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Development of Cotton Fabrics with Prolonged Antimicrobial Action

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Abstract□Cotton xanthate, which was obtained by treating cotton with carbon disulfide in alkaline solution, was treated with the solution of polyvalent metal ions to produce cotton xanthate-metal chelates. This chelation reaction was readily and simply achieved, and antimicrobial agents with suitable structures could subsequently be coupled to the chelate with ease at moderate pH values and in aqueous solution. Metal ions used in present work include Cu(II), Zn(II) and Fe(III). Tetracycline, streptomycin, neomycin and pyrithion were used as antimicrobial/antifungal agents. Antibacterial activities were measured employing ditch plate method against G(+) *Staphylococcus aureus, Streptococcus faecalis,* and G(−) *Escherichia coli, Enterobacter aerogenes,* and the fungus, *Aspergillus niger.* All the cotton xanthate-metal-antimicrobial agent chelates exhibited activities whereas the cotton xanthate-metal chelates themselves were inactive. Considering the extensive washing procedures and results from control experiments, possibility of the involvement of physical adsorption for the binding of drugs could be excluded.

Keywords□Xanthated cotton-metal-antimicrobial agent chelate, antimicrobial activity.

In recent years, a great deal of attention has been paid to the development of modified polymers with desirable properties. Some are directed to develop modified polymers as a drug-delivery system, while others are aimed at the development of polymers with specific activities.[1-10]

In the case of controlled release drug delivery system, the rate of delivery of the bioactive agent would, presumably, be determined by the nature of the bonding between the polymer matrix and the drug molecule. The formation of covalent, hydrolyzable bonds is one of methods which have been employed most frequently. There is, of course, no absolute requirement that this highest energy type of bond should be the linkage used. Conceptually at least, it should be possible to develop controlled release delivery system based on other recognized type of lower energy chemical bonds. These comprise the van der Waals forces which are associated with low energies (ΔH 1 Kcal/mole), ionic bonds (ΔH 10-15 Kcal/mole) and coordinate or chelate linkage (ΔH 50 Kcal/mole). With coordinate or chelate type of bonding, it is possible to creat highly stable, complex organometallic structures and there is vast body of background chemical information defining the stability constants of such entities.[11]

Neogi and Allen[12] reported on the iron and aluminum chelates of 4-amino-3,5,6-trichloropicolinic acid utilizing Dowex A-1 as polymer matrix. Kennedy et al.[6] have reported on the coupling of antibiotics to cellulose, presumably, via chelate bond. Recently, Morris et al.[13] reported that a number of organic antimicrobial agents were durably bound to cotton fabrics in the form of zirconium complexes. These results demonstrate that chelated controlled release systems are feasible and further research is needed to establish the utility of more readily available and low cost backbone polymers which contained suitably oriented potential chelating sites.

The authors have been interested in the modification of naturally occurring fibers such as wool or cotton to give modified properties through the modification of the structure of the polymer matrix by chemical methods. We have previously reported that wool fibers or cotton fabrics can be

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modified successfully to carry dithiocarbamate group or xanthate group, which binds various metal ions tenaciously.[14-16]

In the present study, a number of antimicrobial agents were coupled to cotton xanthate by forming chelate bonds via polyvalent metal ions, expecting that the active agents would slowly and continuously be displaced from the matrix by dissociation in accordance with stability constant of the chelate bond.

EXPERIMENTAL METHODS

Instruments and materials

Adsorbent gauze (K.P.) for medical use were employed as representing cotton matrices for this reaction. The material was cut down about 5 × 5 cm in size, boiled in distilled water and dried before use. Copper (II) nitrate, zinc (II) nitrate, ferric (III) chloride and aluminum (III) chloride were reagent grade.

Determination of copper was performed using Baird atomic absorption spectrometer Model Alpha 3. Tetracycline hydrochloride, streptomycine sulfate, neomycine sulfate, and pyrithion sodium salt were purchased from Sigma Chemical Co., U.S.A., and used without further purification. The Gallenkamp flask shaker and Grant shaking water bath were used. The ingredients of medium for antimicrobial test were received from difco Laboratories, U.S.A.. Microorganisms used were G(+) Staphylococcus aureus, *Streptococcus faecalis,* and G(−) *Escherichia coli, Enterobacter aerogenes* and fungus, *Aspergillus niger.*

Preparation of cotton xanthate

Gauze(1g) was placed in a reaction flask containing 70 ml of 2N-NaOH and 1.2 ml of carbon disulfide was added. It was placed in a shaker bath at 25 °C and was reacted for 24 hours. As the reaction proceeded, the reaction mixture turned to orange color. At the end of the reaction, the material was taken out, washed thoroughly with water, ethanol, and acetone in turn. There was almost no material loss by this procedure.

