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NEW APPROACH FOR IMPARTING ANTIMICROBIAL PROPERTIES FOR POLYAMIDE AND WOOL CONTAINING FABRICS

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Abstract

Direct fiber polymer/antibiotic interaction is a promising means of providing infection resistance textile fabrics. Ionic interaction between cationic reactive groups (antibiotic) and carboxylic groups in wool, wool/polyamide, wool/polyester and polyamide was used as tools to develop desirable durable antimicrobial fabrics. The finishing conditions such as pH, finishing temperature, and time were studied.

The results revealed that pH of the finishing bath and the antibiotic concentration as well as finishing temperature were very critical parameters in affecting exhaustion of the antibiotic by the fabric along with the extent of ionic interactions. Sorption of doxymycin antibiotic follows the descending order: wool>wool blends>polyamide. Zone of inhibition of all treated fabrics is governed by the pH and follows the descending order:

$$pH\ 6.5 > pH9 > pH\ 2.3.$$

FTIR Spectroscopy confirmed the ionic interaction between wool 100 %, wool/polyamide (50%/50%) and doxymycin, because of appearance of new bands at 1641cm^{-1} which attributed to NH_2 deformation amide I and band at 1514 cm^{-1} corresponding to amide II { in case of wool 100 %}.For wool/polyamide blend it is noticed that appearance of new bands at 1415 cm^{-1} corresponding to C-N stretching (amide III band for $CONH_2$,in addition to the appearance of new band at 927 cm^{-1}which corresponding to CO-NH in plane.

Reusing of the treatment bath was tried and proved to be very effective approach to produce infection resistant biomaterials for medical applications.

Key words: wool, wool blends, doxymycin, biomaterial, durability, reusing.

Introduction:

Clothing and textile materials are not only carriers of microorganism such as pathogenic bacteria, odor generating bacteria, fungi, but also good media for growth of microorganisms. Textile in hospitals may be responsible to some cross infection or transmition of dermal disease .At the same time the number of applications for textile materials in medicine and surgery are huge and diverse ranging from a single thread suture to the complex composite structure for bone replacement [1].

Novel textile materials with particular functions that can provide healthy benefits to people have attracted much attention in recent years[2]. Antimicrobial is one of the important functions which can be achieved by chemical finishing .In order to confer this special property on textiles materials, functional finishing must be incorporated into the polymer molecules of the fiber. Some of the specific textiles items in which it is desirable to have antibacterial activity include the following foundation garments, undergarments, outergarment, wash cloths, bandage, and military clothing[3]. The requirements of durability vary quite widely depending on the end use conditions.

The ionic bonding method for achieving durable antibacterial activity is applicable to both anionic and cationic active bactericidal and bacteriostatic agents. There is one step reactions may be used depending on the fiber type. The basic principle employed is formation of salt link between the fiber and antibacterial agents .

$$\text{Fiber:COO}^{-} + \text{R}\overset{+}{\text{N}}\text{H}_3 \rightleftharpoons \text{Fiber.COO}^{-}\,{}^{+}\text{NH}_3\text{R}$$

In case of proteinic and polyamide which have both cationic and anionic groups, both types of antibacterial agents may be employed by adjusting ambient pH condition.[3].

Antimicrobial agents can be either incorporated within the fiber structure at the spinning stage[4], or can be applied on the surface of the fibers, yarn, or fabrics as finishing or coating[5], depending on product type and its intended applications[6]. Incroporation of antibiotic onto the biomaterial surfaces is one option for preventing the surface infection[7].

All tetracyclines have similar antimicrobial spectra with activity against many Gram-positive aerobic cocci including Staphylococci and Streptococci[8] Doxycycline is a long-acting tetracycline that acts against susceptible organisms by inhibiting protein synthesis. It enters bacteria by an energy dependent process and bind reversibly to the 3OS ribosomal sub-units of the bacteria[9].

Wool fiber has not been used for any biomedical applications, probably because of its short fiber length, low strength, and high price. Nevertheless wool is one of the most absorbent fibers available and can be easily made into any of the usual textile structural forms suitable for extracorporeal applications[10] .In addition to conventional biomedical applications, there are ever growing concerns of exposure to bacteria associated with biological warfare and spread of contagious diseases such as severe acute respiratory syndrome (SARS) .These concerns prompt the development of novel infection- resistant biomedical gear [10].

The main goal of this study is to impart an infection resistant functional property, without the use of exogenous binders, via incorporating antibiotic into fiber polymer (wool, wool/polyamide, wool/polyester and polyamide) to increase their applications in medicine and surgery and for other several medical applications.

Experimental

Fabrics

Different fabrics were used in this study, {wool 100 %, wool blend purchased from Misr Co. for spinning and weaving (Mehalla El Kubra)},and polyamide purchased from Misr Rayon Co. Kafr El Dawar, Egypt.Details about these fabrics are given in tables 1,2.

