Dyeing of Wool with Antibiotics to Develop Novel Infection Resistance Materials for Extracorporeal End Use

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ABSTRACT: Two antibiotics, doxycycline (Doxy) and ciprofloxacin (Cipro), were applied under a variety of conditions to wool and to hydrolyzed wool at 40°C. Nylon was used as a synthetic control. Sorption of Doxy was much higher in wool than in nylon, whereas sorption of Cipro was similar in both fibers. FTIR spectroscopy confirmed that a drastic increase in sorption of antibiotics by hydrolyzed wool was attributed to an increase in polar functional groups by peptide scission and in oxidized sulfur groups by cystine oxidation. Both sorption and zone of inhibition (ZOI) values were improved by hydrolysis of wool. Wool hydrolyzed for 20 or 40 min at 40°C and dyed with Doxy at 45°C for 3.5 h maintained around 30 mm of ZOI after 24 h of challenge by a simulated flow of blood. Wool hydrolyzed for 60 min at 40°C and dyed with Cipro at 45°C for 3.5 h also maintained its antibiotic activity for an extended time. For the most part, ZOI values for nylon dyed by both antibiotics were zero within 24 h. This technique produced infection-resistant biomaterials of potential use in extra-corporeal biomedical and biological applications. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 92: 3343–3354, 2004

Key words: wool; doxycycline; ciprofloxacin; biomaterials; absorption; FTIR

INTRODUCTION

Textile and other polymeric materials are ubiquitous in biomedical devices, whether used in implanted (e.g., prosthetic arterial grafts, prothetic valve sewing cuffs), percutaneous (e.g., catheters, sutures), or extracorporeal (e.g., wound dressings, bypass pump tubing) situations.[1] In general, significantly better implanted materials have been produced, but progress in external applications has been limited.[2]

Natural protein materials such as silk and collagen have been used as suture materials for centuries.[3,4] However, as far as we are aware, wool fiber has not been used for any biomedical applications in spite of its similar generic composition. Lack of interest in wool as biomedical material has probably been because of its short fiber length, low strength, and high price. Nevertheless, wool is one of the most absorbent fibers available[5] and can be easily made into any of the usual textile structural forms suitable for extracorporeal applications. Its amino acid composition renders major toxicity, tissue reaction (cellular response), and blood compatibility problems less likely, providing all extraneous matter is removed with a careful scouring process. Furthermore, although wool is still one of the most important natural protein fibers for apparel, its importance in the apparel market is continuously decreasing. Finding alternative uses for wool, therefore, is important for wool producers and the textile industry.

Regardless of their intended application, biomedical materials are prone to a number of potentially catastrophic problems, one of which is bacterial infection.[6–10] Our group has previously examined several techniques for producing infection-resistant vascular graft materials without involving any exogenous additives.[6, 11–15] These studies indicated that the presence of polar functional groups in substrate and antibiotic such as carboxylic and amine groups facilitated uptake of antibiotics by a mechanism akin to that of textile dyes.[11–15] Considering the abundant nature of the polar functional groups in wool, it is logical to examine its sorption of antibiotics, although no prior study has been carried out in this area.

In addition to conventional biomedical applications, there are ever-growing concerns of exposure to bacteria associated with biological warfare, bioterrorism, and the spread of contagious diseases such as severe acute respiratory syndrome (SARS). These concerns also prompt the development of novel infection-resistant biomedical materials for personal protective gear such as masks, gowns, filter for gas masks, socks, gloves, and the like.

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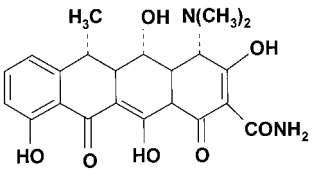
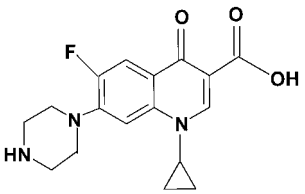


Doxycycline



Ciprofloxacin

Figure 1 Chemical structure of doxycycline and ciprofloxacin.

The current study therefore examines the feasibility of developing infection-resistant wool for external biomedical and biological end uses using a "dyelike" interaction of antibiotics that does not involve any exogenous additives. The two antibiotics selected were doxycycline (Doxy) and ciprofloxacin (Cipro), which are highly active against Gram-negative and Gram-positive bacteria and are the only antibiotics approved for treatment of anthrax (*Bacillus anthracis*) infection by the Centers for Disease Control and Prevention (Atlanta, GA).[16] Doxy is a tetracycline material,[17] whereas Cipro is a fluoroquinolone.[18] The chemical structures are shown in Figure 1. Interaction between antibiotics and wool was studied to examine the relationship between extent of uptake and subsequent release (and concomitant antibacterial activity) to produce novel infection-resistant biomaterials. The sorption of antibiotics on nylon was also examined as a comparison purpose.

EXPERIMENTAL

Materials

Wool garbadine (Type 541, fabric weight 187 g/m^2) and nylon taffeta (Type 306A, fabric weight 59 g/m^2) were purchased from Test Fabrics, Inc. (West Pittston, PA). Doxy and Cipro were donated by Pfizer and Serological Products (Bayer-made), respectively, in pure form and used as supplied. Other chemicals, such as sodium hydroxide, glacial acetic acid, ammonium hydroxide, and methylene blue, were all purchased from Aldrich Chemicals (Milwaukee, WI) in reagent grade.

Dyeing of fibers with Doxy and Cipro

Dyeings were carried out in an Ahiba Polymat (Datacolor International, Lawrenceville, NJ) dyeing machine, in which 2% on the weight of fabric (owf) antibiotic was applied at a liquor-to-fabric ratio of 20 : 1. Experimental parameters were dyeing temperature, time, and dyebath pH. The bath pH values used were 2, 6.5, and 9 for Doxy and 3, 5.5, and 10 for Cipro: the lowest pH in each case was the initial pH of the dyebath containing the antibiotic. Other pH values were controlled by addition of 1% NaOH and 1% acetic acid, monitored using a Corning pH meter 115 (Corning Laboratory Sciences, Corning, NY). Dyeing temperatures and times were 25, 45, 65, 85, and 100°C for 1, 2, and, 3.5 h. After the process was run, the fabric was removed and the amount of antibiotic taken up by the substrate was determined (see analyses below)

To investigate the effect of hydrolysis on sorption of antibiotics, the wool was treated in 1% NaOH for 20, 40, and 60 min at 40°C with 20 : 1 liquor ratio in the Ahiba dyeing machine. Nylon was also hydrolyzed by NaOH for 1, 2, 3, and 4 h at 85°C. After hydrolysis, the tensile strength of the treated fabric was measured using a Q-test (MTS Systems Corp. Eden Prairie, MN) CRE (constant rate of extension) instrument, according to ASTM D5035-95 (raveled strip) with a crosshead speed of 200 mm/min and gauge length of 76.2 mm.

