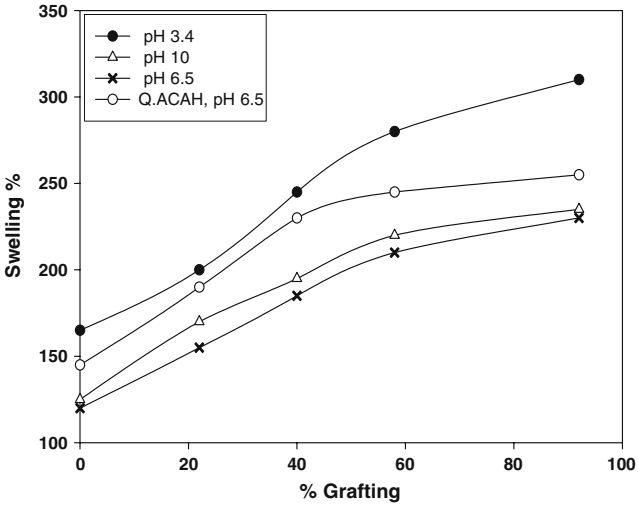

Fig. 6 Swelling curves for the chitosan/ACAH graft copolymers as a function of pH and G%, (o) is a quaternized graft copolymer

Dye uptake of the grafted ACAH/chitosan copolymers

Samples of copolymers with different degree of grafting have been investigated with respect to their ability to absorb dyes. Two types of dyes have been used (EBT), and (MB). From the calibration curve, the amount of the washed dye was estimated. Figure 7 shows the dye uptake of chitosan and the ACAH/chitosan graft copolymer. The dye uptake slightly increases with the increase in grafting percentage with a higher uptake for the acidic dye. Samples treated

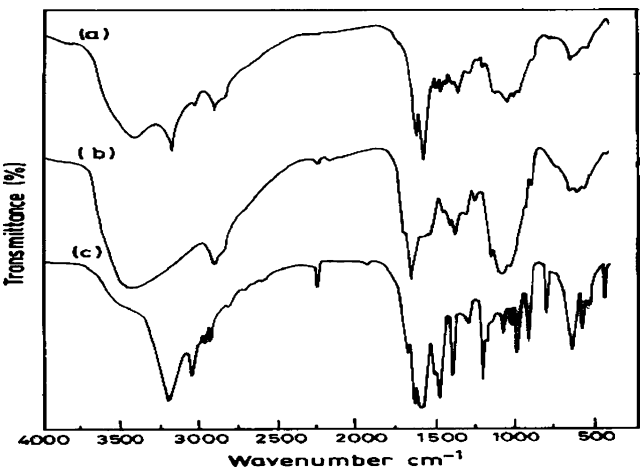

Fig. 5 The FTIR spectrum of (**a**) pure chitosan (**b**) chitosan/ACAH copolymer 21%G, (**c**) 92%G

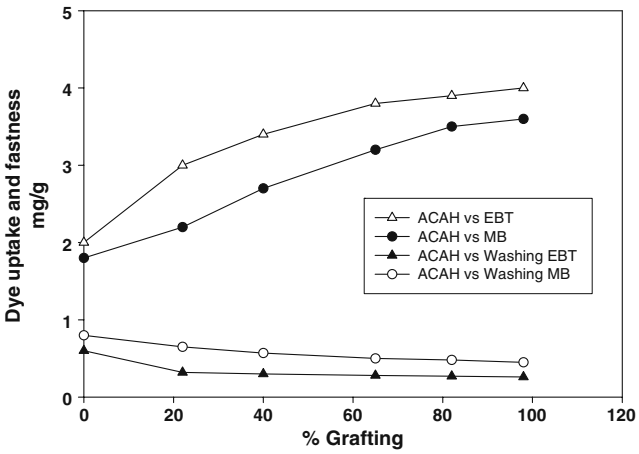

Fig. 7 Dye uptake and washing fastness of chitosan/PACAH graft copolymers, T=37 °C and at pH=6.5

with EBT (acidic dye) shows very small amount of leached dye relative to the samples treated with MB as shown in Fig. 7. The acidic dye was absorbed to higher extent (chemisorptions) as expected since the chitosan has a cationic character attracting thus the acidic molecules of the EBT.

