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Interaction of Antibiotic with PAN and Cationic-Dyeable PET Fibers in Development of Infection Resistant Biomedical Materials

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Abstract: Interaction of a representative antibiotic, doxycycline (Doxy), with commercial poly(acrylonitrile) (PAN) and cationic-dyeable poly(ethylene terephthalate) (PET) fiber was studied in development of infection resistant biomedical materials. Regular PET was also employed for a comparison purpose. Their interactions were investigated at different treatment temperatures, times, and pHs. Fibers were also hydrolyzed by 1 % NaOH for 1 or 2 hours at 85 °C and 100 °C to study effect of hydrolysis on antibiotic sorption. Infection-resistant characteristics of the substrates were evaluated by zone of inhibition (ZOI) test. Results revealed that a significant chemical change occurred in PAN and cationic-dyeable PET due to hydrolysis. Additional functional groups obtained by hydrolysis not only enhanced sorption of the antibiotics but also provided greater ZOI values, indicating substantial improvement in sustained infection resistance properties.

Keywords: Doxycycline, Cationic-dyeable poly(ethylene terephthalate), Poly(acrylonitrile), Infection resistant materials, Hydrolysis

Introduction

Poly(ethylene terephthalate) (PET) fiber has been used as one of the most common biomedical materials and its end-use can be found in many areas such as vascular graft [1-5]. On the other hand, poly(acrylonitrile) (PAN) fiber is generally utilized in biomedical area as hollow-type membrane for biohybrid organs such as biohybrid liver support system [6], artificial kidney [7], and fiber for removal of endotoxin from fluids [8]. Recently, it was found that the pure PAN membrane provided excellent conditions for seeding with fibroblasts in bioartificial skin application [9]. Highly permeable membranes were also prepared from hydrolyzed PAN [10].

Regardless of application of these biomedical materials, they frequently encountered severe problems such as thrombosis and infection. These problems can lead to number of possible disastrous outcomes, including death [4,5,11]. For example, between 2 and 6 % of arterial grafts become infected during the operation, with roughly half of them resulting in death [5, 12,13]. Such problems can be alleviated by applying therapeutic agents, i.e., antibiotics, to the materials [4,5].

Sustained release of antibiotics from infection-resistant biomaterials is very important in implanted or percutaneous devices [2-5]. Conventional dipping of the substrates into antibiotics has proven not to be completely satisfactory because of the rapid release of the agent [2-4]. Previous study revealed that introduction of polar functional groups in PET fibers by using amine treatment could facilitate uptake of a certain antibiotic [5]. Such a material exhibited more sustained release of the antibiotics from the substrate. This result was later confirmed by other fibers containing more polar functional groups such as silk, wool, and nylon [2,3].

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Commercial PAN fibers usually contain 5-10 % of one or more comonomers to improve solvent solubility, reduce structural rigidity, and provide ionic functional groups [14]. In addition, chain terminal sulfate and sulfonic acid groups are also present in PAN fibers due to the redox initiator used during polymerization [12]. Likewise cationic-dyeable PET fiber typically contains S-sulfoisophthalic acid as a modifier to increase basic-dye affinity [15]. The presence of these ionic polar groups in PAN and modified PET expects to influence on sorption of antibiotics. However, no 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 the antibiotic onto these fibers using "dye-like" interaction as a continued effort to develop novel infection resistant biomaterials. The fibers were also hydrolyzed with alkali and effect of the hydrolysis on sorption of antibiotic was investigated. Zone of inhibition 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 135 g/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 polyester, Dacron 54 (plain weave with 171 g/m^2 weight, Type 755), was also used for a comparison purpose. These fabrics were all purchased from Test Fabrics, Inc. (USA). Doxycycline (Doxy), which is a semisynthetic tetracycline, was selected as a representative antibiotic because of its high activity against Gram-negative and Gram-positive bacteria [16].

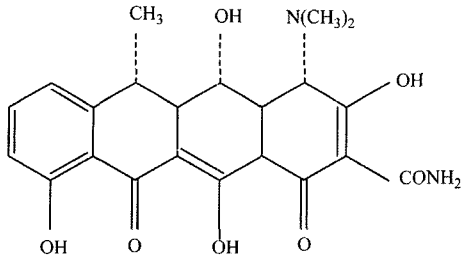

Figure 1. Chemical structure of doxycycline.

