Fluoroquinolon-Type Antibiotic Treatment of PAN and Cationic-Dyeable PET Fibers for Infection Resistant Materials

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ABSTRACT: Commercial poly(acrylonitrile) (PAN) and cationic-dyeable poly(ethylene terephthalate) (PET) were dyed with a fluoroquinolon-type antibiotic, ciprofloxacin (Cipro), to develop infection resistant biomedical materials. Regular PET fiber was also treated as a comparison purpose. Experimental parameters examined were different dyeing temperatures, times, and pHs. To investigate effect of hydrolysis on Cipro sorption, the fibers were hydrolyzed by 1% NaOH for 1 or 2 h at 85°C and 100°C. Regardless of pH conditions, both PAN fibers (Orlon and Acrilan) sorbed high level of Cipro at 100°C for 3.5 h, but zone of inhibition (ZOI) value reached zero at 4 h of wash time. Contrarily, the presence of additional functional groups in PAN and cationic-dyeable PET obtained by hydrolysis not only considerably enhanced sorption of Cipro but also provided much better sustained release, indicated by high ZOI value at 24 h of wash time. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 853–858, 2007

Key words: ciprofloxacin; poly(acrylonitrile); cationic-dyeable poly(ethyleneterephthalate); infection resistant materials; hydrolysis

INTRODUCTION

One of the most common biomedical materials is poly (ethylene terephthalate) (PET) fiber and its end-use can be found in many areas such as vascular graft.[1–7] Utilization of poly(acrylonitrile) (PAN) fiber is also common in biomedical area. The fiber is generally employed as hollow-type membrane for biohybrid organs in biohybrid liver support system, artificial kidney, fiber for removal of endotoxin from fluids, and bioartificial skin.[8–12]

These biomedical materials; however, often encountered severe problems such as thrombosis and infection. Number of disastrous outcomes, including death, can be possible by such problems.[5,7,13] It was reported that between 2 and 6% of arterial grafts became infected during the operation, with roughly half of them resulting in death.[7,14] This infection can be greatly minimized by applying therapeutic agents, i.e., antibiotics, to the materials.[5–7]

For implanted or percutaneous devices sustained release of antibiotics from infection-resistant biomaterials is very important.[2–7] Conventional dipping of the substrates into antibiotics has proven not to be completely satisfactory because of the rapid release of the agent.[2–5] The studies therefore have been made to develop sustained infection resistant materials.[2–7] Advantage of using dyeing technology was first recognized in application of fluoro-quinolone type antibiotics on regular PET fibers.[6] Later it was found that introduction of polar functional groups in PET fibers by using amine treatment could facilitate uptake of a certain antibiotic, resulting in its more sustained release from the substrate.[7] Efficacy of polar functional groups was later confirmed by other fibers such as silk, wool, nylon, and other fibers.[2–4]

Commercial PAN fibers usually contain 5–10% of one or more comonomers to improve solvent solubility, reduce structural rigidity, and provide ionic functional groups.[15] In addition, chain terminal sulfate and sulfonic acid groups are also present in PAN fibers because of the redox initiator used during polymerization.[14] Likewise cationic-dyeable PET fiber such as Dacron 64 typically contains *S*-sulfoisophthalic acid as a modifier to increase basic-dye affinity.[16] The presence of these ionic polar functional groups in PAN and modified PET expects to influence on sorption of antibiotics. However, little study has been done in this area.

Therefore, in this study we selected PAN and cationic-dyeable PET fibers as substrates. Our goal was to investigate sorption of antibiotic onto these fibers

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using ''dye-like'' interaction as a continued effort to develop novel infection resistant biomaterials. The fibers were also hydrolyzed with alkali and its effect on sorption of antibiotic was investigated. Zone of inhibition (ZOI) technique was employed to determine infection resistant characteristics of unhydrolyzed and hydrolyzed substrates.