Determination of metal binding capaacities of cotton Xanthate

Xanthated gauze(1g) was placed in a 100 ml of 5×10^{-3}M solution of various metal ions. It was shaken on bath for 3 hours, and the concentration of metal ion in solution which was not bound was analyzed. The concentration of copper ion was determined by atomic absorption spectrometer employing 327.4 nm line from hollow cathod lamp. The concentration of zinc, aluminum, and iron were determined by EDTA chelate method.[17]

Coupling of antimicrobial agents to cotton xanthate-metal chelate

Cotton xanthate-metal chelate (1g) was washed twice with 30 ml of 0.02 M sodium phosphate buffer (pH 5.1). The samples were shaken with a solution of antibiotic (100mg) in 0.1M sodium phosphate buffer (pH 4.5:50ml) for 2 hours at 4 °C. The material was then washed with five cycles of 0.1M sodium phosphate buffer (pH 5.1:30ml) and 0.5M sodium chloride in the same buffer (30ml), and then washed with distilled water. After drying, samples were washed with ethanol (three times), and diethyl ether (three times) to effect drying and sterilization.

Determination of antimicrobial activity

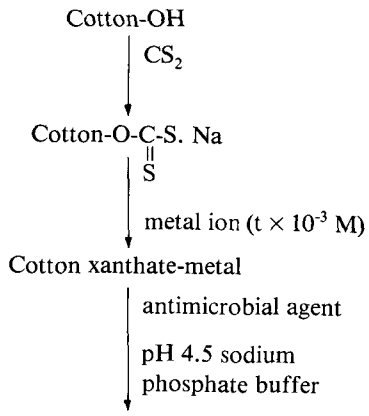
To a culture plate (diameter:90 mm and height: 15 mm) was placed 21 ml of medium I and allowed to harden to give a smooth base layer with uniform 3-4 mm depth. To this plate, 6.0 ml of inoculum was added, and the plate was tilted back and forth to spread the inoculum evenly over the surface. Ditch plates were prepared by placing four stainless steel cylinders (i.d.: 9 mm, o.d.: 100 mm) before solidifying and allowed it to harden. After solidifying, the four steel cylinders and the contents were removed to give four cylindrical ditches, to each of which 30 mg of the sample wetted with a few drops of agar solution was plated. The plate was incubated at 37 °C for 24hours and diameter of the inhibition zone was determined.

To investigate the duration of the antimicrobial activity of each sample, the content of the cylindrical ditch after 24 hours of incubation was removed and transfered to a ditch of freshly prepared plate as described above and incubated at 37 °C for 24 hours. This procedure was continuously repeated until the inhibition of growth was no longer noticed. The same procedure were followed using 0.04 mg of free drugs to compare the antimicrobial activities of free and matrix-bound drugs. For the determination of antimicrobial and antifungal activity, four plates per smaple to be tested were prepared.

RESULTS AND DISCUSSION

Formation of cotton xanthate-metal chelate

Coupling of anatimicrobial agent to cotton xanthate-metal chelate was carried in three steps as shown in Scheme 1.



Scheme 1. Coupling of antimicrobial agent to cotton xanthate-metal chelate.

Xanthation reaction was carried out adoping the method reported in the literation.[18-21] Cotton xanthate is easy to prepare and the mechanical strength did not change at the preparation conditions.

Cotton xanthate was stable as polyvalent ion complex at room temperature for one year stored.

In a series of experiments, copper-bound cotton xanthate fabrics was placed in a solution of pH 4, 6, and 9, respectively at 100 ° for 30 min. Displacement of copper ion was noticed. Even though metal bound xanthate material is stable at room temperature, bound metal ions were displaced at elevated temperature, which might be explained in terms of the instability of xanthate group at elevated temperature.

The extent of reaction occurred was estimated indirectly by measuring the metal binding capacity of the material, since unreacted cotton does not bind metal ions at all. The metal binding capacities of the cotton xanthate are listed in Table I.

The binding of metal ions to the xanthate group has been described as 1:1 and 1:2 complex.[22] However, due to the steric hindrance imposed by polymeric structure of cotton xanthate, it is believed that provability for the formation of 1:2 complex might be considerably reduced and 1:1 complex might be favored, especially so in the present experiments where cotton was treated with exessive ions.

Metal binding to the xanthated material seems to occur via covalent bond between the xanthate group and metal ion, since the bound metal ions were not displaced by the usual stripping methods, where the solution of disodium EDTA was used as stripping agents. It might be expected that some of the metal ions might simply be adsorbed without actual binding through the covalent bond. It turned out that simple adsorption of metal ion was negligible as evidenced from the experiments, where the cotton was treated with alkali in the absence of carbon disulfide.