All fabrics were washed separately in a bath containing sodium carbonate (2 g/l), non-ionic detergents (5 g/L) at 50 C^0 for 1 hour. The fabrics were then thoroughly rinsed with tap water and finally air dried at ambient temperature.

Materials

Doxymycin antibiotic purchased from the Nile Company for pharmaceutical and chemical industries, Cairo, Egypt in pure grade and use as supplied. Other chemicals such as sodium hydroxide, glacial acetic acid purchased from El-Naser pharmaceutical chemical Company in reagent grade, and a non-ionic detergent Hostpal® CVL-EL Clarient of commercial grade.

Procedure

The fabric treatment was done in a laboratory dyeing machine (HTHP Sample dyeing MIC Model LL, R.B- Electronic & Engineering PVT, LTD, India) using Doxymycin concentration (1-4 %) owf at different pH's, temperatures (65 -100^{0}C) and times of treatment. The pH was adjusted by controlled addition of 1 % acetic acid or1 % NaOH. After the treatment process was run, the samples were removed, rinsed thoroughly with tap water and allow to dry in the open air.

Analysis

Determination of the fabric sorption of doxymycin, and the concentration of the residual antibiotic were measured using Shimadzu UV/Visible spectrophotometer at λmax 274nm. The relationships between absorbance and concentration was established at λmax. The extent of exhaustion expressed as E % was calculated according to the following equation:

$$E\% = \frac{Ci - Cf}{Ci} \times 100$$

Where C_i and C_f are the doxymycin concentration before and after treatment respectively.

Lightness L* values of untreated and treated fabrics were evaluated using Hnterlab D25M Optical sensor. The infrared spectra of the untreated wool, wool/polyamide 50/50 were evaluated using Nicolete 380 FTIR spectrophotometer in attenuation total Reflection ATR Mode with Zinc selenium crystal in wavelength Range 650- 4000 cm^{-1}

Durability test

The durability of the treated fabric against repeated laundering were evaluated by washing fabrics according to AATCC test method 124-1996 [11],subjected to 1 and 5 washing cycles in presence of a non ionic detergent.

Reuse of treatment bath

The exhausted baths were reused two times respectively, to treat all fabrics under investigation. Predeterminations of the doxymycin concentration were carried out keeping the material to liquor ratio fixed.

Antimicrobial activity of the treated fabrics
Microorganisms used

Microorganisms used in this study are given in Table3. Five bacterial and one fungal species were subjected to antimicrobial activity test of treated fabrics. These microorganisms were obtained from Microbial Chemistry Department, National Research Center, Cairo, Egypt.

Table 3: microorganism classification

Test microorganism	**Classification**	**Abbreviation**
Bacillus subtilis	Gram-positive bacteria	Bs
Bacillus cereus	Gram-positive bacteria	Bc
Escherichia coli	Gram-negative bacteria	Ec
Pseudomonas aeruginosa	Gram-negative bacteria	Pa
Staphylococcus aureus	Gram-positive bacteria	Sa
Candida albicans	Yeast	Ca

Media used

Nutrient broth/agar medium: contains peptone (5 g/l), beef extract (3g/l). For solid medium (15 g/l) agar was added. Malt broth/agar medium: contains peptone (5 g/l), malt extract (24g/l) . For solid medium (15 g/l) agar was added.

Growth conditions

An inoculums of each bacterial strain was suspended in 25 mL of nutrient broth medium and shaken for 24 h at 37°C. For yeast, malt broth was inoculated with test organism and incubated at 28 ^{0} C for 24 h.

Antimicrobial activity test

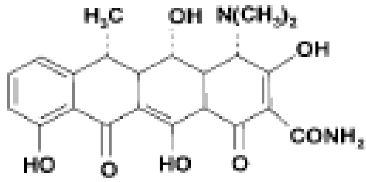
Disc diffusion method with some modification was used for screening the treated fabric samples for antimicrobial activity[12]. Nutrient agar (for bacteria) or malt agar (for yeast) plates were inoculated with 0.1 mL of an appropriate dilution of the tested culture. Fabric samples (1 cm diameter) were placed on the surface of the inoculated plates. The plates were incubated at the appropriate temperature for 24 h. Diameter of inhibition zone (mm) including the disc diameter was measured for each treatment.

Table1: Specification of woven fabrics

Woven Fabric	Fabric specification		
	No. of ends/inch	No. of picks/inch	Fabric weight g.m^{-2}
Wool 100 %	63	58	266
Wool/PA 50/50	48	46	246
Wool/PA 25/75	50	46	246
Wool/PET 40/60	52	48	186

Table 2: Specification of knit fabrics

knitted Fabric	Fabric specification		
	No. of walls	No. of courses	Fabric weight g. m^{-2-}
polyamide	44	52	103



Structure of doxymycin

Results and Discussions

Thermal stability of antibiotic

The colour of aqueous solution of doxymycin tended to darken on standing. The colour change was associated with the change that occurred in the region of a second absorption maximum peak (~ 345nm).