Furthermore, it is one of only two antibiotics approved for treatment of anthrax (*Bacillus anthracis*) infection by the Centers for Disease Control and Prevention in U.S.A. [17]. Doxy was obtained in pure form from Pfizer and used without further purification. Figure 1 shows a chemical structure of Doxy. Sodium hydroxide and glacial acetic acid were reagent grade and purchased from Aldrich Chemicals.

Fiber Treatment with Doxy

Application of Doxy on the substrates was carried out in an Ahiba Polymat (Datacolor International, USA) dyeing machine. Doxy at 2 % on the weight of fabric (owf) was applied at a liquor-to-fabric ratio of 20:1. Experimental parameters were treatment temperature, time, and bath pH. The bath pHs employed were 2, 6.5, and 9 and the pH 2 was the initial pH of the Doxy-containing bath. Other pH conditions were controlled by addition of 1 % NaOH and 1 % acetic acid and monitored using Corning pH meter 115 (USA). Treatment temperatures and times examined were 45, 65, 85 and 100 °C for 1, 2, and 3.5 hours. After the treatment, the fabric was removed, and the amount of antibiotic taken up by the substrate was determined (see below).

To investigate effect of hydrolysis on sorption of the antibiotic, PAN and PET fibers were treated in 1 % NaOH for 1 and 2 hours 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 Doxy by PAN and PET fibers, the concentration of residual Doxy in the treating bath after the treatment was measured using a Cary 50 UV/VIS spectrophotometer (Varian Instruments, USA). The λ_{max} value of Doxy was determined as 274 nm. The relationships between absorbance and concentration were established at λ_{max} for each antibiotic. No pH adjustment was carried out for the bath after treatment since the absorbance at 274 nm and solubility of Doxy were quite consistent at different pHs. 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:

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

where C_o is concentration of antibiotic in blank solution and C_r is residual antibiotic concentration of the bath containing the substrate after treatment.

The CIE lightness (L^*) of Doxy-dyed fabrics were evaluated with a Macbeth ColorEye system along with SLI-Form®/NG software (She Lyn Inc., USA). The infrared spectra of the untreated and hydrolyzed fabric were obtained with a Sense FTIR spectroscope (SensIR Technologies, USA) with an attached diamond ATR in the spectral region of 4000-700 cm^{-1} with 54 scans at 4 cm^{-1} resolution.

A zone of inhibition (ZOI) test determined the antimicrobial activity of the antibiotic-dyed materials [2-5]. A stock solution of *S. epidermidis* was thawed at 37 °C for 1 hour. Upon thawing, 1 μl of this stock was added to 10 m*l* of Trypticase Soy Broth (TSB) and incubated overnight at 37 °C. From this solution, 10 μl was streaked onto Trypticase Soy Agar (TSA) plates. Untreated and antibiotic-treated silk segments were autoclaved, embedded into the streaked TSA plates ($n = 3$ segments/time interval/treatment) and placed into a 37 °C incubator overnight. Standard 5 μg antibiotic Sensi-Discs ($n = 3$) were also embedded at each time interval. The zone of inhibition each piece was determined, taking the average of 3 individual diameter measurements. Meanwhile, pieces of the dyed materials were subjected to a "washing" to simulate blood flow conditions. Samples were removed at intervals and the washing solution replaced each time. The ZOIs of the removed samples were determined, and thus zone size (mm) over time was determined for each parameter evaluated. Samples with no ZOI were transferred to sterile 50 m*l* polypropylene tubes containing 30 m*l* of TSB and sonicated at 60 Hz for 10 minutes in an ice bath. Sonicate solutions (100 μl) were backplated onto TSA plates and examined after 24 hours to determine the presence of adherent bacteria on the segments.