Table I. Metal uptake per gram of cotton xanthate[a]

Metal ion	mg	mmole
Cu(II)	13.6	0.22
Zn(II)	30.4	0.46
Fe(III)	21.2	0.38
Al(III)	12.2	0.45

a) Metal binding experiments were carried in 5 × 10^{-3}M metal solution at pH 5 and room temperature.

Coupling of antimicrobial agents to cotton xanthate-metal chelate

Coupling of antimicrobial agent was carried out easily by treating metal-bound cotton xanthate with aqueous solutions of antimicrobial agents.

In the chelated form of cotton xanthate, a proportion of metal ions are coordinated with molecules or ionic species that are essentially the ligands of the complex ion. More specifically, these ligands may be water molecules or anionic species in the solution or molecules containing electron-donating groups.

This exchangeable nature of residual ligands imparts a reactivity to the derivatized cotton, and on account of the insolubility of cotton provides a matrix suitable for the immobilization of liquid soluble molecules by covalent attachment. The incoming molecules should be in aqueous solution and near-neutral pH is expected to be adequate for the coupling.

The antimicrobial agents presetly studied all possess structures which have electron-donating ability. The chelating groups in neomycin are primary and secondary hydroxy, primary amino groups, in streptomycin they are primary and secondary hydroxy, secondary amino, guanidino and aldehyde groups, in tetracycline they are hydroxy and tertially amino groups and in pyrithion they are mercaptol and tertially amino groups. These groups are effective ligands for metal ions.

Table II. Inhibitory zone diameter (mm) of cotton xanthate-metal-tetracycline[a)]

Micro-organism	Metal	Successive test period (day)				
		1	2	3	4	5
S. aureus	Free	31	26	20	-	
	Cu(II)	35	28	25	22	18
	Zn(II)	40	32	26	-	
	Fe(III)	35	30	25	-	
S. faecalis	Free	25	12	-		
	Cu(II)	28	23	-		
	Zn(II)	29	17	-		
	Fe(III)	28	22	16	-	
E. coli	Free	30	12	-		
	Cu(II)	33	23	-		
	Zn(II)	29	15	-		
	Fe(III)	18	-			
E. aerogenes	Free	22	12	-		
	Cu(II)	28	26	17	-	
	Zn(II)	28	15	-		
	Fe(III)	24	20	-		

a) Free drug, 40 μg or 30 mg of matrix bound drug was used for the test.

Table III. Inhibitory zone diameter (mm) of cotton xanthate-metal-streptomycin[a)]

Micro-organism	Metal	Successive test period (day)				
		1	2	3	4	5
S. aureus	Free	22	17	14	-	
	Cu(II)	24	16	14	-	
	Zn(II)	21	20	18	-	
	Fe(III)	20	21	18	-	
S. faecalis	Free	15	14	-		
	Cu(II)	15	16	-		
	Zn(II)	21	20	20	-	
	Fe(III)	18	20	20	16	-
E. coli	Free	21	18	12	-	
	Cu(II)	23	18	-		
	Zn(II)	22	21	-		
	Fe(III)	18	18	20	20	-
E. aerogenes	Free	22	12	12	-	
	Cu(II)	23	17	14	-	
	Zn(II)	22	17	17	14	-
	Fe(III)	16	17	16	-	

a) Free drug, 40 μg or 30 mg of matrix bound drug was used for the test.

Whereas all these groups are able to act as ligands, the relative extents to which they do so will depend upon their different abilities to donate electrons. Thus, on introduction to the cotton xanthate-metal chelate further complexation occurs in which the stronger electron donor groups of the antimicrobial agents become coordinated to the metal in preference to water molecules. In fact, the driving force of the chelating reaction in this experient is the insolubility of the cotton xanthate-metal chelate and cotton xanthate-metal-antimicrobial agent chelate, which displaces the equilibria in favor of their formation. Although chelation of cotton xanthate with various metal ions may yield the activated matrix in partially ionic form with a distribution of charges along its chain, the possibility that the antimicrobial agents may simply be bound ionically to the matrix is discounted since such a linkage to the matrix would be expected to break down during the washing with solutions of high ionic strength.

Antimicrobial activity of cotton xanthate-metal-antimicrobial agent

Antimicrobial activity of the final product was evaluated employing ditch plate method. Final product (30mg) was used for the test, and the diameter of the inhibition zone reached 20-40 mm range. To compare the activity with that of the free drug, a separate experiment was carried out using 0.04mg of free drug. To investigate the duration of the antimicrobial activity of each sample, the content of the cylinderical ditch was carefully removed and trasfered to a ditch of freshly prepared plate and incubated at 37 °C for 24 hours. This procedure was repeated every 24 hours of incubation period until the diameter of the inhibition zone was decreased within 10 mm (diameter of the ditch).