Analyses

To determine the sorption of antibiotics by wool and nylon, the concentration of residual antibiotic in the dyebath after dyeing was measured using a Cary 50 UV–vis spectrophotometer (Varian Instruments, Palo Alto, CA). The λ_{max} values of Doxy and Cipro were determined as 274 and 276 nm, respectively. The relationships between absorbance and concentration were established at λ_{max} for each antibiotic. No pH adjustment was carried out for Doxy dyebaths after dyeing because the absorbance at 274 nm and solubility of Doxy were quite consistent at different pH values. However, Cipro is insoluble at higher pH values, so the pH of the Cipro dyebath after dyeing was adjusted to pH 3 with acetic acid. The residual baths were also immersed in a water bath at 85°C for 15 min before dilution to ensure complete dissolution of Cipro for the absorbance measurement. The amount of antibiotic taken up by the fiber was determined as the "percentage exhaustion," calculated as follows:

$$\text{Exhaustion (\%)} = [(C_0 - C_r)/C_0] \times 100$$

where C_0 is the concentration of antibiotic in blank solution and C_r is the residual antibiotic concentration of the dyebath containing the substrate after dyeing.[19]

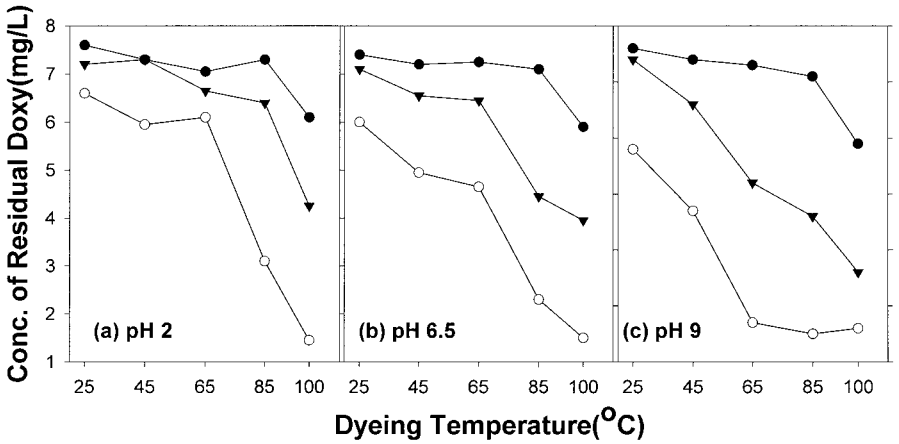


Figure 2 Effect of dyeing temperature on sorption of Doxy on wool and nylon: (a) pH 2, (b) pH 6.5, (c) pH 9. ●: blank; ○: wool; ▼: nylon.

The CIE [Commission Internationale de l'Eclairage (International Commission on Illumination)] lightness (L*) values of Doxy-dyed fabrics were evaluated with a Macbeth ColorEye system along with SLI-Form®/NG software (She Lyn Inc., New Windsor, NY). The infrared spectra of the untreated and hydrolyzed wool and nylon were obtained with a Sense FTIR spectroscope (SensIR Technologies, Danbury, CT) with an attached diamond ATR in the spectral region of 4000–700 cm^{-1} with 54 scans at 4 cm^{-1} resolution.

To quantify anionic groups, hydrolyzed nylon fabrics were dyed with 0.25% owf methylene blue at 50°C for 60 min with 50 : 1 liquor ratio in an Ahiba dyeing machine. Ammonium hydroxide was used to adjust the dye bath to pH 9.5. The free carboxylic acid groups were quantified using the following equation:

$$\text{Carboxylic group density} = (Q/M)/W$$

where Q represents the amount of dye taken up, M is the molecular weight of methylene blue, and W is the weight of the fabric.[12]

The infection-resistant properties of the treated substrates were evaluated by a zone of inhibition (ZOI) test and release of antibiotics from the substrates as described previously.[19]

RESULTS AND DISCUSSION

Dyeing of wool and nylon with Doxy

The effects of dyeing temperature on the sorption of Doxy on wool and nylon fabrics at three pH levels are shown in Figure 2. The concentration of residual Doxy in both wool and nylon dyebaths decreased (i.e., sorption increased) with increase in dyeing temperature at all pH levels. The trend in this residual concentration decrease for both fabrics was similar: the decrease was slow up to 65°C and much faster at 85 and 100°C. This may be attributed to a glass-transition effect in the nylon, and the need for elevated temperatures on wool to facilitate the penetration of the surface cuticle structure. The Doxy sorption at pH 9, shown in Figure 2(c), was quite different for both fabrics from those at other pH values. In dyeing at low temperatures the slope of the decrease was much steeper at pH 9 than that at other pH levels. The lowest residual concentration of Doxy in the bath after dyeing (highest sorption) was shown at 100°C. A certain fraction of this decrease was attributed to decomposition of Doxy, indicated by a decrease in the residual concentration of the blank dyebath at 100°C.

Sorption of Doxy was always higher on wool than on nylon, regardless of pH and dyeing temperature, mainly because of a combined effect of a lower crystallinity and greater abundance of polar functional groups than nylon. Wool is regarded as a highly amorphous polymer with little crystallinity because of its complex amino acid compositions.[5] Sorption of Doxy on nylon increased considerably at 85 or 100°C but its value was still much lower than that of wool.

Percentage exhaustions calculated against the blank solution are tabulated in Table I. Doxy exhaustions in the wool dyebath increased considerably with an increase in temperature and generally were twice as much as those in nylon at the same dyeing condition. It is worth noting that sorption of Doxy on wool at pH 9 and 65°C was comparable to the values at 85°C for other pH values. Therefore, this condition could be used for applying Doxy to wool to minimize its decomposition under high-temperature application. In addition, at high temperature the change in % exhaustion with pH was much less than that at low temperature.