Results and Discussion

Effect of Treatment Temperature

Figure 2 shows effect of temperature on sorption of Doxy on PAN and PET fabrics at pH 2. Concentration of residual Doxy in the bath was consistent and the sorption of Doxy was little at the temperatures up to 85 °C for all fibers. Substantial increase in sorption, i.e., decrease in residual concentration, was shown only at 100 °C. It should be noted that a certain fraction of this decrease was probably due to decomposition of Doxy, indicated by a slight decrease in the residual concentration of the blank bath at 100 °C. Within each fiber category, two types of PAN and PET fibers exhibited almost identical sorption of Doxy, but sorption on PAN was much higher than that on PET fibers. Little sorption of Doxy on PAN and PET at the temperatures up to 85 °C was most likely due to their compact structure, high crystallinity, and

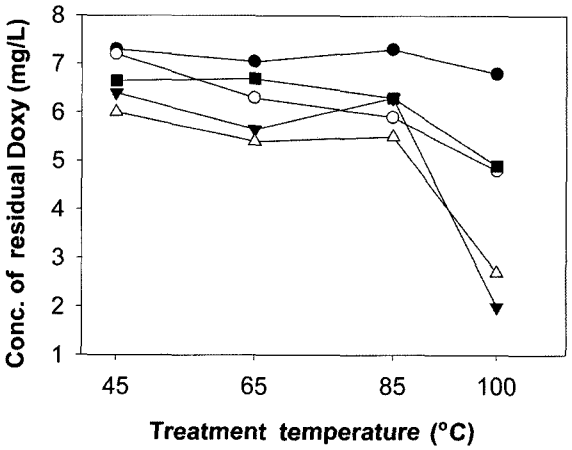

Figure 2. Effect of temperature on sorption of Doxy; (●) Blank, (○) Dacron64, (▼) Orlon, (△) Acrilan, (■) Dacron54.

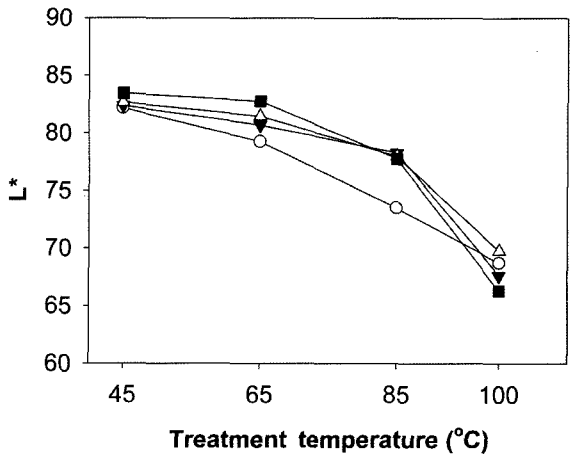

Figure 3. Effect of temperature on lightness; (○) Dacron64, (▼) Orlon, (△) Acrilan, (■) Dacron54.

high glass transition temperature [14]. This effect was apparently more prominent with PET, resulting in lower sorption. Little diffusion of molecules at dyeing temperature lower than 100 °C with PAN and PET is quite common events even in conventional dyeing of both fibers with basic or disperse dyes [14].

Color of pure Doxy powder is yellow. Once aqueous solution is prepared, however, Doxy starts to change its color to brown. Intensity of the aqueous Doxy solution was influenced by various factors such as pH, temperature, and duration of preparation. For example, at alkaline pHs such as pH 9 color change of aqueous Doxy solution occurred instantaneously [2,3]. Therefore, the fabrics were actually dyed by Doxy, producing deep brown colored fabrics especially at high temperature treatment. Decrease in lightness of the treated substrates with increase in treatment temperature is illustrated in Figure 3. Unlike residual concentration the lightness of the treated fabrics tended to decrease with increase in the treatment temperature, showing some deviations from residual concentration.

Effect of Treatment Time

Since there was little sorption of Doxy at other temperatures, effect of treatment time on sorption was studied at 100 °C. As shown in Figures 4 and 5, increase in treatment time improved sorption of Doxy on the substrates accompanied by decrease in their lightness. It is interesting to note that sorption of Doxy was quite similar in each fiber category while values in lightness were somewhat different at different treatment time. This result again substantiated that the decrease in lightness was not solely due to the increase in Doxy concentration on the substrates, confirming the previous study with other substrates [2,3]. Since long treatment time such as 3.5 hours provided great sorption of Doxy, this time was used in further experiments.