A set of experiments was designed to examine the effect of treatment temperature, time and pH on the thermal stability of doxymycin solution within the examined pH range. The results revealed that increasing the treatment duration leads to increasing the decomposition of doxymycin (figure 1) and the degree of the decomposition was higher at pH 9. Increasing the treatment temperature enhances increased the decomposition of doxymycin as shown in (figure 2) and the percent of decomposition increased linearly with increasing temperature.

Extent of exhaustion of Doxymycin

Another set of experiments was designed to examine several factors affecting the extent of exhaustion on all type of fabrics used in this study. Table 4 shows the effect of both pH of the treatment bath and treatment duration on sorption of doxymycin on wool, wool/Polyamide (with different blending ratio), polyamide and wool/polyester .It was found that the exhaustion % of doxymycin by wool was always higher than that for all wool blends and polyamide regardless of pH of the treatment bath .Also increasing the wool content of the used blends leads to an increase in exhaustion % of doxymycin. This may be attributed to the combined effect of lower crystallinity and grater abundance of polar function groups on wool. On the other hand wool and doxymycin are amphoteric in nature i.e. sorption of doxymycin is expected to be largely dependent on the bath pH. The isoelectric points of wool and doxymycin are approximately 5 [10].The results revealed that higher doxymycin sorption % occurred at pH 6.5 than that at other pH’s. This implies that an electrostatic repulsion between the positively charged substrates and doxymycin played an important role on exhaustion of doxymycin under acidic pH, i.e. the positive charges derive from easy protonation of basic nitrogen in wool and polyamide produce a columbic repulsion towards the reactive cationic group in doxymycin[10]. On the other hand under higher pH value, the more easily the nucleophilic substitutive reaction takes place, more free amino groups are available and the fabric surface is electronegative, whereas the same charge was less likely to occur on doxymycin because the ionization of oxygen in hydroxyl would be much more difficult to achieve, resulting in higher exhaustion. On the other hand the higher decomposition % of doxymycin at pH 9 plays an important role in decreasing the sorption of doxymycin on fabrics than at pH 6.5 as shown in (figure 3).

Table 4 Effect of pH, treatment time as well as type of substrates on the extent of doxymycin exhaustion %

Fabric type	pH 2.3				pH 6.5				pH 9			
	Treatment time (hrs)				Treatment time (hrs)				Treatment time (hrs)			
	1	2	3	4	1	2	3	4	1	2	3	4
Wool 100 %	3.4	8.1	11.6	15.9	55.4	59.1	61.6	65	31.66	32	34.3	38
wool/polyamide 50%/50%	2.6	6.33	8.1	8.4	49	50	51	51	22.5	24	26.5	27
wool/polyamide 25 %/75 %	2.1	3.6	4.8	6.5	35.8	38.7	41.7	42	20	22	21	23
Polyamide	1.6	1.65	1.8	4.93	16	18.9	19.4	25	10.44	10.4	12	17.8
wool/polyester 40% / 60%	2.5	2.9	4.5	4.12	28.5	32.1	33	34.5	14	14.3	15.7	16.8

Treatment condition: Treatment temperature 85^0C, 2 % (owf) doxymycin concentration, material to liquor ratio 1:60

Another set of experiments was designed to examine the effect of treatment temperature on the exhaustion % of doxymycin (figure 4). Increasing treatment temperature leads to increasing the doxymycin exhaustion which may be explained on the basis of increasing the swelling and depending on the polymer structures thereby incorporating doxymycin molecules onto and/or into the polymer matrix followed by fixation through ionic bonds. Whereas for wool and wool/polyamide blends raising the treatment temperature more than 65°C (which is higher than Tg of polyamide) has no significant effect on the exhaustion %.At 100°C the higher % of decomposition leads to decrease in doxymycin exhaustion. Depending on the above results applying doxymycin to wool at lower temperature is preferable to minimize doxymycin decomposition at higher temperature.

Diffusion of doxymycin into fabrics depends on its concentration in the treatment bath. To study the effect of doxymycin concentration, set of experiments was designed at optimum pH. It was found that increasing doxymycin up to 4 % leads to a decrease in extent of exhaustion of doxymycin, (figure 5). This trend is common for all types of fabrics used in this study. This can be attributed to the limited available active sites COOH groups on and/or wthin the treated substrates[13]. This might indicate that 1 % (owf) is enough to create sufficient exhaustion for all types of fabric.

Extent of lightness of treated fabrics.