EXPERIMENTAL

Materials

PANs used in this study were Orlon 75 (plain weave with 135g/m^2 weight, Type 864) and Acrilan 16 (knit with 143 g/m^2 weight, Type 867). Cationic-dyeable PET was Dacron 64 (plain weave with 163 g/m^2 weight, Type 763). Regular PET, Dacron 54 (plain weave with 171g/m^2 weight, Type 755), was also used for a comparison purpose. These fabrics were purchased from Test Fabrics, (West Pittston, PA). Ciprofloxacin (Cipro) was obtained in pure form Serological Products (Bayer-made) and was used without further purification. Cipro is a fluoroquinolone-type antibiotic, which is a first generation DNA gyrase inhibitor.[17,18] Cipro was selected as an antibiotic because of its high activity against Gram-negative and Gram-positive bacteria. It is also one of only two antibiotics approved for treatment of anthrax (*Bacillus anthracis*) infection by the Centers for Disease Control and Prevention.[19] Sodium hydroxide and glacial acetic acid were reagent grade and purchased from Aldrich Chemicals.

Dyeing of fibers with cipro

Dyeing of antibiotic on the substrate was carried out in an Ahiba Polymat (Datacolor International) dyeing machine. Detailed dyeing procedure can be found in the previous study.[2–4] Experimental parameters were dyeing temperature, time, and dyebath pH. The bath pHs employed were 3, 5.5, and 10 and the pH 3 was the initial pH of the dyebath containing Cipro. Other pHs were adjusted by adding 1% NaOH and 1% acetic acid. Dyeing temperatures and times examined were 45, 65, 85, and 100°C for 1, 2, and 3.5 h. After the dyeing, the fabric was removed, and the amount of antibiotic taken up by the substrate was determined (see analyses later). All the treatment was carried out twice.

To investigate effect of hydrolysis on sorption of antibiotics, PAN and PET fibers were treated in 1% NaOH for 1 and 2 h at 85°C and 100°C with 40 : 1 liquor ratio in the Ahiba dyeing machine. After hydrolysis, the fabrics were washed with glacial acetic acid and rinsed with deionized water until rinsing water became neutral.

Analyses

To determine sorption of antibiotics by PAN and PET, the concentration of residual antibiotics in the dyebath after dyeing was measured using an UV/VIS spectrophotometer (Mecasys Instruments, Korea). The λ_{max} value of Cipro was determined as 276 nm. Since Cipro was insoluble at higher pHs, so the pH of the dyeing solutions for analysis was controlled to pH 3. The solutions were also immersed in a water bath at 85°C for 15 min prior to appropriate dilution to ensure a complete dissolution of Cipro for the absorbance reading. The data were analyzed in terms of this residual concentration. In addition, the amount of antibiotic taken up by the substrate was determined as the ''percent exhaustion,'' calculated as follows:

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

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

The infrared spectra of the untreated and hydrolyzed fabrics were obtained by a FTIR spectroscope (JASCO Model FT/IR-4100) with an attached ATR in the spectral region of 4000–650 cm^{-1} with 100 scans at 4 cm^{-1} resolution. Effects of hydrolysis on fabric whiteness were also evaluated by using Microflash spectrophotometer by using CIE whiteness.[2,3]

Amounts of anionic groups were quantitatively determined by using Methylene Blue uptake. Methylene blue at pH 9.5 was applied on hydrolyzed fabrics at 45°C for 1 h in Ahiba dyeing machine. Number of anionic groups ($-COO^-$ and $-SO_3^-$, etc.) was calculated according to the following equation:[5]

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

where Q is amount of dye taken up, M is formular weight of the Methylene Blue, and W was weight of the fabric.

A ZOI test determined the antimicrobial activity of the antibiotic-dyed materials.[2–7] A stock solution of *S. epidermidis* was thawed at 37°C for 1 h, then 1 μL of this stock was added to 10 mL of trypticase soy broth and incubated overnight at 37°C. From this solution, 10 μL was streaked onto trypticase soy agar plates. Untreated and antibiotic-treated fiber segments were autoclaved, embedded into the streaked trypticase soy agar plates ($n = 3$ segments/time interval/treatment), and placed overnight in a 37°C incubator. Standard 5 μg antibiotic Sensi-Discs ($n = 3$) were also embedded at each time interval. The ZOI of each piece was determined, taking the average of three individual diameter measurements. Meanwhile, pieces of the dyed materials were subjected to a ''washing'' to simulate blood flow conditions.[6] Samples were removed at intervals and the washing solution replaced each time.