Because both wool and Doxy are amphoteric in nature, sorption of Doxy on wool is expected to be

TABLE I
Percentage Exhaustion of Doxycycline on Wool and Nylon

Material	Bath pH	Dyeing temperature and time (°C/h)						
		25/3.5	45/3.5	65/3.5	85/1	85/2	85/3.5	100/3.5
Wool	2	13.2	18.5	13.5	18.6	32.6	57.5	76.2
	6.5	18.9	31.3	35.9	27.1	43.7	67.6	74.6
	9	23.7	36.5	63.0	29.9	38.4	64.8	55.0
Nylon	2	5.3	0	5.7	—	—	12.3	30.3
	6.5	4.1	9.0	11.0	—	—	37.3	33.1
	9	2.6	10.8	28.8	—	—	35.2	39.0

largely dependent on the bath pH. The isoelectric points of wool and Doxy are approximately 5.[5,20] At low dyeing temperatures (45 and 65°C), the exhaustion of Doxy was higher at pH 6.5 and much higher at pH 9, as listed in Table I. This pH effect was much less significant at 85 and 100°C. This implied that an electrostatic repulsion between the positively charged substrate and antibiotic played an important role on Doxy sorption at low temperature and acidic pH. The positive charges derive from easy protonation of basic nitrogens in wool and Doxy in acidic pH. On the other hand, wool was negatively charged at pH 9, whereas the same charge was less likely to occur in Doxy because the ionization of oxygen in hydroxyl group would be much more difficult to achieve (Fig. 1), resulting in high exhaustion at high pH. However, high-temperature dyeing could minimize the effect of pH on exhaustion. Nylon showed a similar trend, but the high exhaustion at pH 9 appeared up to 85°C, probably because of its higher glass-transition temperature than that of wool.

Pure Doxy is a yellowish powder. Aqueous solutions of Doxy tended to darken on standing. It has been found that the rate at which solutions darkened increased with simultaneous increase in temperature, pH, and duration of preparation.[19] The color change of aqueous solution was associated with a change that occurred in the region of a second absorption maximum peak (~ 345 nm).[19] Nevertheless, absorbance at the first absorption maximum (274 nm) was relatively consistent with such variations, with the exception of high-temperature application such as 100°C for 3.5 h, which was interpreted to mean that Doxy is stable under these conditions, despite the dark color produced.

Doxy "dyed" substrates showed a light to dark brown color. CIE lightness (L*) values for pristine wool and nylon are 82.9 and 78.8, respectively, and the lightness of Doxy-dyed fabric was normalized against that of pristine fabric. Normalized lightness of the Doxy-dyed continuously decreased with increase in the treatment temperature for both wool and nylon at all three pH values, as illustrated in Figure 3. The range of colors produced at different temperatures was readily apparent by simple visual analysis.

To examine the effect of dyeing time on Doxy sorption, wool was dyed by Doxy at 85°C, where consistent residual concentrations of blank dyebaths showed that Doxy was stable (Fig. 4). A continued decrease in residual Doxy concentration of the dyebath indicated that a long dyeing time would allow for increased concentrations of Doxy on wool. Alternatively, the initial applied concentration could also be increased: the two different strategies would likely result in a different distribution of the same amount of Doxy within the wool, and possibly a different pattern of subsequent release. Again, sorption difference was not substantial at different pH values in the 85°C dyeing, as previously shown in Figure 2 and Table I.

Dyeing of wool and nylon with Cipro

As illustrated in Figure 5, the residual concentration of Cipro in the blank dyebath was consistent over various dyeing temperatures, indicating the better thermal stability of Cipro than that of Doxy. Cipro is known as a highly thermally stable antibiotic.[21] In addition, there was no observable color change among Cipro-dyed substrates.

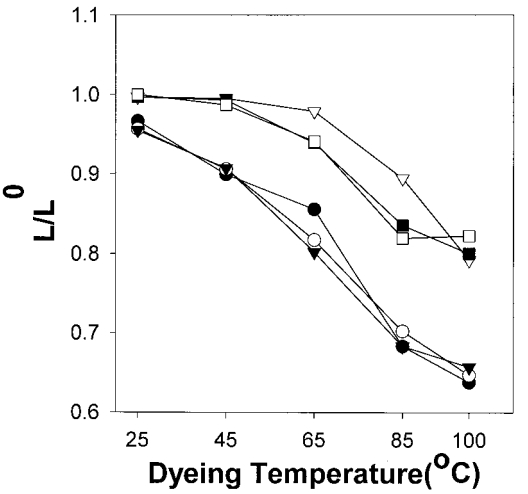


Figure 3 Effect of dyeing temperature on normalized lightness of Doxy-dyed wool and nylon: ●: wool 2; ○: wool 6.5; ▼: wool 9; ▽: nylon 2; ■: nylon 6.5; □: nylon 9.

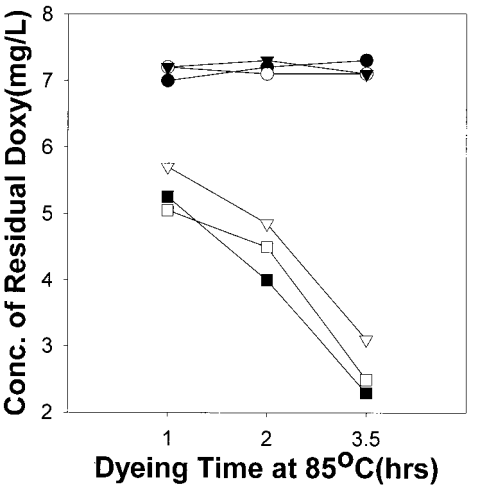


Figure 4 Effect of dyeing time on sorption of Doxy on wool: ●: blank 2; ○: blank 6.5; ▼: blank 9; ▽: wool 2; ■: wool 6.5; □: wool 9.

An increase in dyeing temperature tended to facilitate sorption of Cipro onto the substrates, but the effect was much smaller than that of Doxy, as shown in Figure 5 and Table II. Low residual concentrations (or high %exhaustion) of Cipro in dyebaths at 45°C with pH values of 5.5 and 10, as illustrated in Figure 5(b) and (c), were attributed to the low aqueous solubility of Cipro at high pH values and low temperature. At 45°C Cipro was soluble only in water at pH 3 among the three pH levels. At the concentrations used here, Cipro readily formed precipitates at pH values of 5.5 and 10 during dyebath preparation. Therefore, undissolved, precipitated Cipro was physically trapped within the fabric structure, resulting in low measured concentration of residual Cipro in the bath.