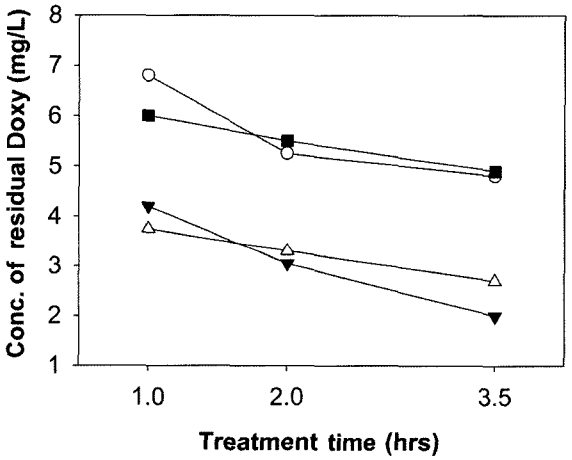

Figure 4. Effect of treatment time on sorption of Doxy; (○) Dacron64, (▼) Orlon, (△) Acrilan, (■) Dacron54.

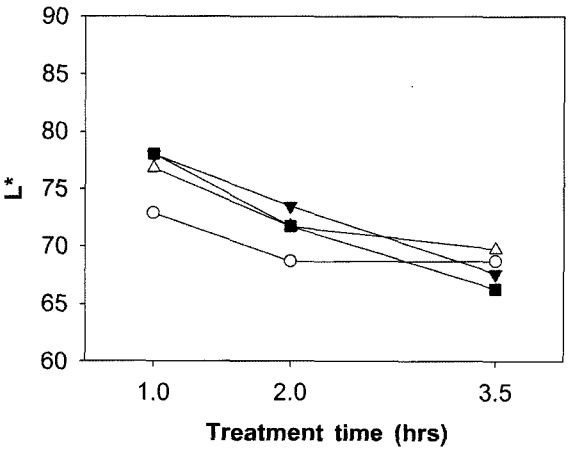

Figure 5. Effect of treatment time on lightness; (○) Dacron64, (▼) Orlon, (△) Acrilan, (■) Dacron54.

Effect of Treatment pH

Effect of pH on sorption of Doxy for PAN and PET fibers at two different treatment temperatures is illustrated in Figures 6 and 7. This result indicated that absorbance of Doxy at λ_{max} was stable at three pH levels. But its second maximum absorbance at around 345 nm changed considerably with variations in pH, temperature, and duration of preparation [2,3]. Such deviations caused change in color of Doxy solution. Sorption of Doxy on PET was generally consistent with pH changes at both temperatures whereas that on PAN changed significantly especially at 100 °C treatment. The effect of pH was not consistent with the substrates examined, for example, Orlon showed greatest sorption at pH 9 and 85 °C while the greatest sorption of Doxy on both PAN fibers at 100 °C showed on pH 2.

Analysis of Sorption

Effects of temperature, time, and pH on sorption of antibiotic could be more easily evaluated by using %exhaustion values, which were calculated against concentration of blank bath in each specific condition applied (Table 1). These data revealed that sorption of Doxy on PAN and PET was quite complex and no single factor dominated its sorption. At acidic pH, increase in treatment temperature increased %exhaustion, but the trend was reversed at other pHs. This was the same for both PAN. At 85 °C, %exhaustion was higher at neutral or alkaline pHs. Furthermore, both PET fibers showed some increase in %exhaustion with increase in treatment temperature and time, but variations were relatively much smaller than those of PAN.

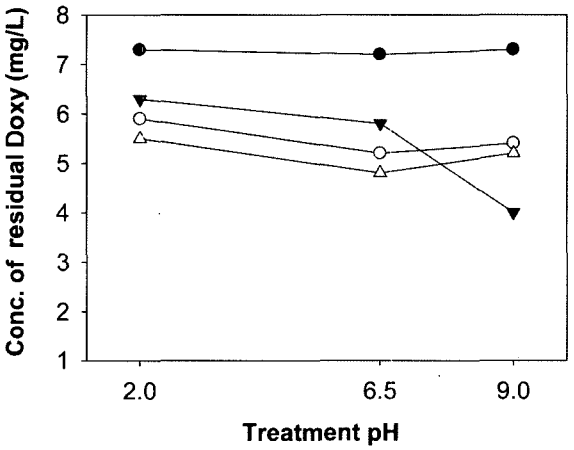

Figure 6. Effect of pH on sorption of Doxy at 85 °C for 3.5 hours; (●) Blank, (○) Dacron64, (▼) Orlon, (△) Acrilan.

