Grafting of Cellulose–Thiocarbamate with Vinyl Monomers: Antimicrobial Activity

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Synopsis

Grafting of vinyl monomers onto cellulose–thiocarbamate was carried out using ceric ammonium sulfate (CAS) as an initiator. The graft yield was found to depend on the amount of thiocarbamate groups, initiator, and monomer concentrations as well as temperature. The graft yield increased with increasing (CAS) concentration. The reactivity of vinyl monomers studied followed the order ethyl acrylate > acrylonitrile. A comparison between the graft yields obtained with the modified cullulose indicated that cellulose thiocarbamates having less than 1.1% nitrogen showed lower graft yields than the unmodified cellulose. Above this, cellulose thiocarbamate was much more amenable to grafting than the unmodified cellulose. The grafted cellulose thiocarbamates exhibited high antifungal activity and had no effect on gram-negative, gram-positive bacteria and yeast. The maximum zone of inhibition was obtained after grafting with 2 h which resulted in 43 and 50% add-on polymer in the cases of acrylonitrile and ethyl acrylate, respectively. Grafted cellulose thiocarbamates with acrylonitrile had higher potency for antifungal activity than that grafted with ethyl acrylate.

INTRODUCTION

It has been reported that urea reacts at high temperatures with cellulose to yield mainly cellulose carbamate,[1,2]

In this work cotton cellulose (cell—OH) reacted with thiourea to yield essentially cellulose thiocarbamate:

$$\text{Cell}-\text{OH} + \text{NH}_2-\overset{\overset{\text{S}}{\|}}{\text{C}}-\text{NH}_2 \cdots \text{Cell}-\text{O}-\overset{\overset{\text{S}}{\|}}{\text{C}}-\text{NH}_2 + \text{NH}_3$$

The cellulose thiocarbamate and native cellulose were grafted with ethyl acrylate and acrylonitrile using ceric ammonium sulfate as initiator; grafting of synthetic polymers to cellulose is an effective method for modifying its properties.[3]

Jovanovic and Jaksevic studied the effect of grafting with vinyl monomers on the resistance of cellulose fibers to the action of microorganisms. Some antifungal compounds have with chemical applications, e.g., in the control of plant pathogens. A detailed survey of this aspect was given by Thirumalachar.[5] The present work deals with the antimicrobial activity of grafted thiocarbamate of cellulose with vinyl monomers prepared at different temperatures.

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MATERIALS AND METHODS

Preparation of Cellulose Thiocarbamate

Cellulose carbamate was prepared by impregnating the cotton linter in an aqueous thiourea solution (60%) for 10 min at room temperature. Then the sample was squeezed to give a wet pickup of about 100%. It was followed by air drying and heat treatment at 180°C for 30 min, washing thoroughly with deionized water and finally air drying. Modified celluloses having various amounts of carbamate groups were obtained by changing the thiourea concentration.[1] The amount of thiocarbamate groups is expressed as percent of nitrogen. The latter was determined by the Kjeldahl method according to Cole and Parks.[6]

Grafting polymerization was carried out under purified nitrogen in a well-stoppered glass bottle of 50 mL capacity where 0.5 g of purified cellulose (oven dry basis) was placed together with ceric ammonium sulfate (CAS), 0.08 g/100 mL 1% sulfuric acid at a 40:1 liquor to cellulose ratio. Monomer was added in a ratio of 3 mL to 1 g cellulose. The monomers used were acrylonitrile and ethyl acrylate. The reaction period ranged from 0.25 to 4 h at a reaction temperature of 30 and 60°C. At the end of the reaction period, the samples were washed thoroughly by extraction with several portions of water, dried, and weighed. The crude grafting yield C was calculated as follows:

$$C = B - Z/Z \quad (\%)$$

where Z is the weight (g) of cellulose and B is the weight (g) of the grafted product before extraction.

The crude grafted celluloses were extracted for a period of 72 h with suitable solvents for the removal of the homopolymer, namely dimethyl formamide (DMF) for polyacrylonitrile (PAN) and tetrahydrofuran (THF) for poly(ethyl acrylate) (PEA). However, washing with distilled water followed. Finally, the samples were dried and weighed. The true grafting yield A was calculated as follows:

$$A = G - Z/Z \quad (\%)$$

where G is the weight of grafted product/weight of the grafted cellulose after extraction.