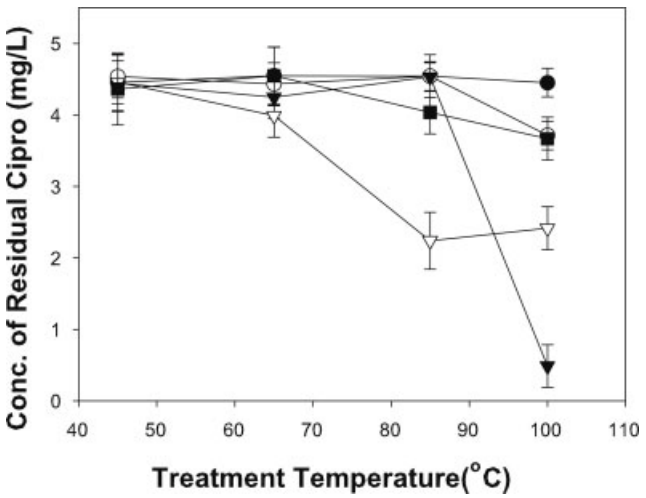

Figure 1 Effect of temperature on sorption of Cipro on PAN and PET fibers (pH 3 and 3.5 h). ●; Blank, ○; Dacron64, ▼; Orlon, △; Acrilan, ■; Dacron54.

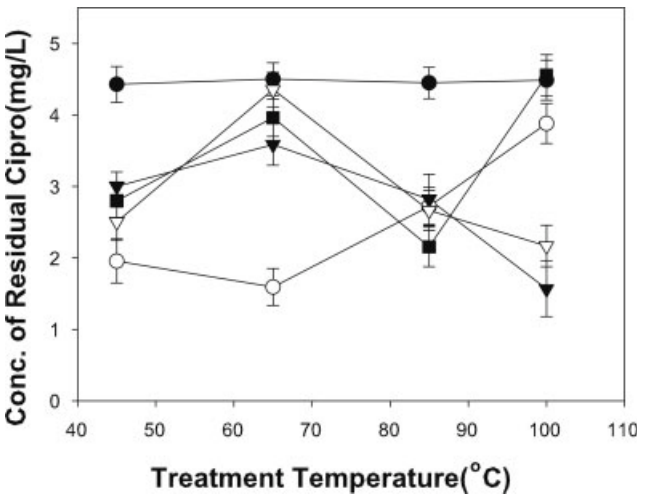

Figure 3 Effect of temperature on sorption of Cipro on PAN and PET fibers (pH 10 and 3.5 h). ●; Blank, ○; Dacron64, ▼; Orlon, △; Acrilan, ■; Dacron54.

The zones of inhibition of these samples were determined, and thus zone size (mm) over time was determined for each parameter evaluated.

RESULTS AND DISCUSSION

Sorption of Cipro

Sorption of Cipro on PAN and PET fibers was shown at different temperatures and three pH levels in Figures 1–3. Cipro showed exceptionally high thermal stability as exhibited by its consistent absorbance in blank solution even after the treatment at 100°C for 3.5 h. Its high thermal stability is advantageous over other antibiotics such as tetracycline type (i.e. doxycycline), especially when the dyeing technology is used to apply it to the substrates.

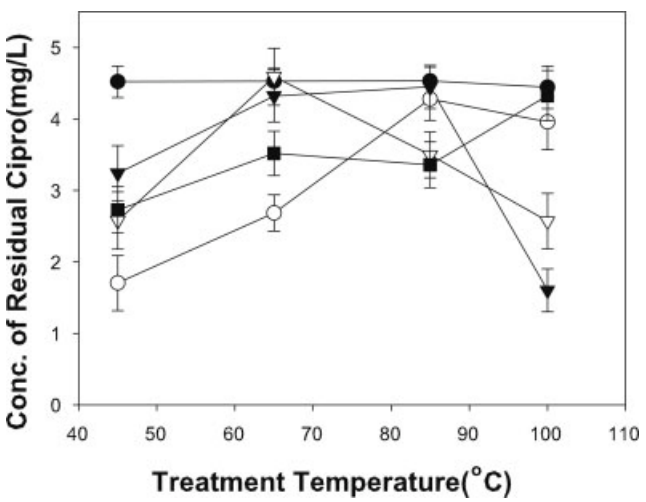

Figure 2 Effect of temperature on sorption of Cipro on PAN and PET fibers (pH 5.5 and 3.5 h) ●; Blank, ○; Dacron64, ▼; Orlon, △; Acrilan, ■; Dacron54.