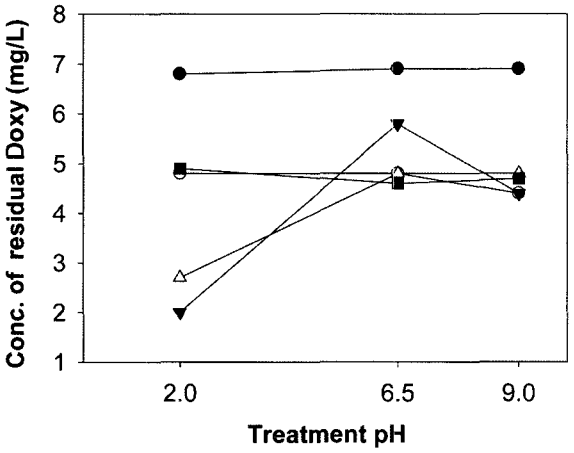

Figure 7. Effect of pH on sorption of Doxy at 100 °C for 3.5 hours; (●) Blank, (○) Dacron64, (▼) Orlon, (△) Acrilan, (■) Dacron54.

PAN and cationic-dyeable PET fibers used in this study contain various ionic functional groups (carboxylic, sulfonate, sulfate, etc.) by copolymerizaton and redox initiators used in polymerization [14]. In addition, Doxy also contains a single carboxylic group and basic nitrogen, which can be dissociated or protonated at appropriate pH range (Figure 1). It is known that dissociation of weak acid groups such as carboxylic acid is very sensitive to pH variation, but strong acidic groups such as sulfonic acid group is dissociated over a wide range of pH [14]. At pH 2 therefore one could expect an electronic attraction between positively-charged Doxy and negatively-charged sulfonic group-containing substrates such as PAN and Dacron 64. Carboxylic group in Doxy is less likely ionized at this pH. To form ionic bonding however Doxy molecules must be situated in close proximity to ionic groups of the substrates.

At the temperature below 100 °C high T_g and crystallinity of PAN and Dacron 64 hindered diffusion of Doxy, resulting in no or little sorption. Contrarily, at 100 °C diffusion of Doxy into PAN was sufficiently high generating greater ionic interaction that led to considerable increase in sorption of Doxy at acidic pH. However, in the case of Dacron 64 the treatment at 100 °C was probably not high enough to cause any substrate-antibiotic electronic interaction, exhibiting no

Table 1. Percent exhaustion of Doxy at different treatment conditions

Fiber	pH	Treatment temperature and time (°C/hrs)					
		45/3.5	65/3.5	85/3.5	100/1	100/2	100/3.5
Orlon	2	12.3	22.6	13.7	42.5	53.1	67.2
	6.5			18.3			1.7
	9			43.7			25.4
Acrilan	2	17.8	23.4	24.7	48.6	49.2	55.7
	6.5			32.4			18.6
	9			26.8			18.6
Dacron64	2	1.37	10.6	19.2	6.9	19.2	21.3
	6.5			28.8			18.6
	9			26.0			25.4
Dacron54	2	8.9	5.0	11.0	17.8	15.4	19.7
	6.5						22.0
	9						20.3

significant increase in uptake of Doxy at pH 2, compared to other pH levels.

At alkaline pH both PAN and Doxy molecules are now negatively-charged, causing electronic repulsion that consequently led to reduction in sorption of Doxy at the temperature above T_g such as 100 °C for PAN. Doxy is in its zwitterionic state at around pH 5 [2] and its ionic interaction at around this pH would be much more complex. Different degree of dissociation of weakly acidic (carboxylic) and strongly acidic (sulfonic) groups in the substrates even further leads to complex interactions against Doxy. Furthermore, high exhaustion values of Doxy at 85 °C at neutral or alkaline pH tended to suggest that other binding forces such as dispersion force and dipole force by cyano groups in PAN could play a role during Doxy treatment. In addition, hydrogen-bonded interaction between carboxylic or hydroxyl groups of hydrolyzed Acrilan and carbonyl oxygen or hydroxyl groups in Doxy molecule should not be ruled out.