Doxymycin treated substrates showed light to dark brown color. Lightness of treated fabrics was normalized against that of pristine fabric. The colour produced at different temperature, at different pH's, and duration of treatment was readily apparent by visual analysis (figures 6-8). Normalized lightness L* of doxymycin treated fabrics is continuously decreased (increasing the darkness) with the increase in treatment temperature .Also it was found that the decreased Lightness (increased color change) occurred as a result of increasing the treatment time and pH of the treatment bath. This trend is held true for all types of fabrics used.

Reusing of treatment bath

The exhausted treatment baths were reused two times successively in an attempt to get two targets. The first one is to maximize the benefit of doxymycin solution as possible, and the second one is decreasing the amount of antibiotic released to waste water. The amount of doxymycin in solution was determined before and after each use for each fabric sample individually. (Figure 9) and table 5 demonstrate that reusing of doxymycin solution could be selected according to the exhaustion % of doxymycin and its antimicrobial activity. Table 5 shows that the antimicrobial activity of wool, wool/polyamide blends and polyamide (treated with reused bath for two times successively). It was found that slightly decreased of antimicrobial activity comparing with those treated with freshly prepared doxymycin solutions and still have higher antimicrobial activity against gram +ve, gram –ve bacteria and yeast. Table 8 revealed that reusing of doxymycin solution could be selected according to antimicrobial activity of the treated substrates.

Table 5: Antimicrobial activity of different fabrics treated with 2 % (owf) doxymycin and reuse of the treatment bath two times successively.

Microorganism tested	Antimicrobial activity (Zone of Inhibition mm)														
	First treatment					First reuse					Second reuse				
	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa
Wool 100 %	30	28	34	38	38	28	26	30	36	26	22	24	20	38	26
wool/polyamide 50%/50%	32	34	34	40	40	30	32	32	42	36	28	26	30	38	28
Wool/polyamide 25 %/75 %	30	28	34	40	38	30	32	30	40	36	30	30	24	40	34
Polyamide	28	30	34	38	34	24	24	26	38	34	28	26	26	38	32

Treatment conditions: Temperature 85 ^{o}C, Time 1 hr, pH 6.5, Material to liquor ratio 1:60

Normalized lightness of doxymycin treated substrates (either treated with freshly prepared solution or treated with reused treatment bath i.e. two times successively), decrease with the first reuse than either normalized lightness of doxymycin treated substrates treated with freshly prepared solution or that treated with the second reuse treatment bath, (figure 10). This may be attributed to the decreasing of the concentration of the second reuse treatment bath, than the first reuse treatment bath.

Antimicrobial Assessment

Sustained release of antimicrobial agent from infection resistance biomaterials is very important in implanted or percutaneous devices, but also in extra-corporate situation[10].Effiacy of antibiotic treated substrates can be analyzed by use of the zone of inhibition (ZOI) test. ZOI of treated fabrics at doxymycin concentration 2 % and at temperature 85^{o}C showed the following order : pH 6.5>pH 9>pH 2.3 this agree with other work[10], this may indicate that the order of ZOI was correlated to the sorption of doxymycin.

Measurement of doxymycin released from the treated fabrics at the studied three pH level revealed the effectiveness of the antimicrobial activity against Bc, Sa, Ec, Ca and Pa for the used fabrics (table 6). Treated wool shows slightly higher ZOI than wool blends .This may be attributed to the higher content of polar function groups thereby enhancing antibiotic sorption[15].

All treated fabrics in this investigation showed antimicrobial activity against all type of microbes used in this study. It was noticed that there is partially correlation between the extent of exhaustion of doxymycin and ZOI. Table 7 demonstrates that 4 % owf gives the lowest ZOI, this trend is the same for all types of fabrics. This can be explained on the basis that increasing doxymycin concentration in the treatment bath might exceed available reactive groups on the fibers, since at concentration 4 %, the exhaustion % of doxymycin is about less than half that at 2 %(owf) [13], and this clearly reflects on ZOI.

Table 6: Antimicrobial activity of different fabric treated with different pH

Microorganism tested	Antimicrobial activity (inhibition Zones on mm)														
	pH .3					pH 6.5					pH 9				
	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa
Wool 100 %	17	22	34	27	30	32	32	34	38	40	29	32	36	49	40
wool/polyamide 50%/50%	20	15	26	27	32	32	31	32	47	46	22	29	32	37	36
Wool/polyamide 25 %/75 %	24	24	29	35	36	32	32	28	43	43	32	34	20	37	45
Polyamide	20	10	10	35	27	30	30	30	41	41	16	10	14	33	29
Wool/polyester 40% / 60%	22	10	10	30	30	24	30	3	34	36	26	24	20	34	36

Table 7: Antimicrobial activity of different fabric treated with different doxymycin concentration