The dye uptake and the dyeability should be highly sensitive to the variation of the pH therefore the dye uptake was investigated at three pH namely acidic, neutral and basic conditions. The following figures represent the effect of pH on the dyeability of chitosan/PACAH graft copolymer. Figure 8 indicates that at pH 3.4 the dye uptake is higher than at pH 10 or 6.5 here the amino groups are mostly protonated and have higher ability to absorb both dyes. At pH 10 as shown in Fig. 9 the amino groups are free amines and dye uptake is lowered, meanwhile the dye fastness shows a minimum leaching at the lower pH according to the same arguments.

X ray analysis

X-Ray curves for copolymer samples prepared by heterogeneous grafting indicated that the peak at around 2θ=10° (characteristic for chitosan [21]) disappears after grafting which implies a slight decrease in the percent crystallinity.

Thermal analysis

The thermo-gravimetric analysis of chitosan, PACAH and one graft copolymer is shown in Fig. 10. The unmodified chitosan shows a small weight loss before 100 °C due to the loss of water followed by one major decomposition band with a maximum temperature at 314 °C, while poly (ACAH) homopolymer shows major decomposition at 226.28 °C. On the other hand, the grafted sample (92% G) showed the same water loss then a small decomposition at around 273.04 °C probably due to the loss of the ACAH side chain followed by the major decomposition band at 305.45 °C. Figure 10 shows also a slight declining in thermal characteristic of the PACAH/chitosan graft copolymer over pure chitosan but improvement over poly (ACAH) homopolymer.

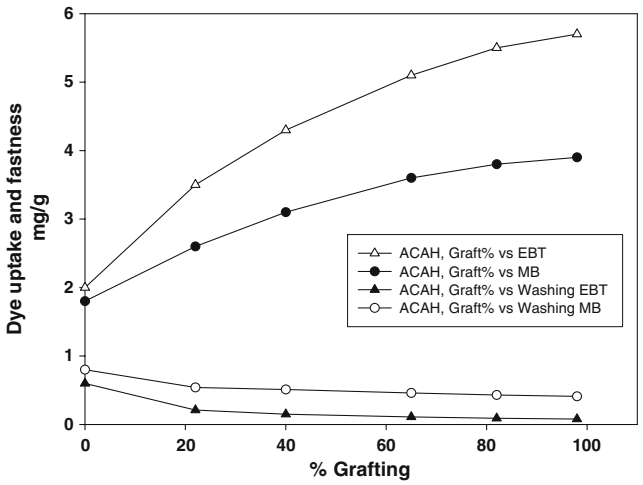

Fig. 8 Dye uptake and washing fastness of chitosan/PACAH graft copolymers, T=37 °C and at pH=3.4

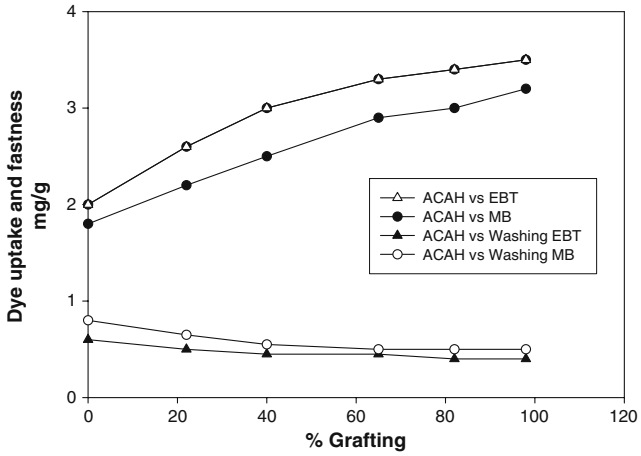

Fig. 9 Dye uptake and washing fastness of chitosan/PACAH graft copolymers, T=37 °C and at pH=10