Sorption of Doxy at 100 °C was generally more difficult to cationic-dyeable PET fiber than to PAN fiber. Much less variation in sorption of Doxy was also observed in Dacron 64 than PAN fibers. It was known that the diffusion rate of basic dyes in cationic-dyeable PET was less than that in unmodified PAN even with presence of carrier [14]. Higher sorption of Doxy on PAN than Dacron 64 was believed to be due to the presence of various comonomers in PAN that not only reduced the structural regularity of the polymer, thereby reducing T_g value from around 105 °C to 75-85 °C, but also providing ionic functional groups [14]. Nevertheless, variation in sorption of Doxy on Dacron 64 was still greater than that on regular PET (Dacron 54) due to its ionic nature.

Effect of Hydrolysis on PAN and PET

Modification of polymer surface is an area of considerable technological and academic importance. By modification, other applications such as immobilization of enzymes or antibiotics on the modified surfaces can be possible [18]. A main objective of hydrolysis of PAN and PET fibers was to introduce additional functional groups within the fibers. This could result in improvement in sorption of antibiotics at lower temperature application.

As shown in Figure 8, weight loss of Dacron 64 was the greatest, showing high sensitivity of cationic-dyeable PET. High sensitivity of the modified PET against hydrolysis has been previously reported [14]. Contrarily, in PAN fiber main reaction during the hydrolysis in PAN fiber was expected to be a conversion of cyanide (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 in regular PET was slight greater than those of

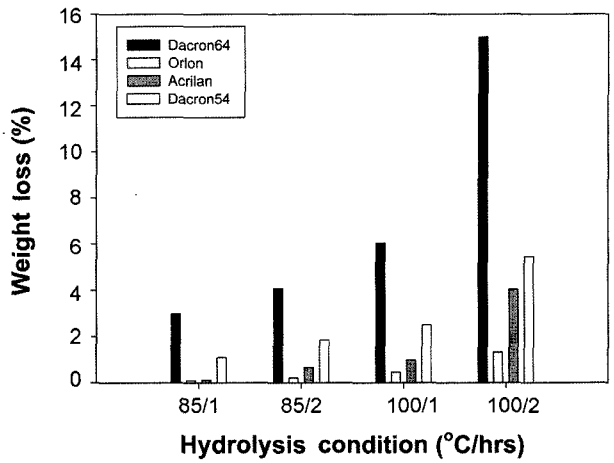

Figure 8. Effect of hydrolysis on weight loss.

Table 2. Height changes in absorption peaks for cyano and ester carbonyl groups in Orlon and Acrilan fibers

Hydrolysis condition	Height of absorption peaks ($\times 10^{-4}$)			
	Orlon		Acrilan	
	2242 cm^{-1}	1731 cm^{-1}	2243 cm^{-1}	1735 cm^{-1}
Unhydrolyzed	49.7	40.6	58.8	52.6
1 hr/85 °C	36.2	29.1	35.3	35.4
2 hr/85 °C	31.6	26.1	36.4	32.2
1 hr/100 °C	33.6	26.9	31.7	27.5
2 hr/100 °C	26.1	22.7	38.6	28.7

Acrilan at each hydrolysis condition applied.

Effect of hydrolysis on chemical change was investigated by FTIR analysis as shown in Table 2. Changes in FTIR absorption peaks occurred in Orlon were most significant on two peaks; 2242 cm^{-1} for cyano group and 1731 cm^{-1} for ester carbonyl group. Heights for both peaks decreased considerably with increase in severity of hydrolysis condition. In the case of Acrilan, however, changes in heights of absorption peaks due to hydrolysis were much more diverse than those of Orlon. The same absorption peaks for cyano and ester carbonyl decreased whereas the peaks at 3346 cm^{-1}, 1670 cm^{-1}, and 1568 cm^{-1} increased substantially. These results disclosed that hydrolysis treatment of PAN fibers brought in considerable change in their chemical structure, resulting in increase in number of polar functional groups. Not much 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 Doxy on Hydrolyzed PAN and PET