Microorganism Tested	Antimicrobial activity (inhibition Zones on mm)														
	1 %					2 %					4 %				
	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa	Bc	Sa	Ec	Ca	Pa
Wool 100 %	34	32	34	41	48	32	32	34	44	49	28	38	39	47	37
wool/polyamide 50%/50%	32	38	29	44	49	37	31	32	47	48	29	32	32	43	49
Wool/polyamide 25 %/75 %	32	42	33	49	43	32	32	38	43	43	32	33	30	38	43
Polyamide	30	35	25	41	44	30	30	30	41	41	23	28	25	41	41
Wool/polyester 40% / 60%	25	41	27	48	48	24	30	30	34	36	20	30	27	43	34

Durability to laundering

Durability of antimicrobial activity to washing is one of the major concerns of textiles researchers and users because textiles are subjected to frequent laundering. Table 9 depicts the durability of antimicrobial after five times repeated home laundering. The results revealed that no significant difference between ZOI of unwashed and washed sample (one wash and five washes), which means that the treated samples do not lost their biocidal properties after five washes which give indication that the strong ionic interaction between doxymycin and anionic carboxylate on the polymer confers such durable antimicrobial function[1] .Also treatment the substrates with reused bath gives high antimicrobial properties i.e. ZOI ,as there is no deference between ZOI for unwashed and washed one ,which means the possibility to use this technique to get antimicrobial properties as well as to maximize the benefits from the residual in the treatment bath as shown in (figure 11).

Table 8: Antimicrobial activity of different fabrics treated with 2 %(owf) doxymycin and reuse of the treatment bath two times successively

Microorganism tested	Antimicrobial activity (Zone of Inhibition mm)														
	First use					**Second use**					**Third use**				
	Bc	**Sa**	**Ec**	**Ca**	**Pa**	**Bc**	**Sa**	**Ec**	**Ca**	**Pa**	**Bc**	**Sa**	**Ec**	**Ca**	**Pa**
Wool 100 %	34	32	34	44	40	36	24	28	46	26	36	26	20	44	28
wool/polyamide 50%/50%	37	31	32	47	40	39	31	32	49	36	33	33	30	49	28
Wool/polyamide 25 %/75 %	30	32	28	43	43	30	32	24	43	41	30	28	26	43	39
Polyamide	34	30	30	41	41	38	38	26	41	41	34	30	20	41	32

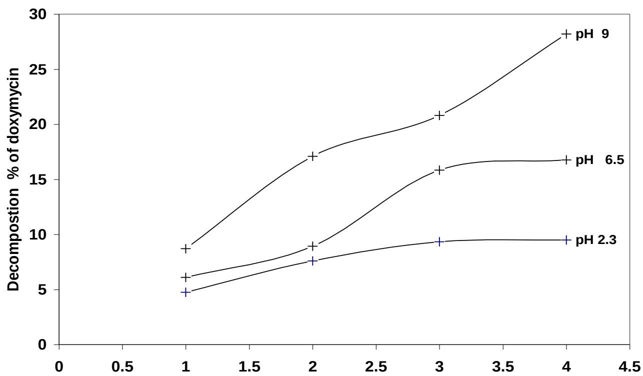
Treatment conditions: Temperature 85 ^{o}C, Time 1 hr, pH 6.5, Material to liquor ratio 1:60

Table 9: Antimicrobial activity of different treated fabrics treated with doxymycin after successive washings (AATCC laundry)

Microorganism tested	Antimicrobial activity (inhibition Zones on mm)									
	1 wash cycle					**5 wash cycles**				
	Bc	**Sa**	**E**	**Ca**	**Pa**	**Bc**	**Sa**	**E**	**Ca**	**Pa**
Wool 100 %	28	36	34	30	34	30	30	30	40	34
wool/polyamide 50%/50%	26	32	34	40	36	28	34	34	38	34
Wool/polyamide 25 %/75 %	20	34	30	42	36	26	32	34	40	14
Polyamide	20	24	22	36	26	22	22	26	38	26

FTIR Analysis

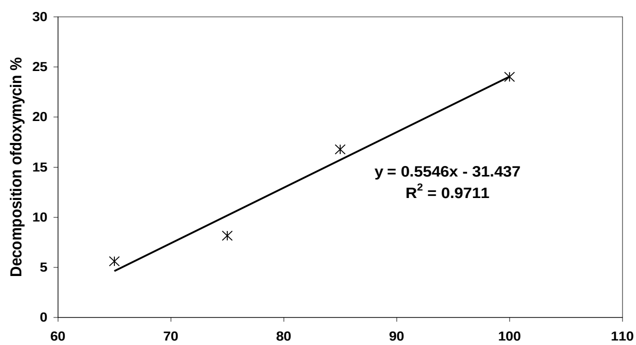
FTIR spectroscopic analysis was used to investigate the effect of treatment of fabrics (wool, wool/polyamide 50/50) with doxymycin, (figure 12). With respect to wool the most important differences was found is the appearance of new band at 1641 cm^{-1} which may be attributed to NH_2 deformation amide I i.e. $–CONH_2$, also appearance of new band 1514 cm^{-1} corresponding to amid II, this give evidence to the ionic interaction between doxymycin and carboxylate anion of the polymer[16]. With respect to wool/polyamide 50%:50%, it was found that, appearance of new bands at 1415 cm^{-1} which correspond to C-N stretch (amid III band for $CONH_2$). Rang between 1100-900 cm^{-1} is the most interesting. It is also called fingerprint region, characteristics for each individual molecule. The comparison of original spectra already indicates the appearance of new band at 927 cm^{-1} which corresponding to CO-NH in plane.