Results for cotton xanthate-metal-tetracycline, -neomycin, -streptomycin, and -pyrithion are summarized in Table II, III, IV, and V, respectively. Even though these data do not represent the results on the quantitative basis, general trend and durability of the antimicrobial activities of the material can be deduced. All of the product exhibits antimicrobial activity, which shows that antimicrobial agents have successfully bound to cotton xanthate. As expected, the antimicrobial activi-

Table IV. Inhibitory zone diameter (mm) of cotton xanthate-metal-neomycin[a)]

Micro-organism	Metal	Successive test period (day)				
		1	2	3	4	5
S. aureus	Free	16	15	14	14	14
	Cu(II)	21	16	15	-	
	Zn(II)	19	20	18	20	17
	Fe(III)	17	17	20	20	19
S. faecalis	Free	15	15	14	15	15
	Cu(II)	20	15	15	14	15
	Zn(II)	19	20	20	20	18
	Fe(III)	16	16	24	22	18
E. coli	Free	15	15	15	15	15
	Cu(II)	23	15	14	-	
	Zn(II)					
	Fe(III)	18	20	20	18	-
E. aerogenes	Free	21	15	15	15	15
	Cu(II)	25	15	14	14	14
	Zn(II)	19	20	19	17	17
	Fe(III)	17	17	22	21	19

a) Free drug, 40 mg or 30 mg of matrix bound drug was used for the test.

Table V. Inhibitory zone diameter (mm) of cotton xanthate-metal-pyrithion[a)]

Micro-organism	Metal	Successive test period (day)					
		1	2	3	4	5	6
S. aureus	Free	32	12	12	-		
	Cu(II)	25	22	20	19	20	20
	Zn(II)	24	23	22	20	-	
	Fe(III)	27	23	27	20	20	15
S. faecalis	Free	32	12	12	-		
	Cu(II)	20	18	17	17	20	18
	Zn(II)	29	27	27	23	18	-
	Fe(III)	18	20	20	16	-	
E. coli	Free	38	16	-			
	Cu(II)	23	23	22	20	22	20
	Zn(II)	32	29	30	-		
	Fe(III)	22	24	26	25	21	17
E. aerogenes	Free	35	12	12	-		
	Cu(II)	21	17	17	17	20	19
	Zn(II)	29	28	26	22	-	
	Fe(III)	21	22	23	19	22	17
A. niger	Free	27	12	-			
	Cu(II)	22	20	21	19	20	18
	Zn(II)	28	29	28	25	17	-
	Fe(III)	22	18	20	20	21	18

a) Free drug, 40 mg or 30 mg of matrix bound drug was used for the test.

ty of each sample decreased gradually on each successive test period, for both free and bound drug.

In the case of neomycin, however, it is noticed that the degree of the decrease in activity on each successive test was not significant. Since the same phenomena were observed for both free and bound drug, this can be attributed to the fact that the diffusion of the active drug across the agar medial is slow, and that the rate-controlling step is the diffusion rather than the dissociation of bound drug from the matrix.

Somewhat different situation occurs in cotton xanthate-metal-pyrithion as shown in Table V. It is noticed that the duration of the antimicrobial activity is significantly increased for bound drug compared with that of free drug. This may occur if the rate-controlling step is the dissociation of pyrithion from the matrix rather than the diffusion of active ingredient across the media.

To clarify the binding nature and the possibility of the involvement of simple adsorption on drug binding, a series of control experiments were carried out by treating cotton with the solution of metal ion and the drug successively, in the same procedure that employed in the preparation of cotton xanthate-metal-antimicrobial agent chelates.

None of the product exhibited antimicrobial activity, which proved indirectly that simply adsorbed free drugs were almost completely removed by the washing procedures in the present experiments. In a separate control experiment it showed that cotton xanthate or cotton xanthate-metal chelate themselves did not possess antimicrobial activities. Therefore, binding of the drug to the matrix could be indirectly proved by the exhibition of the antimicrobial action of the product.

It is believed that the antimicrobial activity of cotton xanthate-metal-antimicrobial agent chelate is manifested by the free, dissociated form of the drug in the agar media and the stability constant of the metal-drug chelate may play an important role in binding and releasing of the drug to and from the matrix.

CONCLUSION

The conversion of cotton fabrics to cotton xanthate-metal chelate was readily and simply achieved with little damage to the matrix, and antimicrobial agents with electron-donating ligands could successfully be coupled to the chelate. The products exhibited antimicrobial activities. The technique provide a novel form of sterility for sheets and other cotton-based fabrics and gauze, and for treating athlete's foot and infected root canals in teeth before root filling.

Cotton xanthate-metal-antimicrobial agent chelate possessed good storage characteristics showing no observable loss of activity on storage at room temperature at least for 6 months.

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