Effect of hydrolysis on sorption of Doxy on PAN and PET fibers was determined as shown in Figure 9. Doxy was applied on its natural pH, i.e., pH 2, at 85 °C for 3.5 hrs. Sorption of Doxy increased in hydrolyzed PAN fibers, but

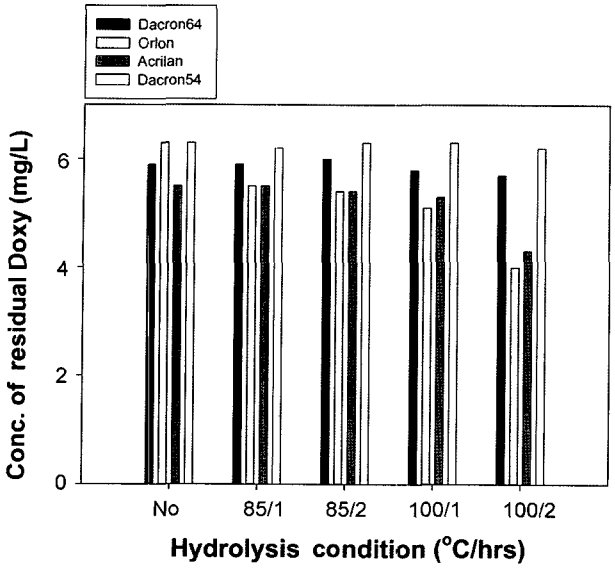

Figure 9. Effect of hydrolysis on sorption of Doxy treated at pH 2 and 85 °C/3.5 hrs.

the increase was the greatest at the most harsh hydrolysis condition (2 hours/100 °C). Contrarily, hydrolysis did not improve sorption of Doxy on cationic-dyeable and regular PET fibers.

Since treatment of the substrates with Doxy was carried out in acidic condition, increase in carboxylic groups in hydrolyzed fibers did not expect to increase level of ionic interaction between the antibiotic and substrate. That is probably why hydrolysis did not enhance sorption of Doxy on both PET fibers. Therefore, we believe that improvement in sorption of Doxy 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 cationic-dyeable PET and (+)-charged antibiotics due to conformational change of the fiber brought by hydrolysis reaction must be also considered. Further study is needed to analyze chemical and conformational changes in the hydrolyzed fibers more detail.

Infection Resistance Properties of the Antibiotic-treated PAN and PET

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

Figures 10 and 11 showed ZOI values for PAN and PET fibers treated with Doxy. Results indicated that hydrolysis of PAN and cationic-dyeable PET fibers provided better sustained release of Doxy from the substrates. On the other hand, unhydrolyzed fibers showed initial infection resistant property, but Doxy sorbed was quickly released from the substrates,

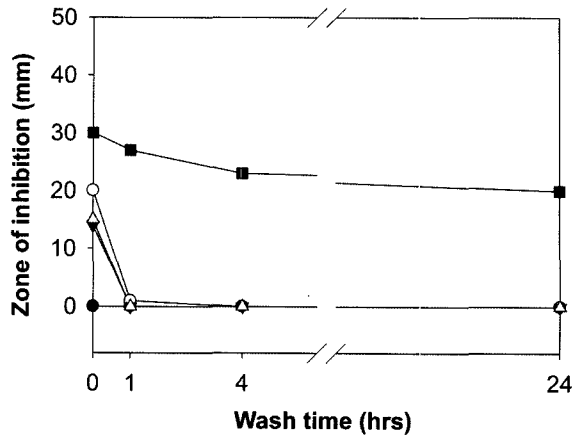

Figure 10. Zone of inhibition of Dacron64 fibers treated by Doxy at pH 2; (●) untreated, (○) treated at 45 °C, (▼) 85 °C, (△) 100 °C, (■) hydrolyzed and treated at 100 °C.