Treatment time (hr)

Figure 1: Effect of treatment time & pH of the treatment bath on the thermal stability of doxymycin

Treatment Condition: time 4 hr., doxymycin concentration 2 %(owf) , Material to liquor ratio 1:60



Treatment temperature (^oC)

Figure 2: Effect of treatment temperature on the thermal stability of doxymycin
Treatment Condition: time 4 hr., doxymycin concentration 2 % (owf), pH 6.5, Material to liquor ratio 1:60

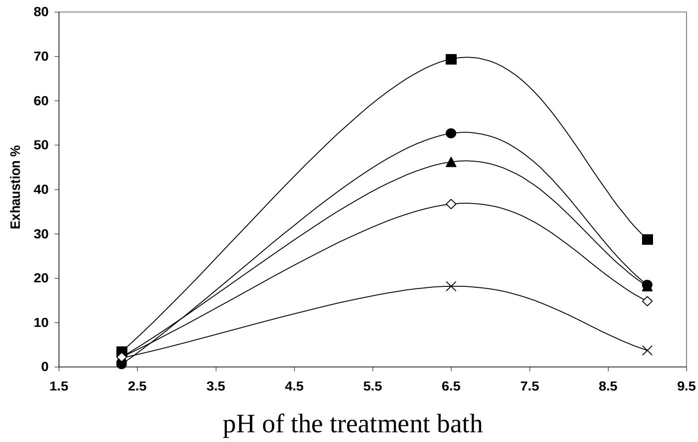


Figure 3 : Effect of pH of the treatment bath on the exhaustion % of doxymycin
Treatment Conditions: Treatment temperature 85 ^{0}C, treatment time 4 hrs, 2 % owf doxymycin , Material to liquor ratio 1:60

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%
▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide
◊----◊ Wool/PET 40% 60 %

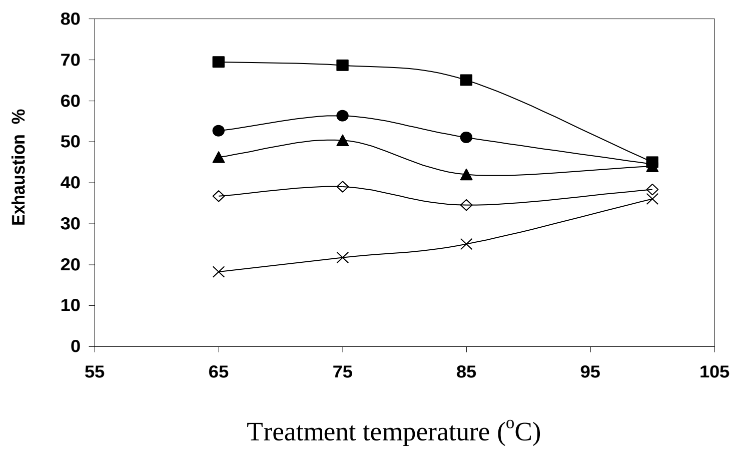


Figure 4: Effect of treatment temperature on the Exhaustion % of doxymycin for different substrate
Treatment Condition: Concentration of Doxymycin 2 % owf ,pH 6.5, Material to liquor ratio 1:60 ,time 4 hr

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%
▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide
◊----◊ Wool/PET 40% 60 %

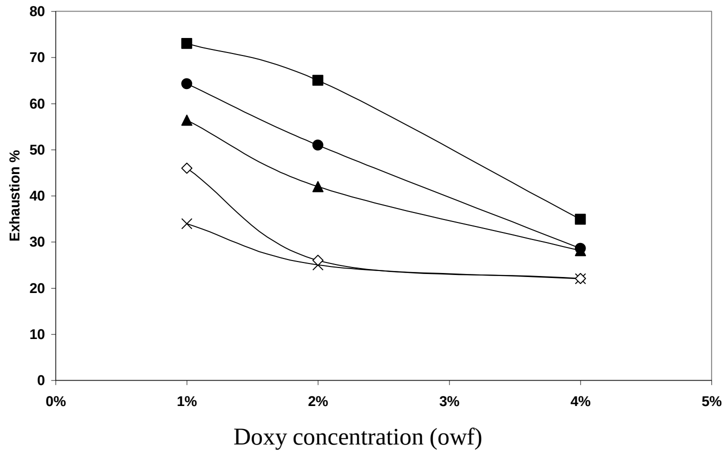


Figure 5: Effect of Doxy concentrations on the Exhaustion % of treated fabric
Treatment condition Treatment temperature 85 ^{0} C, Time 4 hrs, M: R 1:60