As shown in Figure 1, Cipro sorption on Acrilan at pH 3 considerably increased at 85°C whereas the same drastic increase occurred at 100°C for Orlon. No or little sorption of Cipro observed with two types of PET at the same pH. Contrarily, the shapes of the sorption curves appeared to be changed completely at higher pHs as shown in Figures 2 and 3. Careful observation, however, revealed that with the exception of treatment at 45°C the sorption of Cipro on Orlon and Acrilan generally increased with increase in the dyeing temperature as similar as pH 3. Unexpected low residual concentration of Cipro bath at 45°C or sometimes at 65°C with neutral and alkaline pHs for all substrates was mainly because of incomplete or partial solubility of Cipro at these pHs and temperatures.

Percent exhaustion on Cipro was also calculated and tabulated in Table I for detailed evaluations of effect of pH and temperature on its sorption. In most cases, sorption and exhaustion of Cipro were higher with Acrilan up to 85°C than those with Orlon, but the trend was reversed at 100°C. Especially, at pH 3 and 100°C almost 90% of Cipro sorbed to Orlon. Such high exhaustion is greatly advantageous while considering high cost of the antibiotic. Difference in sorption and exhaustion in two types of PAN was most likely due to their different comonomer composition.

Effect of hydrolysis on PAN and PET

Modification of polymer surface is an area of considerable technological and academic importance. A main objective of hydrolysis of PAN and PET fibers in this study was to introduce additional polar functional

TABLE I
Percent Exhaustion Values of Cipro on PAN and PET Fibers

		Treatment temperature(°C) for 3.5 h			
Acrylics	pH	45	65	85	100
Orlon	3	0.3	6.7	0.3	89.0
	5.5	28.3	4.6	1.9	63.9
	10	32.2	20.4	36.6	65.1
Acrilan	3	0.0	12.3	50.7	45.7
	5.5	43.0	0.0	22.8	42.1
	10	43.4	3.2	40.1	51.7
Dacron64	3	0	2.6	0	16.6
	5.5	62.3	40.7	5.6	10.9
	10	55.8	64.6	38.8	13.5
Dacron54	3	2.1	0.1	11.2	17.8
	5.5	39.6	22.3	25.9	2.6
	10	36.7	12.0	51.6	1.5

groups within the fibers. This could result in improvement in sorption of Cipro at lower temperature application. Furthermore, other applications such as immobilization of enzymes or antibiotics on the modified surfaces can be possible.[20]

As shown in Table II, weight loss of Dacron 64 was the greatest, showing high sensitivity of cationic-dyeable PET as previously reported.[15] Contrarily, in PAN fiber a main reaction during hydrolysis of PAN fiber was expected to be a conversion of nitrile (—C≡N) to carboxylic acid (—COOH) and hydrolysis of comonomers. Hydrolysis reactions of comonomers in PAN fibers, such as vinyl acetate and methyl acrylate, and methyl methacrylate, could produce vinyl alchohol, acrylic acid, and methacrylic acid moiety, respectively. Since both reactions involved little main chain scission, relatively low weight loss of PAN fiber was expected. Furthermore, weight loss of Dacron54 was somewhat higher than those of PAN fibers at each hydrolysis condition applied. With hydrolysis PAN fibers substantially changed their color to yellow, especially in Acrilan as indicated by extremely low CIE whiteness index value (Fig. 4).

To determine amount of anionic groups in the hydrolyzed PAN and PET quantitatively, Methylene Blue technique was used as shown in Figure 5. Carboxylic and sulfonate groups are some of possible anionic groups presented within these fibers. As expected, regular PET showed very low number of anionic groups even with 2 h of hydrolysis at 100°C. According this calculation, hydrolyzed PAN and Dacron64 fibers contain about three to four times of anionic groups than that of regular PET. This again substantiated higher susceptibility of cationic-dyeable PET on hydrolysis than regular PET. Among all the fibers examined, Acrilan showed great number of anionic groups and concentrations of anionic groups in Orlon and Dacron 64 after hydrolysis reaction were quite similar to each other. In addition, concentration of anionic groups tended to level off at the PAN and PET fibers hydrolyzed for 2 h at 85°C.