Contrarily, at 85 and 100°C dyeing, where Cipro was completely soluble in water at all pH values,

TABLE II
Percentage Exhaustion of Ciprofloxacin on Wool and Nylon

Material	Bath pH	Dyeing temperature (°C) for 3.5 h			
		45	65	85	100
Wool	3	0.0	11.8	20.9	18.3
	5.5	52.8	8.7	21.8	20.0
	10	15.6	5.4	35.5	40.6
Nylon	3	17.1	6.9	9.7	8.8
	5.5	51.4	3.1	17.8	12.6
	10	32.6	2.4	36.5	42.3

sorption of Cipro on both wool and nylon was higher at pH 10. Sorption of Cipro at pH 3, however, did not significantly vary at different dyeing temperatures and the exhaustion of Cipro on wool was only 18.3%, even at 100°C (Table II). Low sorption of Cipro at pH 3 was again mainly attributed to electronic repulsion produced by the positively charged substrate and Cipro. Electronic repulsion between the negatively charged substrate and Cipro was less significant at pH 10 and high temperature, resulting in higher sorption. The only plausible explanation for this was that at high temperature and pH 10 an ionic repulsion may not be important as it is at low temperature, resulting in higher sorption of Cipro onto wool or nylon than that at pH 3. The same phenomena were also observed in our previous studies on silk[19] and polyurethane film.[6]

It should be also noted that at pH values of 5.5 and 10, wool and nylon showed very similar pH effects, but their apparent sorption of Cipro was the same, which indicated that nylon was dyeable as much as wool by Cipro at high temperature and pH. Producing blend fabrics containing wool and nylon therefore could also be considered if the end use requires high strength. Cipro is fluorescent, and visual observation

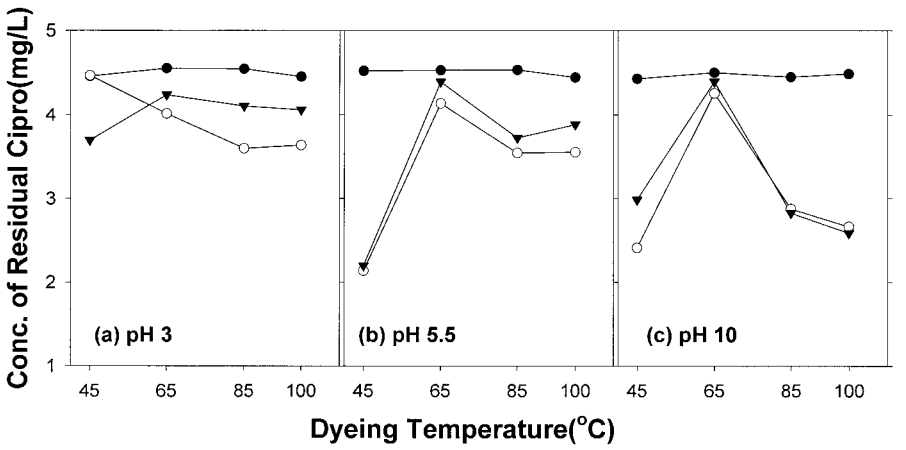


Figure 5 Effect of dyeing temperature on sorption of Cipro on wool and nylon: (a) pH 3, (b) pH 5.5, (c) pH 10. ●: blank; ○: wool; ▼: nylon.

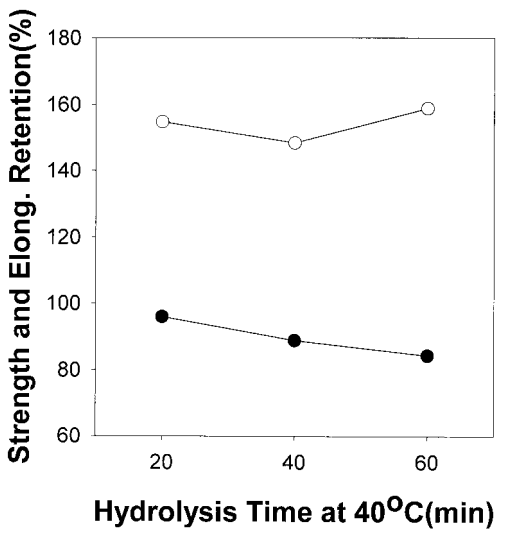


Figure 6 Retention of strength and elongation of hydrolyzed wool fabrics: ●: retention of peak load (%), ○: retention of elongation (%).

of the Cipro-dyed substrates under UV revealed that Cipro in wool was diffused into the interior of fiber, whereas on nylon Cipro appeared confined to the surface. The disparity in location of antibiotics within the fiber would eventually result in different behavior in antibiotic release during end use.

Dyeing of antibiotics on hydrolyzed substrates

To investigate the effect of hydrolysis on sorption of antibiotics by wool, we hydrolyzed wool at 40°C for different times. The weight loss of wool increased with increase in time of hydrolysis: 4.2, 4.7, and 5.7% for 20, 40, and 60 min, respectively. Figure 6 shows fabric strength retentions and elongation after these treatments. The fabric strength decreased only slightly: the most severe hydrolysis (60 min) reduced strength by less than 20%. At the same time the elongation of hydrolyzed fabric increased considerably. Therefore, highly extensible, compact wool structure, resembling "parchment," can be obtained by a mild hydrolysis such as 20 min at 40°C. Such modified wool could be very useful in producing biomaterials for medical applications such as a wound dressing that requires high extensibility.

The hydrolysis of wool considerably increased the sorption of both Doxy and Cipro, as listed in Table III. Surprisingly, the effect of hydrolysis was greater for both antibiotics under low-temperature dyeing conditions, making the process more attractive to use. Sorption of antibiotics generally increased with an increase in hydrolysis time, but the increase was most substantial with the first 20 min of hydrolysis. Dyeing at 45°C, wool hydrolyzed for 60 min at 40°C showed 89% exhaustion of Doxy and 85.2% of Cipro. These high exhaustions could be extremely beneficial given the high cost of antibiotics. The treated material, however, must be judged on its effectiveness in infection resistance, which involves considerably more than just uptake (see below).