Microbiological Measurements

Microbial Strains

The antimicrobial potency of the substance was measured against a wide set of test organisms including:

a. Gram-negative bacteria *Escherichia Coli.*

b. Gram-positive bacteria *Bacillus cereus, Bacillus subtilis* and *Sarcina lutea.*

c. Yeasts: *Candida albicans* and *Saccharomyces cervisiae.*
d. Fungi: *Fusarium oxyporium V. vasifectum, Deplodia ohryzae, Aspergillus flavus, Botrylis alii,* and *Helminthosporium turcicum.*

Media

Bacterial test organisms were cultivated on nutrient agar, yeasts on malt extract agar, and fungi on sucrose Czapek Dox agar.[7]

Assay of Antimicrobial Substances

The methods used for bioassay in the present work were as follows.[8,9]

Antibacterial and Antiyeast Potency

The growth intensities of the test organism in liquid nutritive media, containing different concentrations of the tested antimicrobial substance were determined and plotted against the concentrations of the antimicrobial substance. Such standard curves were applied for estimating the concentration of the antimicrobial substance in other preparation.

Antifungal Potency

The assay was carried out using a modification of the two layer diffusion method. A layer of water agar was poured in a large Petri dish; after solidification another layer of Czapek-dox agar layer was poured. A disc of full grown active slant 48-h-age-old of the tested fungi were suspended into 10 mL water. The second dox agar layer was heavily inoculated with 1 mL of the fungal test spores. Thereafter discs of the grafted cotton were asceptically placed on the agar. The diameter of zones of inhibition of test organism were measured after 24–48 h of incubation.

RESULTS AND DISCUSSION

Cotton cellulose was treated with different aquous thiourea solution to investigate, in particular, the effect of its concentrations on the extent of the reaction. Figure 1 shows that maximum nitrogen content occurred at 60% thiourea concentration while higher thiourea concentrations resulted in lower nitrogen content, due to the release of ammonia as a product.[10]

In order to investigate the effect of the degree of modification of cellulose on grafting, cellulose thiocarbamate sample with nitrogen content ranging 0.1–1.6% as well as the control were grafted with acrylonitrite under similar condition. The obtained results are given in Figure 2. Cellulose–thiocarbamates having an N_2 content less than 1.1% showed lower graft yields compared to control. The opposite holds true for cellulose thiocarbamates having a nitrogen content of 1.2% or more.

However, it seems reasonable to state that the introduction of small amounts of thiocarbamate groups into the cellulose molecules reduces grafting whereas larger amounts increase it.

The IR spectrum of grafted cellulose thiocarbamate and non modified cellulose is illustrated in Figure 3. The spectra showed a strong and sharp

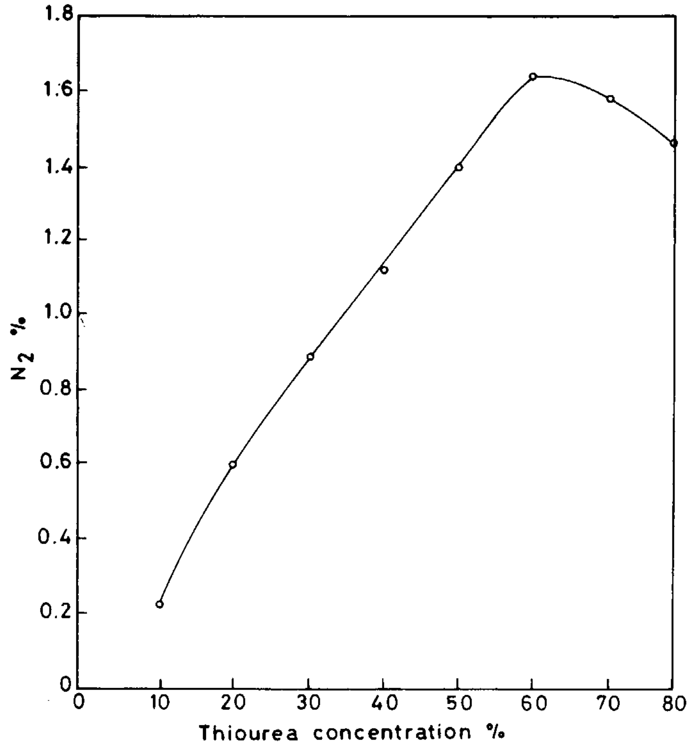

Fig. 1. Effect of thiourea concentration on the rate and extract of the reaction.