Effect of hydrolysis on chemical change was also investigated by FTIR-ATR technique as shown in Tables III and IV. The changes in FTIR absorption

TABLE II
Percentage Weight Loss in the Hydrolyzed Fibers

Hydrolysis condition (h/°C)	Dacron64	Orlon	Acrilan	Dacron54
1/85	2.98	0.1	0.13	1.10
2/85	4.09	0.21	0.67	1.85
1/100	6.05	0.46	0.99	2.49
2/100	15.0	1.32	4.04	5.46

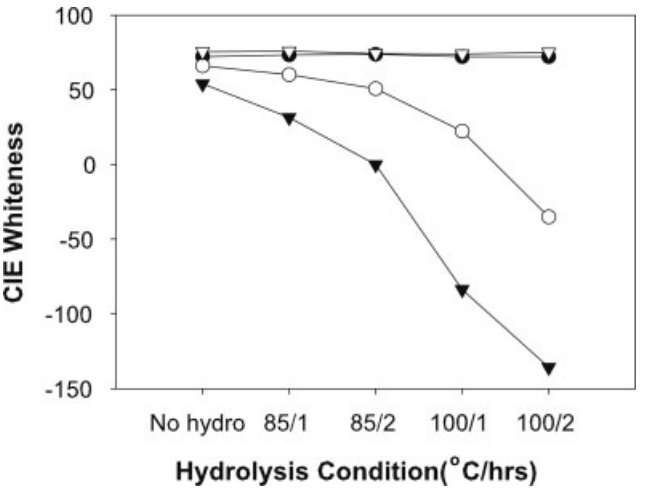

Figure 4 Effect of hydrolysis on CIE Whiteness Index of PAN and PET fibers. ●; Dacron64, ○; Orlon, ▼; Acrilan, △; Dacron54.

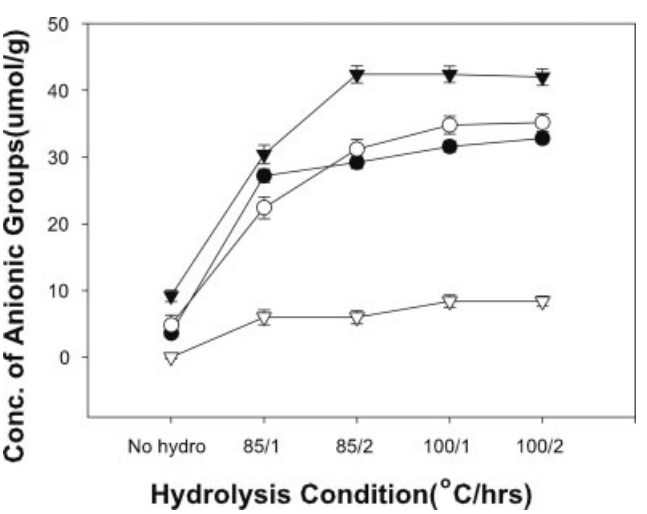

Figure 5 Effect of hydrolysis on concentration of anionic groups of PAN and PET fibers. ●; Dacron64, ○; Orlon, ▼; Acrilan, △; Dacron54.

TABLE III
Height Changes in Absorption Peaks in Orlon

Hydrolysis condition (h/°C)	Height of absorption peaks ($\times 10^{-4}$)	
	2242 cm^{-1}	1731 cm^{-1}
No Hydro	49.7	40.6
1/85	36.2	29.1
2/85	31.6	26.1
1/100	33.6	26.9
2/100	26.1	22.7

peaks occurred in Orlon fibers were most significant at two peaks; 2242 cm^{-1} for nitrile group and 1731 cm^{-1} for ester carbonyl group. Heights for both peaks decreased considerably with increase in harshness of the hydrolysis condition applied. In the case of Acrilan; however, changes in heights of absorption peaks because of hydrolysis were much more diverse than those of Orlon. The same absorption peaks for nitrile and ester carbonyl decreased whereas the additional peaks at 3346, 1670, and 1568 cm^{-1} increased substantially. This indicated that the hydrolysis treatment generated additional polar groups such as hydroxyl and carboxylic acid. Moreover, the peak at 1568 cm^{-1} was probably due to formation of conjugated —C=*N*—C=N— systems, which could cause significant yellowing of Acrilan as shown previously.

Even though it was not shown here, a little variation was shown in FTIR spectra of two PET fibers hydrolyzed at these conditions probably due to the presence of the same functional groups even after hydrolysis.