We also hydrolyzed nylon at 85°C for different times. The weight loss on hydrolysis was low (<2% at 4 h) and change in strength was also minimal. However, the carboxylic group content of hydrolyzed nylon as determined by methylene blue uptake increased from 37.2 μmol/g of fabric for pristine nylon to 42.8, 43.6, 43.6, and 43.7 μmol/g for nylons hydrolyzed at 1, 2, 3, and 4 h, respectively. Therefore, the percentage increase in methylene blue uptake of nylon hydrolyzed at the most severe condition was merely 17.4%. This suggested that hydrolysis caused some cleavage of polyamide chains but the level of chain scission might not be very substantial. As shown in Table III, the sorption of Doxy was slightly greater on hydro-

TABLE III
Dyeing of Antibiotics on Hydrolyzed Wool and Nylon[a]

Material	Antibiotic	Dyeing temperature (°C)	Residual concentration at different hydrolysis times (μg/mL)				
			0	20 min	40 min	60 min	
Wool	Doxy	85	3.1 (57.5)	2.7 (63.0)	2.5 (65.8)	2.1 (71.2)	
		65	6.1 (13.5)	3.1 (56.0)	2.5 (64.5)	1.2 (83.0)	
		45	6.0 (17.8)	1.9 (74.0)	1.6 (78.1)	0.8 (89.0)	
	Cipro	85	3.597 (20.9)	2.190 (51.8)	2.188 (51.9)	1.812 (60.1)	
		65	4.013 (13.4)	3.094 (32.0)	2.219 (51.2)	1.369 (69.9)	
		45	4.467 (0.0)	1.876 (57.9)	1.082 (75.7)	0.658 (85.2)	
			0	1 h	2 h	3 h	4 h
Nylon	Doxy	85	6.4 (12.3)	5.8 (20.5)	5.5 (24.7)	5.4 (26.0)	5.4 (26.0)
	Cipro	85	4.102 (9.7)	2.586 (43.1)	2.578 (43.3)	2.60 (42.8)	2.595 (42.9)

[a] Hydrolysis of wool and nylon was carried out at 40 and 85°C, respectively. The substrates were dyed for 3.5 h by Doxy and Cipro at pH 2 and 3, respectively. The values in parenthesis represent %exhaustion against the blank dyebath.

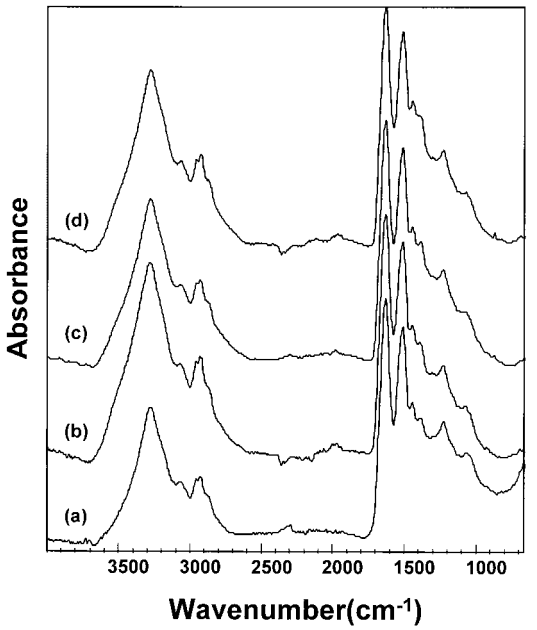


Figure 7 FTIR spectra of hydrolyzed wool: (a) pristine wool, (b) 20 min, (c) 40 min, (d) 60 min.

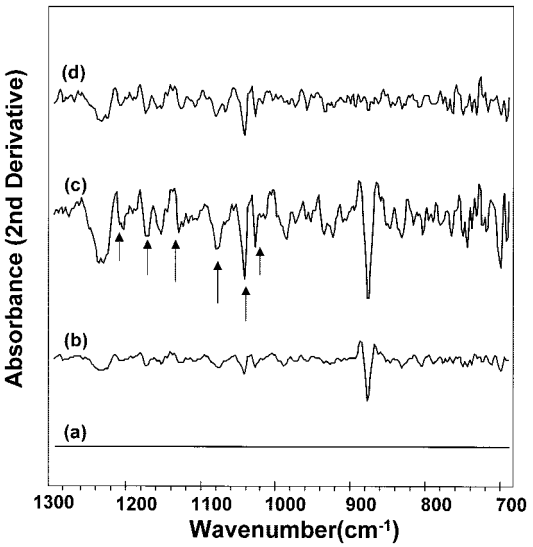


Figure 8 Second derivative spectra of hydrolyzed wool: (a) pristine wool, (b) 20 min, (c) 40 min, (d) 60 min.

lyzed nylon, whereas sorption of Cipro was substantially increased by hydrolysis for 1 h and then leveled off. Nevertheless, sorption of Cipro was always lower in hydrolyzed nylon than that in hydrolyzed wool.

FTIR analyses

FTIR spectroscopic analysis was used to investigate the effect of hydrolysis on the structure of wool and its subsequent sorption of antibiotics. As a typical polypeptide fiber, wool showed major absorption bands at 3281 cm^{-1} (N–H stretch), 1636 cm^{-1} (amide I), 1517 cm^{-1} (amide II), and 1235 cm^{-1} (amide III), as illustrated in Figure 7.[22,23] Hydrolysis of the peptide linkages produces free carboxyl and amino groups, reflected in the increased height of the corresponding absorption bands, as shown in Table IV. However, the increase generally reached maximum at 40 min hydrolysis. At 60 min hydrolysis, the height of absorption peaks decreased considerably, to even less than that of pristine wool, indicating severe chain cleavage at this condition and, consequently, loss of polymer chains.

The second-derivative technique was used to investigate these chemical changes in more detail with better resolution.[24] The spectral range was limited to 1200–1000 cm^{-1}, where the most important differences could be associated with the sulfur–oxygen bands. As shown in Figure 8, the peaks attributed to sulfur–oxygen groups such as 1201 and 1025 cm^{-1} for cysteine-*S*-sulfonate (Bunte salt), 1170 and 1040 cm^{-1} for cysteic acid, 1126 cm^{-1} for cystine-*S*-dioxide, 1077 cm^{-1} for cystine-*S*-monoxide, and 875 cm^{-1} for –S–O

TABLE IV
Peak Heights of FTIR Absorption of Hydrolyzed Wool ($\times 10^{-3}$)[a]

Peak (cm^{-1})	Peak assignment	Hydrolysis time at 40°C (min)			
		0	20	40	60
3281	NH stretch	5.60	9.92	9.73	6.35
3074	Amide A (NH stretch + amide II overtone)	0.33	0.52	0.55	0.39
2955	–CH stretch	0.25	0.40	0.38	0.31
2928	–CH stretch	0.53	1.12	0.93	0.74
1636	Amide I	9.99	13.69	14.45	7.92
1517	Amide II	5.44	7.25	8.05	4.47
1449	CH_2 deformation	0.63	0.89	0.94	0.52
1396	CH_3 deformation	0.36	0.83	0.85	0.39
1235	Amide III	1.17	1.55	1.60	0.93
1079	CH_2 deformation	0.35	0.45	0.45	0.29
875	Sulfinic acid	0.01	0.52	0.34	0.02

[a]From Refs. 22–26.