absorption peak in the 5.85–5.9 μm region and a weak one in the region of 6.15–6.20 μm. These two peaks correspond very closely in location and relative intensity to the similar ones in the spectra of the cellulose products. However the 5.9 μm absorption has been assigned to a joint esteramide carbonyl group and 6.2 μm mode to the amide band.[11]

Although the rest of thiocarbamate absorption in the 7–9.5 μm region are lacking in the spectra of the cellulose reaction products, these could be masked by the strong absorption of cellulose in this region.

Figure 4 indicates the effect of acrylonitrile concentration on the graft yields obtained with cellulose thiocarbamate and nonmodified cotton. It is evident that, within the range studied, the increase of acrylonitrile concentration caused a considerable increase in the graft yield. This is observed with both substrates. However, the graft yields for the modified cotton were higher than those of the nonmodified cotton in accordance with the discussed results. This is due to the fact that introducing thiourea in the cellulose molecule in a large amount increases the susceptibility of cellulose towards grafting while introducing a small amount of thiourea in the cellulose molecule lowers the suceptibility of cellulose towards grafting under the condition studied.

The increment in grafting by increasing monomer concentration is rather expected. At higher monomer concentrations, more acrylonitrile would be available in the polymerization medium for diffusion in, absorption on, and

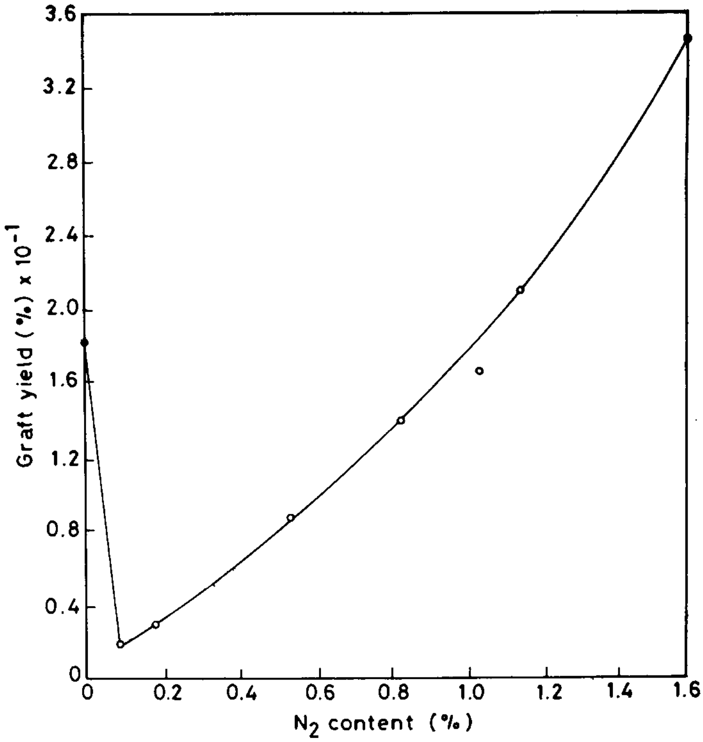

Fig. 2. Standard curve of influence of thiocarbamate groups (expressed as percent N_2) on the percentage of the graft yield.

reaction with cellulose macromolecules thereby enhancing the grafting degree.