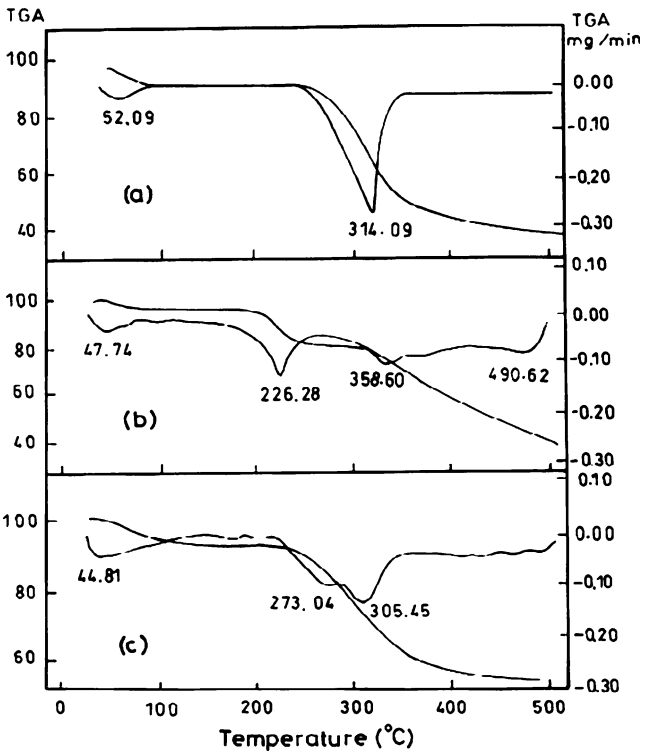

Fig. 10 TGA of (**a**) chitosan, (**b**) PACAH and (**c**) chitosan/ACAH graft copolymer 92% G

Table 1 Effect of the chitosan/PACAH graft copolymers (G% 116) concentrations on the percent germination (G), average length of hyphal extension (Lh), dry mass yield (Dm), production of sclerotia of *Rhizoctonia solani, Sclerotium rolfsii* and macrconidia of *Fusarium solani* at 27 °C

μg ml^{-1}	*R. solani*					
	G (%)	Lh (μm)	Dm (mg)	Initial pH	Final pH	Number of sclerotia/plate
Control	56.1	843.3	1,020.1	5.8	7.9	63
10	19.6	360.1	311.1	5.7	7.4	39
20	12.2	299.2	218.3	5.7	7.4	19
30	6.2	65.3	86.2	5.6	7.2	6
40	0	0	0	5.6	7.0	0
LSD						
1%	3.8	13.6	17.1	–	–	6.3
5%	1.4	8.7	9.2	–	–	2.7
	S. rolfsii					
Control	53.1	662.4	612.1	5.8	3.3	623
10	17.3	310.4	244.3	5.7	3.7	310
20	9.4	140.1	109.9	5.7	3.7	84
30	2.9	44.3	83.1	5.6	3.8	49
40	0	0	0	5.6	4.2	0
LSD						
1%	3.6	12.8	17.4	–	–	6.4
5%	1.2	7.4	9.6	–	–	2.9
	F. solanii					
	G (%)	Lh (μm)	Dm (mg)	Initial pH	Final pH	Number of macroconidia $\times 10^4$ ml^{-1}
Control	63.2	12.4	357.2	5.8	4.3	314.7
10	30.1	6.0	193.2	5.7	4.2	200.1
20	21.6	5.0	100.4	5.7	4.2	186.4
30	12.4	3.3	68.3	5.6	4.1	96.1
40	0	0	0	5.6	4.0	0
LSD						
1%	7.9	2.5	12.0	–	–	18.8
5%	5.3	1.6	3.6	–	–	7.5

Biological activity of the quaternized graft copolymers

Preliminary results of the biological activity measurements show that grafting with ACAH and quaternization of the copolymer produce inhibiting effect on the micro organisms. The inhibition of growth of the fungus will be useful for the plant, decreasing the diseases it causes.