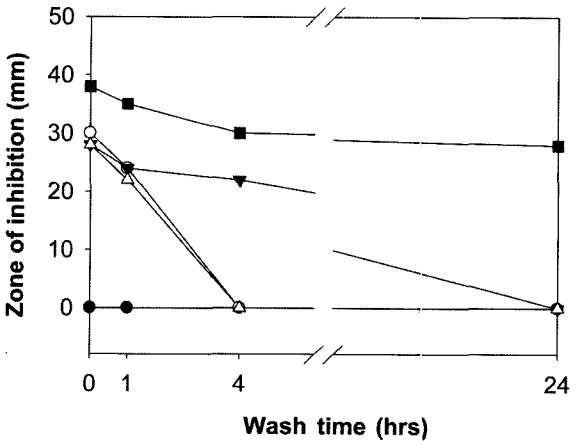

Figure 11. Zone of inhibition of Orlon fibers treated by Doxy at pH 2; (●) untreated, (○) treated at 45 °C, (▼) 85 °C, (△) 100 °C, (■) hydrolyzed and treated at 100 °C.

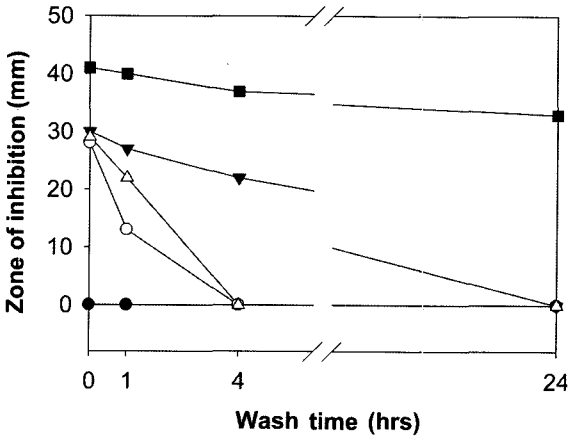

Figure 12. Zone of inhibition of Acrilan fibers treated by Doxy at pH 2; (●) untreated, (○) treated at 45 °C, (▼) 85 °C, (△) 100 °C, (■) hydrolyzed and treated at 100 °C.

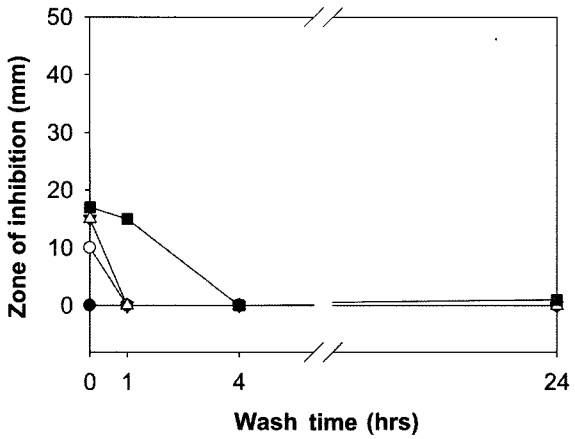

Figure 13. Zone of inhibition of Dacron54 fibers treated by Doxy at pH 2; (●) untreated, (○) treated at 45 °C, (▼) 85 °C, (△) 100 °C, (■) hydrolyzed and treated at 100 °C.

resulting in zero ZOI values within four hours wash time. It should be noted that in cationic-dyeable PET improvement of Doxy sorption due to hydrolysis was not considerable, but the hydrolyzed fabric exhibited much greater ZOI value at 24 hours of wash time, suggesting sustained infection resistance characteristics. In the case of regular PET, however, hydrolysis did not improve both ZOI values and sustained release of antibiotics.

Conclusions

A representative antibiotic, doxycycline (Doxy), 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 both PAN fibers sorbed considerable amounts of Doxy at 100 °C for 3.5 hrs in acidic condition (pH=2). Sorption of Doxy on cationic-dyeable PET was much lower than that on PAN fiber. However, zone of inhibition (ZOI) values of the unhydrolyzed fibers approached zero within four hours wash time, indicating rapid release of the antibiotics from the substrates. On the other hand, the hydrolyzed PAN fibers showed greater sorption of antibiotic as well as higher ZOI values at 24 hours wash time. Cationic-dyeable PET fiber showed little increase in sorption of Doxy by hydrolysis, but the hydrolyzed fiber exhibited greater ZOI values. Contrarily, no improvement was shown in both sorption and ZOI value of the hydrolyzed regular PET fiber at the condition applied. 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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