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%
▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide
◊----◊ Wool/PET 40% 60 %

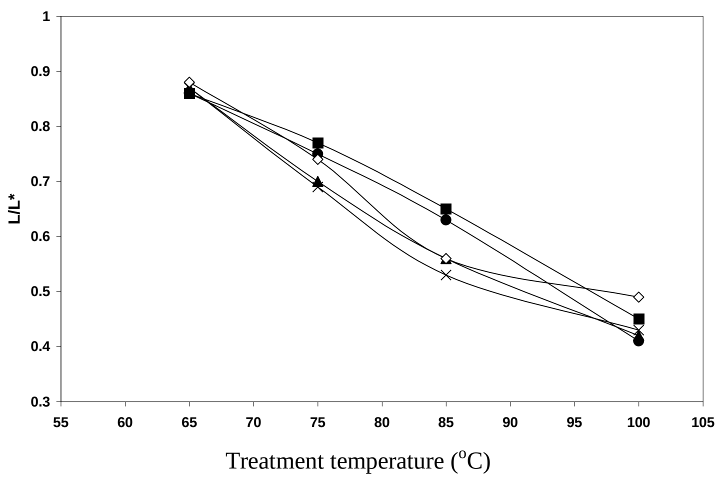


Figure 6: Effect of treatment temperature & type of substrates on the lightness of the fabric
Treatment Condition: Temperature 85 ^{0}C, pH 6.5, 2 % doxymycin (owf) Material to Liquor ratio 1:60

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%
▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide
◊----◊ Wool/PET 40% 60 %

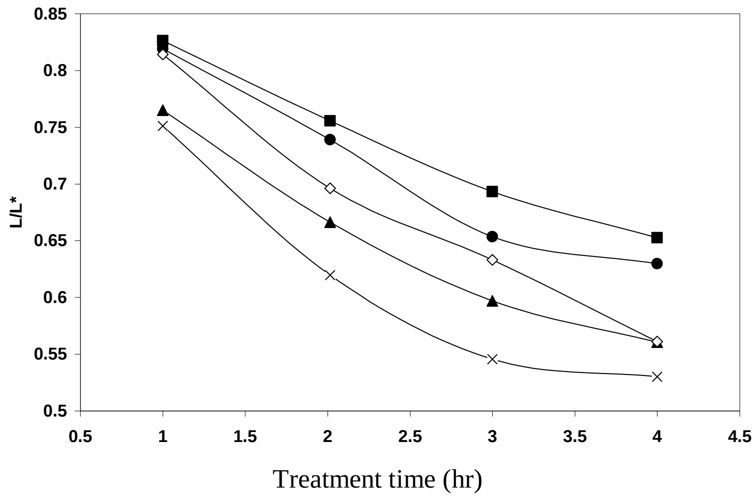


Figure 7: Effect of treatment temperature & type of substrates on the lightness of treated fabrics

Treatment Condition: Temperature 85 ^{0}C, pH 6.5, 2 % doxymycin (owf) Material to Liquor ratio 1:60

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%

▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide

◊----◊ Wool/PET 40%/ 60 %

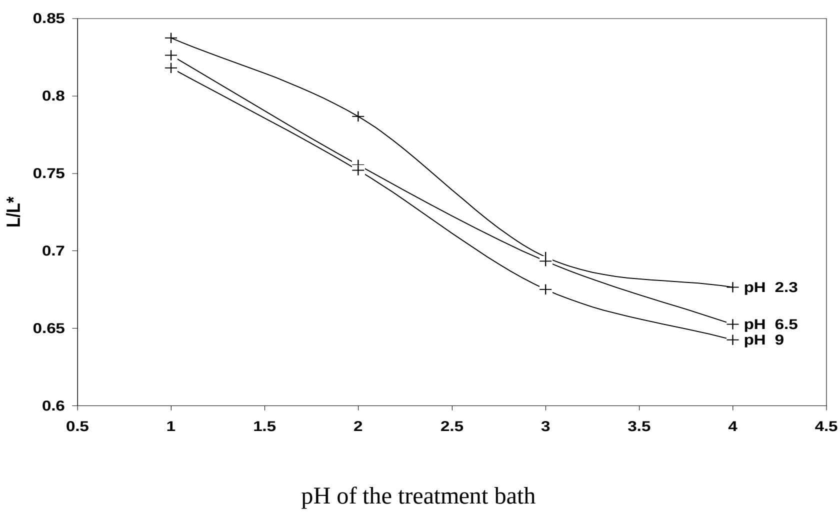


Figure 8: Effect of pH of the treatment bath on the lightness of wool 100 % treated fabric