Variation of grafting magnitude (i.e., graft yield of ethyl acrylate and acrylonitrile of cellulose–thiocarbamate with reaction time is shown in Figures 5 and 6. The same relation for the nonmodified cotton cellulose is also presented in the same figures for comparison. It is clear from Figures

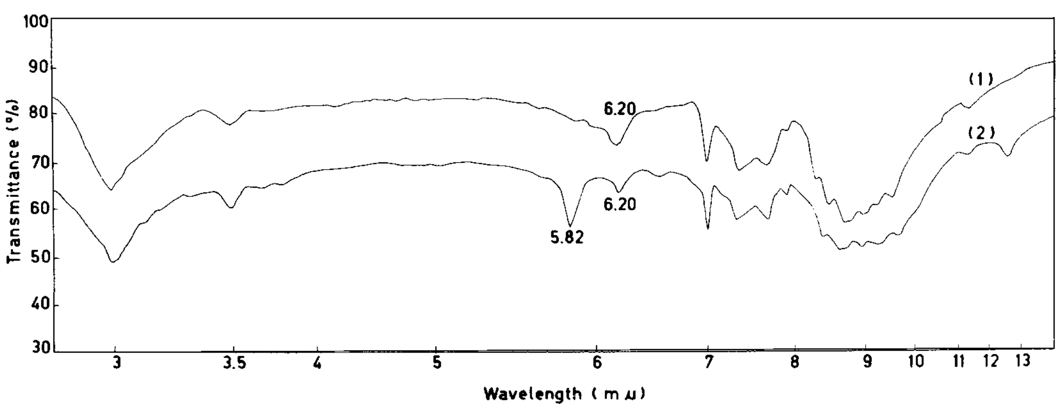

Fig. 3. IR spectra of nonmodified cotton (1) and cellulose thiocarbamate (2).

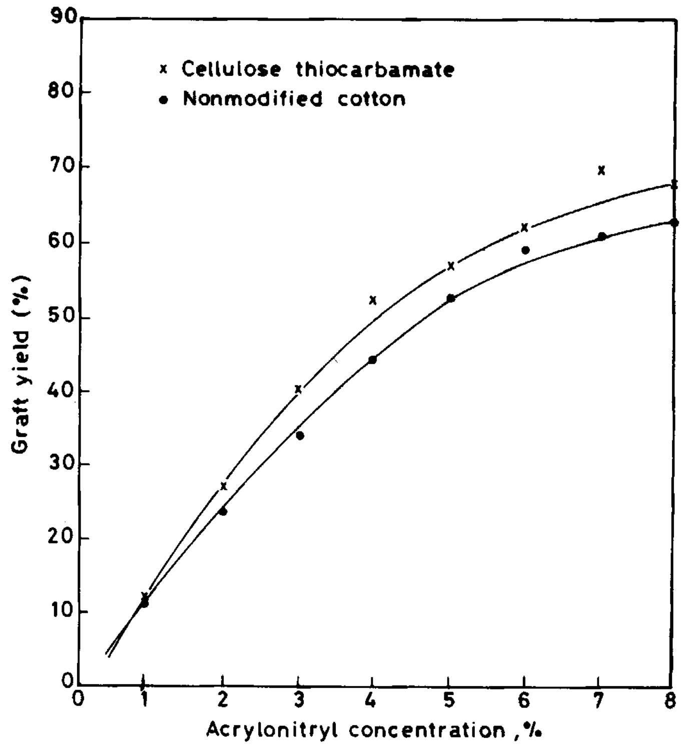

Fig. 4. Effect of monomer concentration on the graft yield of acrylonitryle: (x) cellulose thiocarbamate; (●) nonmodified cotton.

5 and 6 that nonmodified cotton gave lower grafting yield than the cellulose thiocarbamate while the nonmodified cotton gave homopolymer more than the cellulose thiocarbamate did, i.e., the extent and rate of homopolymer formed during grafting of the modified cotton are considerably lower than for the nonmodified cotton.

Higher grafting occurred at 30°C in case of acrylonitrile rather than in either 40 or 60°C. In the case of ethyl acrylate higher grafting yield occurred at 60°C rather than at 30 or 40°C.

Antimicrobial Activity

It is clear from Table I that grafted cellulose thiocarbamates exhibited high potency against the different stains of fungi but did not affect either gram-negative, gram-positive bacteria or yeasts.

It was found that the maximum zone of inhibition was obtained after grafting with 2 h. The cellulose thiocarbamate grafted with acrylonitrile had higher antifungal activity than cellulose–thiocarbamate grafted with ethyl acrylate.