TABLE V
Peak Heights of FTIR Absorption of Hydrolyzed Nylon ($\times 10^{-3}$)[a]

Peak (cm^{-1})	Peak assignment	Hydrolysis time at 85°C (h)				
		0	1	2	3	4
3295	NH stretch	22.95	32.59	36.61	31.54	32.96
3065	Amide A (NH stretch + amide II overtone)	2.49	3.27	3.78	3.18	3.27
2931	–CH stretch	13.24	17.07	18.79	16.31	16.62
2861	–CH stretch	6.09	8.39	9.26	8.16	8.19
1631	Amide I	22.01	32.63	35.98	31.01	32.28
1531	Amide II	17.20	22.16	24.98	22.27	23.23
1468	CH_2 deformation (scissoring)	2.50	3.37	3.74	3.30	3.44
1417	CH_2 deformation	1.93	3.94	4.27	3.78	3.77
1368	Amide III + CH_3 end-group deformation	2.68	3.24	3.55	3.16	3.31
1269	Amide III	4.85	5.62	6.22	5.47	5.83
1194	Amide III, crystalline band	3.41	3.94	4.41	3.96	4.19
1143	CH_2 deformation	0.69	0.90	0.97	0.85	0.80
932	–C–CO stretch "crystallinity band"	2.13	2.73	3.06	2.75	2.88
727	Amide V (out-of-plane of secondary amide)	0.65	1.07	1.17	1.06	1.07

[a] From Refs. 22, 23, and 28–30.

group in sulfinic acid,[24–26] became more intense in hydrolyzed wool than in pristine wool. The increase in peak height attributed to sulfinic acid was also shown in regular FTIR spectra (Table IV). This confirmed that cystine molecules were attacked during the hydrolysis, generating various oxidized sulfur groups. The same effect i.e., maximum intensity of the peaks for sulfur–oxygen groups in wool hydrolyzed for 40 min was observed here. It is known that with diluted sodium hydroxide, up to 50% of cystine is readily attacked, but only a little of the remaining 50% reacts even after prolonged treatments.[5] The increase in polar functional groups by peptide scission and in oxidized sulfur groups by oxidized cystine therefore substantially facilitated sorption of antibiotics onto the hydrolyzed wool. The generation of strong acidic groups such as sulfonate ($-SO_3^-$) could particularly enhance the electronic attraction between wool and positively charged antibiotics at acidic pH.[27] An increase in acid sorption was also previously reported in the hydrolyzed wool.[5]

Hydrolyzed nylon fabrics were also analyzed by using FTIR, and heights of the absorption peak are tabulated in Table V. Major amide absorption peaks in nylon, such as 1631 cm^{-1} for amide 1, 1531 cm^{-1} for amide II, 1269 cm^{-1} for amide III, and 727 cm^{-1} for amide V, tended to increase their heights with hydrolysis.[28–30] This could explain an increase in the sorption of antibiotics onto hydrolyzed nylon. Nevertheless, high thermal stability and crystallinity of nylon limited chemical and conformational change and the associated increase in peak height was much less than that found in wool.

Infection-resistant characteristics of antibiotic-dyed substrates

Sustained release of antibiotics from infection-resistant biomaterials is very important in implanted or

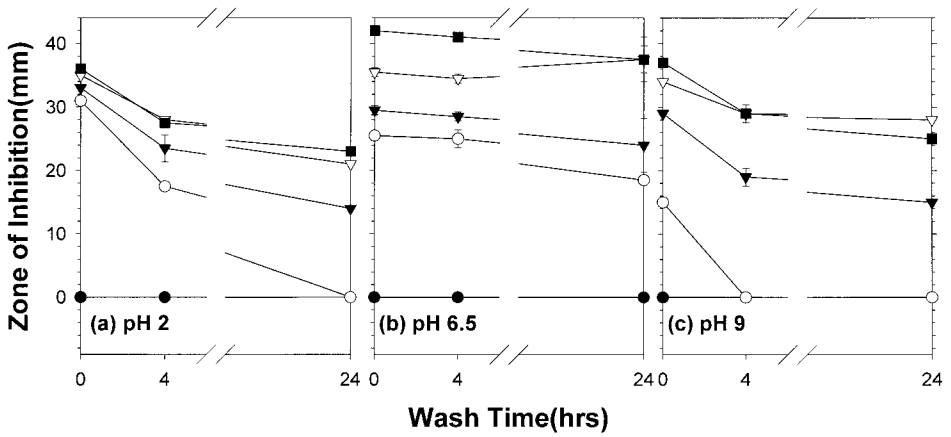


Figure 9 Zone of inhibition of Doxy-dyed wool at different temperatures and three pH levels: (a) pH 2, (b) pH 6.5, (c) pH 9. ●: control; ○: 100°C; ▼: 85°C; ▽: 65°C; ■: 45°C.