From Tables 1, 2 and 3 one can see that the percent germination of sclerotia of *R. solani* and *S. rolfsii* decreased with increasing the chitosan and the three chitosan graft

Table 2 Effect of different chitosan/ACAH graft copolymers on *A. niger, C. herbarum, and F. moniliformae* at 28 °C by the disc plate method (mean values of the diameters of the incubation zones in mm)

Type of compound	% G	*A. niger*	*C. herbarum*	*F. moniliformae*	LSD	
					5%	1%
Chitosan	0	5.1	5.3	5.5	0.24	0.6
Chitosan/ACAH	22	5.5	5.7	5.9	0.21	0.51
	46	5.8	6.2	7.3	0.32	0.62
	78	6.4	6.9	7.9	0.36	0.71
	92	7.0	7.4	8.6	0.25	0.63

Table 3 Minimal inhibitory concentration (MIC) at $\mu g ml^{-1}$ of the grafted chitosan/ACAH copolymers upon *A. niger, C. herbarum* and *F. moniliformae*

Type of compound	% G	*A. niger*	*C. herbarum*	*F. moniliformae*	LSD	
					5%	1%
Chitosan	0	925	800	750	7.1	15.6
Chitosan/ACAH	22	325	300	275	8.4	13.5
	46	300	275	250	7.6	16.3
	78	275	250	250	5.8	9.5
	92	250	250	225	7.1	15.6

copolymers concentrations. The average length of the hyphal extension and the dry mass yield was affected similarly, decreasing proportionally to the polymers concentration. The pH of the growth medium was shifted toward alkalinity for the *R. solani.* The pH increase in the culture medium during fungal growth may have been caused by differential uptake of cations and anions. Transport of anions such as phosphates may act as the hydroxide exchange system with the medium becoming more basic [22]. The rapid decline in the initial pH of the culture was probably due to the production of organic acids (oxalic acid) through the oxidation of carbon source [23].

The numbers of sclerotia produced by *R. solani* and *S. rolfsii* (column 6 in the Tables) at chitosan concentration ranging from 10 to 50 $\mu g\ ml^{-1}$ were reduced proportionally to the chitosan concentration. No sclerotia were produced by all the three graft copolymers at concentration of 40 $\mu g\ ml^{-1}$. Small number of sclerotial production was observed for pure chitosan at concentration of 40–50 $\mu g\ ml^{-1}$. The macroconidia of *F. solani* germinated in a wide range of polymer concentration 10–50 $\mu g\ ml^{-1}$, decreased steadily with an increase in the chitosan and its graft copolymers concentration. Maximum inhibition for pure chitosan was recorded at a concentration of 50 $\mu g\ ml^{-1}$ while no macroconidia was observed at a concentration of 40 $\mu g\ ml^{-1}$ for the four graft copolymers. Similar results were obtained when measuring the length of the germ-tube after 9 h incubation. Maximum reduction in the germ-tube length was obtained at pure chitosan concentration of 50 $\mu g\ ml^{-1}$ (from 12.4 to 3.6 μm) reaching zero μm (no germination) for the graft copolymers at a concentration of 40 $\mu g\ ml^{-1}$. Dry mass yield estimations showed that the mycelial tolerance to chitosan concentration was highest for *F. solani* and lowest for *R. solani* and *S. rolfsii*. For *F. solani*, the results recorded at chitosan concentrations 10–50 $\mu g\ ml^{-1}$ were significantly different from the control. The same trend was found for the investigated copolymers.

From the tables it can be seen that grafting affect strongly both the inhibition zones and the MIC, the latter decreases almost by a factor of four by grafting with ACAH.

The graft copolymers with chitosan have the ability of forming metal complexes; this will be investigated in a future work.

Conclusions

Chitosan was grafted with ACAH under heterogeneous conditions (as suspension in water). 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 are slowly soluble in hot diluted acids, therefore one can exclude crosslinking reaction between the chains but an increased hydrogen bonding making the solubility more difficult. Dye uptake of the grafted samples was studied using acidic and basic dyes. It has been shown that the acidic dye uptake is much higher than that for the basic dye which is due to the basic character of chitosan. 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. X-ray analysis of samples prepared by heterogeneous grafting showed that crystallinity did decline with increasing grafting as expected. 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/PACAH copolymer with dimethyl sulfate in basic medium. Quaternization with three moles of dimethyl sulfate led to the preparation of soluble graft copolymer with a film forming ability, the antibacterial properties of this system show that increasing G% leads to higher inhibition values for bacteria and fungi. The thermal stability of the graft copolymer did not show deterioration compared to the original chitosan; it increases slightly with percent grafting.