On the other hand, the tested compounds exhibited no antibacterial activity when bioassayed against gram-positive test organisms, e.g., *Bacilus subtilis, Bacillus cereus,* and *Sarcina lutea,* and gram-negative ones, e.g.,

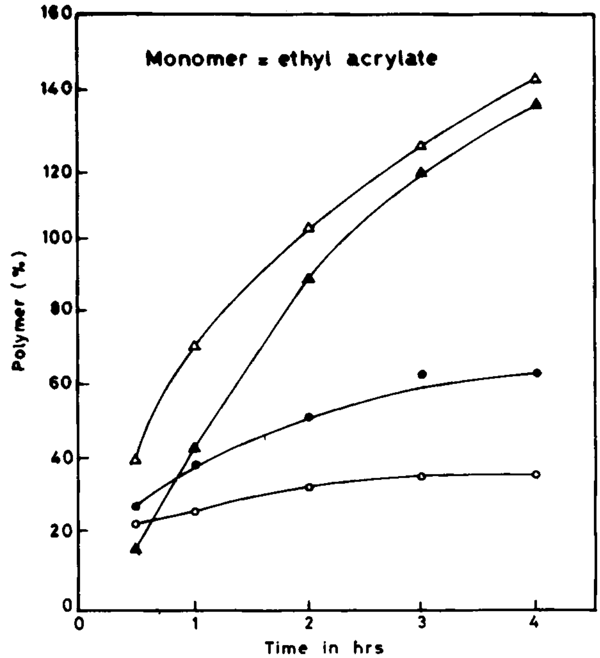

Fig. 5. Rate curve for homopolymerization (△, ▲) and grafting (○, ●) of nonmodified cotton and cellulose thiocarbamate, respectively.

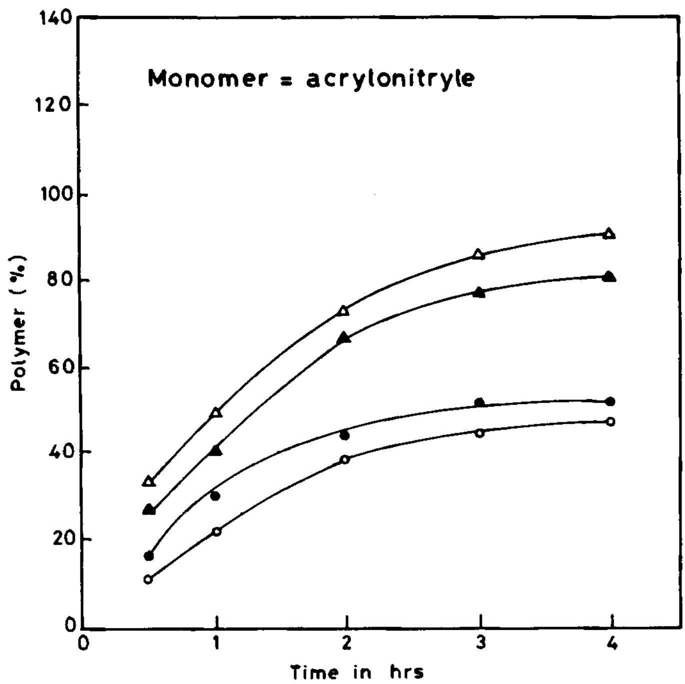

Fig. 6. Rate curve for homopolymerization (△, ▲) and grafting (○, ●) of nonmodified cotton and cellulose thiocarbamate, respectively.

TABLE I
Antimicrobial Potency of the Cellulose Thiocarbamates Grafted with Acrylonitrile and Ethylacrylate at Different Reaction Times, Percentages of Add-on Polymer, and Zones of Inhibition (mm)

Sample	Time of grafting (h)	% add-on polymer	Fusarium oxysporum V. vasinfectum	Deplodia ohryzae	Asperigillus flavus	Botrytis allii	Helminthosporium turicum
Cellulose-thiocarbamate	1	30	28	28	29	25	24
grafted	2	43	35	30	30	26	27
with AN	3	50	25	29	28	21	25
Cellulose-thiocarbamate	1	39	24	25	20	—	—
grafted	2	50	28	26	25	—	—
with EA	3	61	27	25	24	—	—
Cellulose- thiocarbamate	—	—	—	—	—	—	—

Echerichia Coli. These results reflect the high specificity of this compound against fungi and deserve further studies.

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