Sorption of Cipro on hydrolyzed PAN and PET

Effect of hydrolysis on sorption of the antibiotics on PAN and PET fibers was determined as shown in Figure 6 for Cipro. The antibiotic was applied on its natural pH, i.e., pH 3 at 85°C for 3.5 h. Effect of hydrolysis on Cipro sorption was more significant on Acrilan than any other fibers examined. But these effects were only observed in the fibers hydrolyzed at 100°C. Regular PET showed no variations on sorption of Cipro with hydrolysis treatment, whereas other two fibers such as Orlon and Dacron 64 exhibited similar behavior with showing more effect on Orlon. Since dyeing

TABLE IV
Height Changes in Absorption Peaks in Acrilan

Hydrolysis condition (h/°C)	Height of absorption peaks ($\times 10^{-4}$)				
	3346 cm^{-1}	2243 cm^{-1}	1731 cm^{-1}	1670 cm^{-1}	1568 cm^{-1}
No Hydro	0.17	58.8	40.6	0	0
1/85	1.87	35.3	29.1	0	1.32
2/85	7.53	36.4	26.1	0.68	4.50
1/100	8.46	31.7	26.9	2.71	8.75
2/100	22.42	38.6	22.7	8.98	22.69

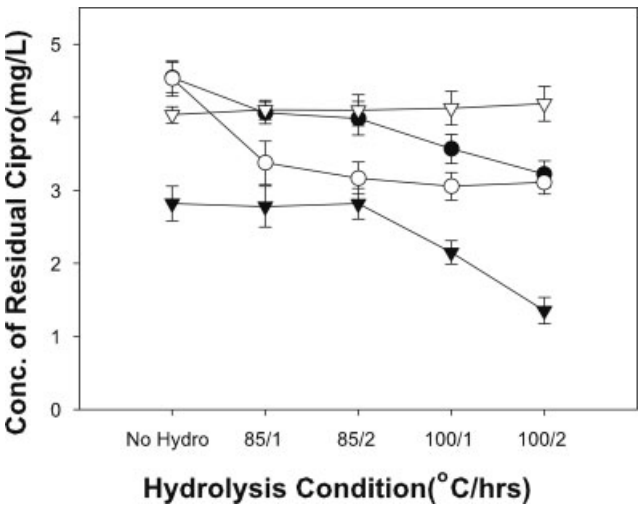

Figure 6 Effect of hydrolysis on sorption of Cipro of PAN and PET fibers (pH 3 and 85°C/3.5 h). ●; Dacron64, ○; Orlon, ▼; Acrilan, △; Dacron54.

of the substrates with Cipro was carried out in acidic condition, increase in carboxylic groups in hydrolyzed fibers did not expect to increase level of ionic interaction between the antibiotics and substrates. Therefore, we believe that considerable improvement in sorption of Cipro in hydrolyzed PAN was mainly due to the presence of hydrogen bonds between polar functional groups of the antibiotic and substrate. However, increase in ionic interaction between strong acidic groups (e.g., sulfonate) in PAN and anionic-modified PET and (+)-charged antibiotics due to conformational change of the fiber brought by hydrolysis reaction must be also considered.

Therefore, it is concluded that large chemical changes in Acrilan brought by hydrolysis were re-

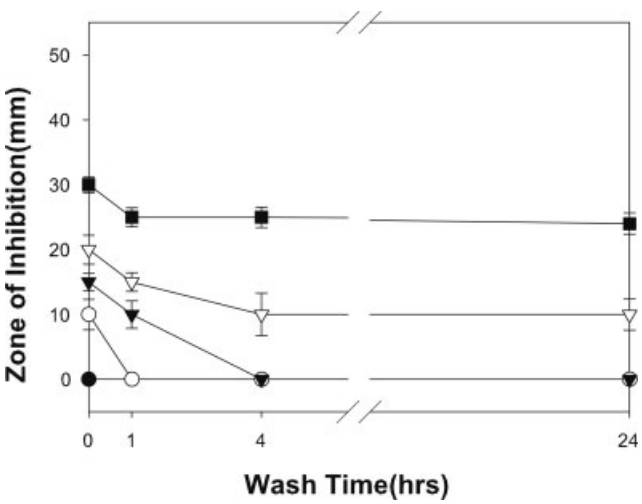

Figure 7 Zone of inhibition of Dacron64 fibers treated by Cipro (pH 3). ●; untreated, ○; treated at 45°C ▼; 85°C, △; 100°C, ■; hydrolyzed fiber (treated at 100°C).