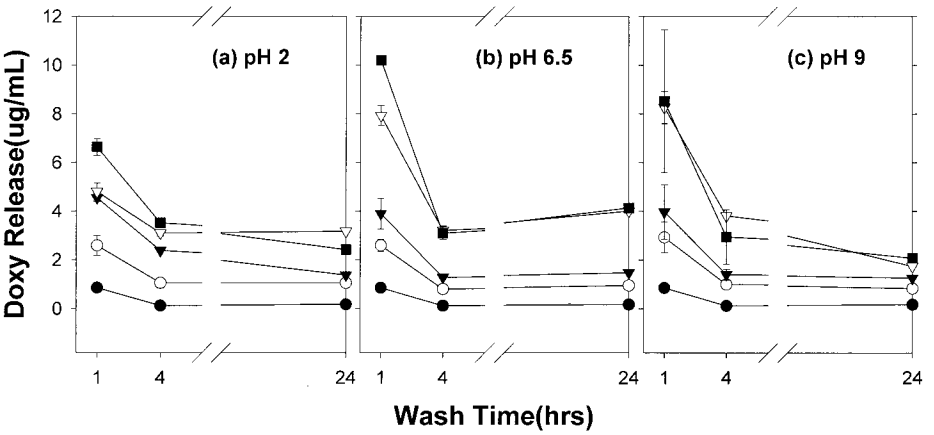


Figure 10 Release of Doxy from wool: (a) pH 2, (b) pH 6.5, (c) pH 9. ●: control; ○: 100°C; ▼: 85°C; ▽: 65°C; ■: 45°C.

percutaneous devices, but also in extracorporeal situations. Conventional dipping of the biomedical material into antibiotic has proven not to be completely effective because of the excessively fast release of the agent.[31] Efficacy of antibiotic-dyed substrates can be analyzed by use of the zone of inhibition (ZOI) test.

Figure 9 shows ZOI values for wool fabric dyed with Doxy at four different temperatures and three pH values. Interestingly, wool fabrics dyed by Doxy at lower temperatures, such as 45 and 65°C, showed consistently greater ZOI than that of fabrics dyed at high temperatures. The fabric dyed at 100°C was the least effective in ZOI at all three pH levels used. ZOI of wool dyed with Doxy at three pH levels and 45 or 65°C was in the order of pH 6.5 > pH 9 > pH 2. The order of ZOI values was only partially correlated to the sorption of Doxy previously shown in Figure 2. This result again confirmed that high sorption of antibiotic is important, but the antibiotics sorbed into the fiber must have an adequate fastness so that the antibiotic can be released in a sustained manner over a long period of time: alternatively, an antibiotic that is too firmly attached to the substrate may not be released at all (although if the biomaterial is used long term and degrades, a high concentration of antibiotic may still be ultimately useful).

Measurement of Doxy release from wool shown in Figure 10 directly corresponds to the ZOI values in Figure 9. The data also indicated that low temperature dyed wool resulted in better sustained release of Doxy than high temperature dyed wool. Low release of the antibiotic at wool dyed at high temperature was attributed to greater penetration of Doxy into the wool at high temperature, making Doxy difficult to release from the fiber interior.

Figures 11 and 12 show ZOI values and release of antibiotics from nylon. With few exceptions, ZOI values for nylon dyed by Doxy and Cipro were zero within 24 h of wash time. Doxy release from nylon in Figure 12 was also much lower than that from wool in Figure 10. The sorption of antibiotics on nylon may be comparable to that on wool, but the resulting infec-

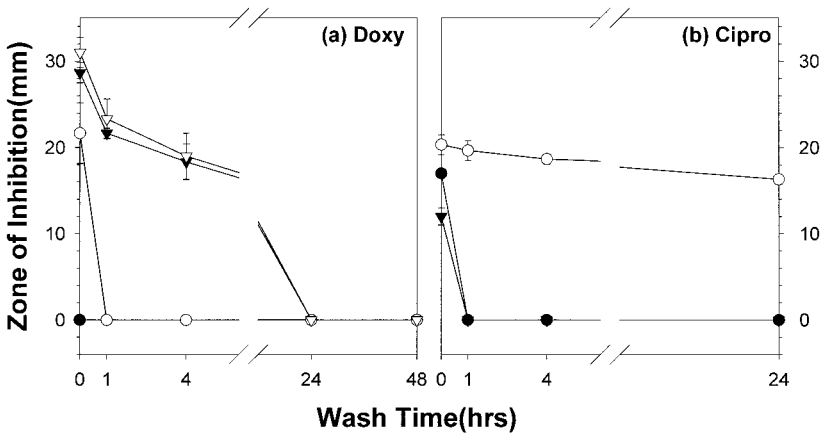


Figure 11 Zone of inhibition of nylon dyed with Doxy and Cipro: (a) Doxy, ●: control, ○: 100°C, ▼: 65°C, ▽: 45°C; (b) Cipro, ●: pH 3, ○: pH 5.5, ▼: pH 10.

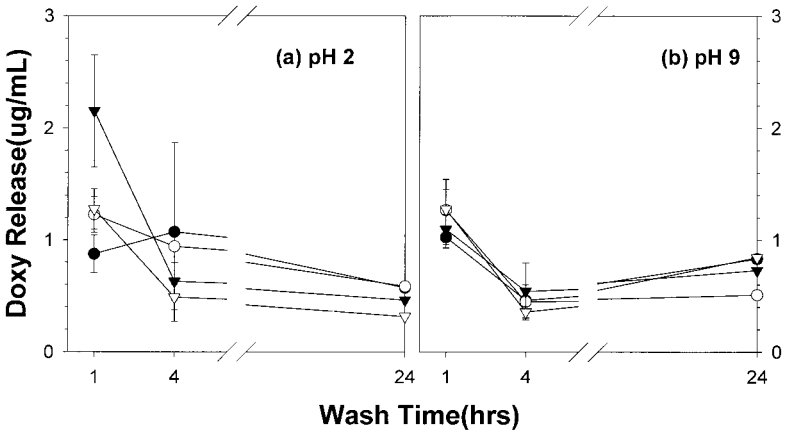


Figure 12 Release of Doxy from nylon: (a) pH 2, (b) pH 9. ●: 100°C; ○: 85°C; ▼: 65°C; ▽: 45°C.

tion-resistant properties were much lower than those of wool, and did not provide as sustained release.

A greater ZOI of wool dyed at lower temperature was also shown in hydrolyzed wool treated with antibiotics at pH 2. Figure 13 shows that ZOI was consistently greater with wool dyed at 45°C than wool dyed at 65 or 85°C at all hydrolysis conditions. This reinforces the previous postulation of strong bonding (or deeper penetration) at higher temperature described above. Regardless of wash times, ZOI was greater with wool hydrolyzed at 20 and 40 min than at 60 min, indicating the efficacy of short hydrolysis times (Fig. 14). Furthermore, comparison of Figures 9(a) and 13 revealed that at pH 2, the ZOI of hydrolyzed wool dyed with Doxy was greater than that of unhydrolyzed wool at 24 h of wash time. Given that in the ZOI test the greater ZOI at longer wash time corresponds to better sustained release, hydrolysis of wool not only improved sorption of Doxy, but also enhanced its sustainable infection-resistance property.