References

1. Muzzarelli RAA (1977) Chitin. Pergamon, New York
2. Muzzarelli RAA, Jeauniax C, Gooday GH (1988) Chitin in nature and technology. Plenum, New York
3. Abdou ES, Nagy KSA, Elsabee MZ (2008) Extraction and characterization of chitin and chitosan from local sources. Bioresour Technol 99:1359
4. Skajak-Braek G, Anthonsen T, Standford P (eds) (1989) Chitin and chitosan. Elsevier, Applied Science, London, 1367
5. Day WH (1991) In: Chum HL (ed) Polymers from biobased materials, vol 81. Noyes Data Corporation, New Jersey
6. Wang J-P, Chen Y-Z, Zhang S-J, Han-Qing Yu (2008) A chitosan-based flocculants prepared with gamma-irradiation-induced grafting. Bioresour Technol 99:3397–3402
7. Konaganti VK, Kota R, Patil S, Madras G (2010) Adsorption of Anionic Dyes on Chitosan grafted Poly(alkyl methacrylate)s. Chemical Engineering Journal accepted manuscript
8. Don TM, King CF, Chiu WY (2002) Preparation of chitosan graft-poly(vinyl acetate) copolymers and their adsorption of copper ion. Polym J 34:418–425
9. Miretzky P, Fernandez Cirelli A (2009) Hg(II) removal from water by chitosan and chitosan derivatives: a review. J Hazard Mater 167(1–3):10–23
10. Elkholy SS, Khalil KD, Elsabee MZ, Eweis M (2007) Grafting of vinyl acetate onto chitosan and biocidal activity of the graft copolymers. J Appl Polym Sci 103:1651–1663
11. Mourya VK, Inamdar Nazma N (2008) Chitosan-modifications and applications: opportunities galore. React Funct Polym 68 (6):1013–1051
12. Jenkins DW, Hudson SM (2001) Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers. Chem Rev 101:3245–3273
13. Jayakumar R, Prabaharan M, Reis RL, Mano JF (2005) Graft copolymerized chitosan—present status and applications. Carbohydr Polym 62(2):142–158
14. Varma AJ, Deshpande SV, Kennedy JF (2004) Metal complexation by chitosan and its derivatives: a review. Carbohydr Polym 55 (1):77–93
15. Jia Z, Shen D, Xu W (2001) Synthesis and antibacterial activities of quaternary ammonium salt of chitosan. Carbohydr Res 333:1–6
16. Sajomsang W, Gonil P, Tantayanon S (2009) Antibacterial activity of quaternary ammonium chitosan containing mono or disaccharide moieties: preparation and characterization. Int J Biol Macromol 44(5):419–427
17. Eweis M (1998) PhD thesis Cairo University, pp 184
18. Yazdani-Pderam M, Retuert M, Quijada JR (2000) Hydrogels based on modified chitosan, 1. Synthesis and swelling behavior of poly (acrylic acid) grafted chitosan. Macrom Chem Phys 201:923–930
19. Silverstein RM, Webster FX, Kiemle DJ (2005) Spectroscopic identification of organic compounds, 7th edn. Wiley, New York
20. Sabaa MW, Mohamed NA, Mohamed RR, Khalil NM, Abd El Latif SM (2010) Synthesis, characterization and antimicrobial activity of poly (*N*-vinyl imidazole) grafted carboxymethyl chitosan. Carbohydr Polym 79(4):998–1005
21. Abdou ES, Nagy Kh, Elsabee MZ (2008) Extraction of chitosan from local sources. Bioresour Technol 99:1359–1367
22. Griffin DH (1994) Fungal physiology. Wiley, New York
23. Punja ZK, Jenkins SF (1984) Influence of medium composition on mycelial growth and oxalic acid production in Sclerotium rolfsii. Mycologia 76:947–995