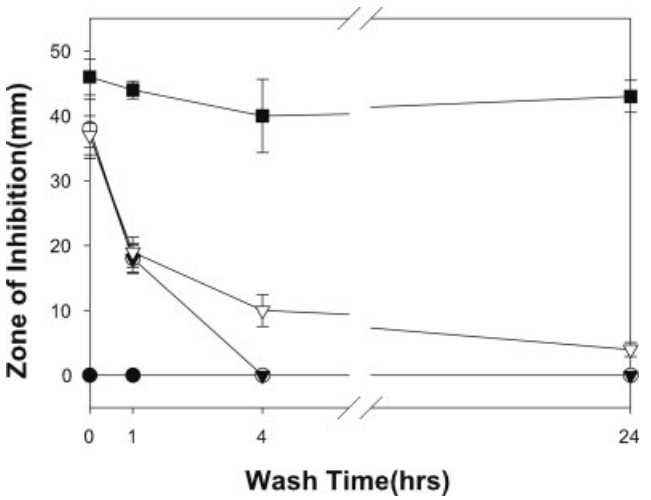

Figure 8 Zone of inhibition of Orlon fibers treated by Cipro (pH 3). ●; untreated, ○; treated at 45°C, ▼; 85°C, △; 100°C. ■; hydrolyzed fiber (treated at 100°C).

sponsible for higher sorption of Cipro. These changes were confirmed by Methylene Blue and FTIR.

Infection resistance properties of the antibiotic-treated PAN and PET

Efficacy of antibiotic-treated substrates can be analyzed by use of the ZOI test. The greater ZOI value at longer wash time corresponds to better sustained release of the antibiotics.

Effect of hydrolysis on better sustained release of the antibiotics was even greater with the substrates treated by Cipro (Figs. 7–9). The same three fibers, such as Orlon, Acrilan, Dacron64, all showed not only much improved initial ZOI values by the hydrolysis, but also much greater ZOI values at 24 h wash time, i.e., the better sustained release. It should be also noted that after 24 h washing visual observation under fluorescent indicated that Cipro was still present within the Cipro-treated PAN. This implied that the Cipro could be further released at even longer wash time. Again regular PET did not show any adequate ZOI values even in the Cipro-treated hydrolyzed substrate (not shown).

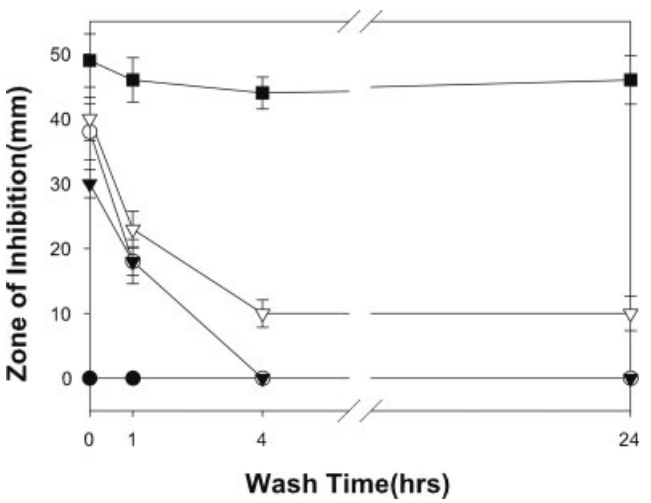

Figure 9 Zone of inhibition of Acrilan fibers treated by Cipro (pH 3). ●; untreated, ○; treated at 45°C ▼; 85°C, △; 100°C, ■; hydrolyzed (treated at 100°C).

CONCLUSIONS

Fluoroquinolon-type antibiotic, ciprofloxacin (Cipro), was applied on unhydrolyzed and hydrolyzed fibers of PAN (Orlon and Acrilan) and PET (cationic-dyeable PET and regular PET) to develop infection resistant biomedical materials. Results indicated that hydrolysis substantially enhanced sorption of the antibiotics. However, ZOI values of the unhydrolyzed fibers approached zero within 4 h wash time, indicating rapid release of the antibiotics from the substrates. In contrast, the hydrolyzed fiber (excluding regular PET) showed greater sorption of antibiotics as well as higher ZOI values at 24 h wash time. This substantiated that the hydrolysis of PAN and cationic-dyeable PET was beneficial in obtaining greater and longer sustained release of antibiotics.

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