CONCLUSIONS

To produce infection-resistant biomaterials for potential use in extracorporeal applications, wool fabric was "dyed" with two common antibiotics, Doxy and Cipro, under a variety of temperatures, times, and pH values. Nylon was used as a synthetic control.

Antibiotic sorption, measured by residual concentration and %exhaustion, increased on both wool and nylon with increase in dyeing temperature at all pH levels, but the effect was much smaller in Cipro than in Doxy. Increase of Doxy sorption at temperatures up to 65°C was generally slow but much faster at 85 and 100°C. Unexpectedly, low residual concentrations of Cipro at 45°C with pH values of 5.5 and 10 were attributed to low aqueous solubility of Cipro at these conditions. Sorption of Doxy was much higher on wool than on nylon, whereas sorption of Cipro was similar on both wool and nylon at high temperature and pH. Continuous decrease in residual Doxy in the

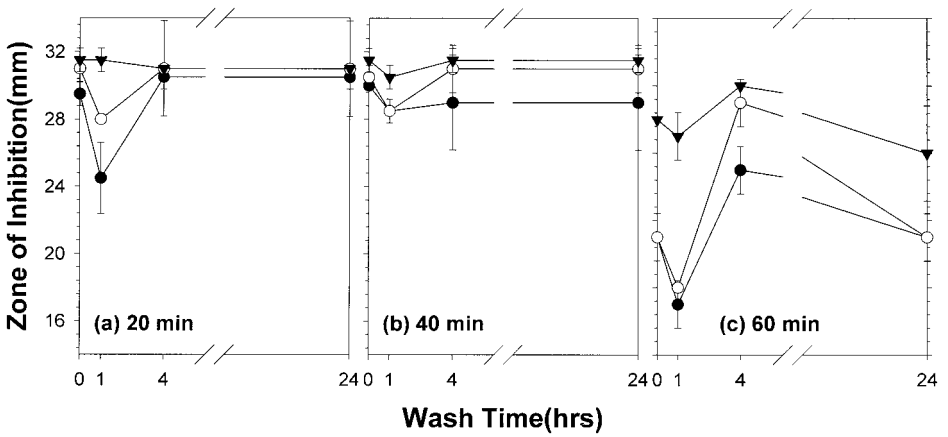


Figure 13 Zone of inhibition of hydrolyzed wool dyed with Doxy at pH 2 for 3.5 h: (a) 20 min hydrolysis, (b) 40 min, (c) 60 min. ●: 85°C; ○: 65°C; ▼: 45°C.

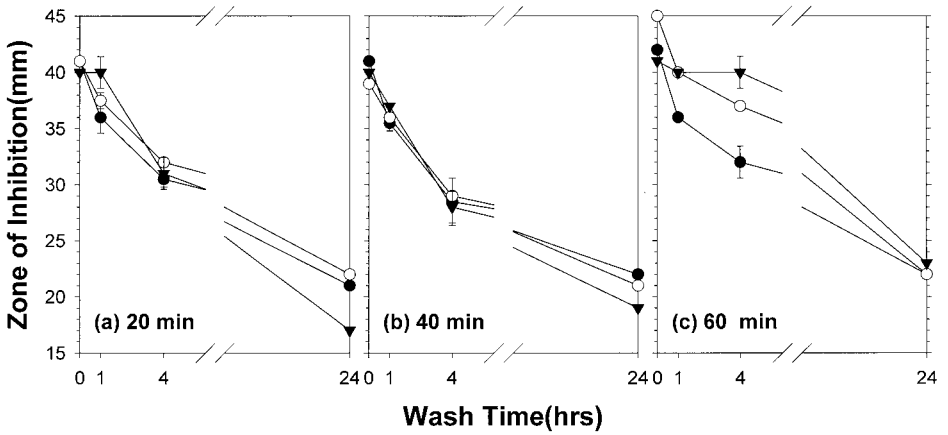


Figure 14 Zone of inhibition of hydrolyzed wool dyed with Cipro at pH 3 for 3.5 h: (a) 20 min hydrolysis, (b) 40 min, (c) 60 min. ●: 85°C; ○: 65°C; ▼: 45°C.

dyebath corroborated that a long dyeing time such as 3.5 h was needed to obtain a sufficient level of Doxy on wool.

In acidic condition and low dyeing temperature, sorption of both antibiotics on wool was hindered because of electrostatic repulsion between positively-charged substrate and antibiotic. High temperature dyeing minimized the effect of pH on exhaustion. Nylon showed a similar trend.

With sorption of Doxy the color of the substrates was changed. The colors produced at different temperatures were readily apparent. The thermal stability of Cipro was better than that of Doxy, as indicated by the consistency of both the color of the treated fabric and the absorbance of aqueous solutions.

Hydrolysis of wool at 40°C produced a highly extensible, compact material with high sorption of both antibiotics. Increase in sorption of both antibiotics was more significant at low temperature, making the process potentially more attractive. The increase in antibiotic sorption was most substantial for the first 20 min of hydrolysis. Hydrolysis of nylon at 85°C up to 4 h slightly improved Doxy sorption but the increase was greater in Cipro sorption. However, sorption of Cipro was always lower in hydrolyzed nylon than in hydrolyzed wool. FTIR spectroscopic analysis substantiated that the increase in polar functional groups by peptide scission and oxygen–sulfur groups by oxidized cystine substantially facilitated sorption of antibiotics onto the hydrolyzed wool. Evidence of an increase of polar functional groups was also shown in hydrolyzed nylon by FTIR spectroscopy.

Both regular and hydrolyzed wool fabrics dyed by Doxy at lower temperatures showed consistently greater ZOI than that of fabrics dyed at high temperatures. ZOI was greater with wool hydrolyzed for a shorter time. Measurement of Doxy released from wool directly corresponded to ZOI values. The ZOI of hydrolyzed wool dyed at pH 2 with Doxy was greater than that of unhydrolyzed wool at 24 h wash time, which indicated that hydrolysis of wool improved both sorption and ZOI values.

The ZOI of wool dyed with Doxy at three pH levels and 45 or 65°C was in the order of pH 6.5 > pH 9 > pH 2. ZOI values for nylon dyed by Doxy and Cipro were generally zero within 24 h of wash time. In addition, Doxy release from nylon was much lower than that from wool. Sorption of antibiotics on nylon, therefore, may be comparable to that on wool, but corresponding infection-resistant properties were inferior.

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