Treatment Condition: Temperature 85 ^{0}C, pH 6.5, 2 % doxymycin (owf) Material to Liquor ratio 1:60

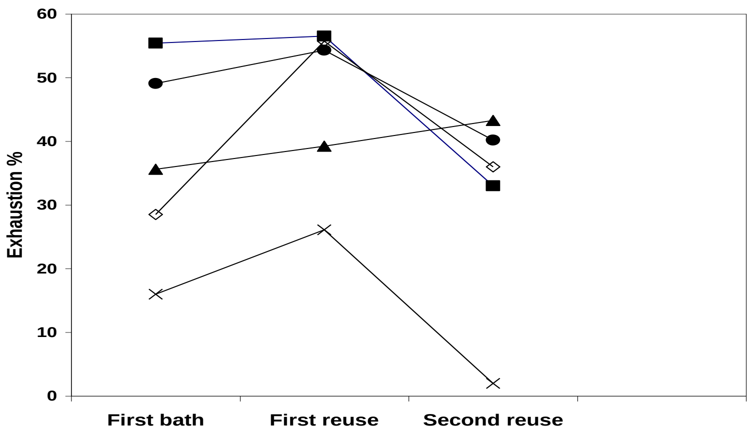


Figure 9: Effect of treatment and reused treatment bath on the lightness of treated fabrics

Treatment Condition: Temperature 85 ^{0}C, pH 6.5, 2 % doxymycin (owf) Material to Liquor ratio 1:60

■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%

▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide

◊----◊ Wool/PET 40%/ 60 %

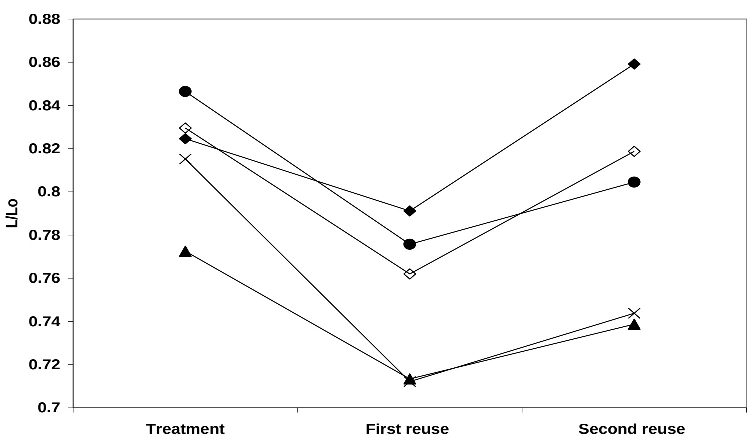


Figure 10: **Effect of treatment and reused treatment bath on the lightness of treated fabrics**

Treatment Condition: Temperature 85 ^{0}C, pH 6.5, 2 % doxymycin (owf) Material to Liquor ratio 1:60

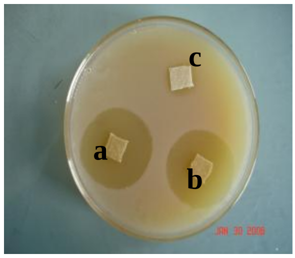
■ -----■ 100 % wool ●---● wool / polyamide 50 %/50%

▲ ---- ▲ Wool / polyamide 25 %/ 75 % ×------× 100 % Polyamide

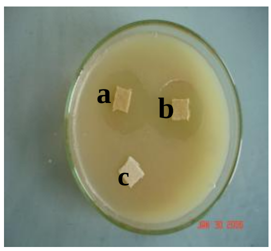
◊----◊ Wool/PET 40%/ 60 %

Figure 11: Zone of Inhibition of washed and unwashed treated substrate

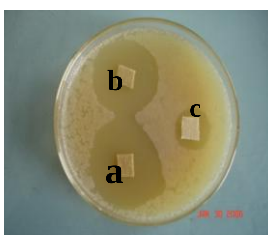
ZOI of wool/polyamide (50/50) treated with doxymycin.
(a} unwashed treated sample (b) five times washed treated sample (c) untreated sample against (Gram –ve bacteria Pa)



ZOI of Wool/PA treated with doxymycin
(a} unwashed treated sample (b) five times washed treated sample (c) untreated sample against (Gram +ve bacteria Sa)



ZOI of Wool/PA(50/50) treated with doxymycin
(a} unwashed treated sample (b) five times washed treated sample (c) untreated sample against Ca



Treatment Condition: Temperature 85 ^{o}C, Time 4 hr., pH 6.5, M: L 1:60, 2 % doxymycin (owf)

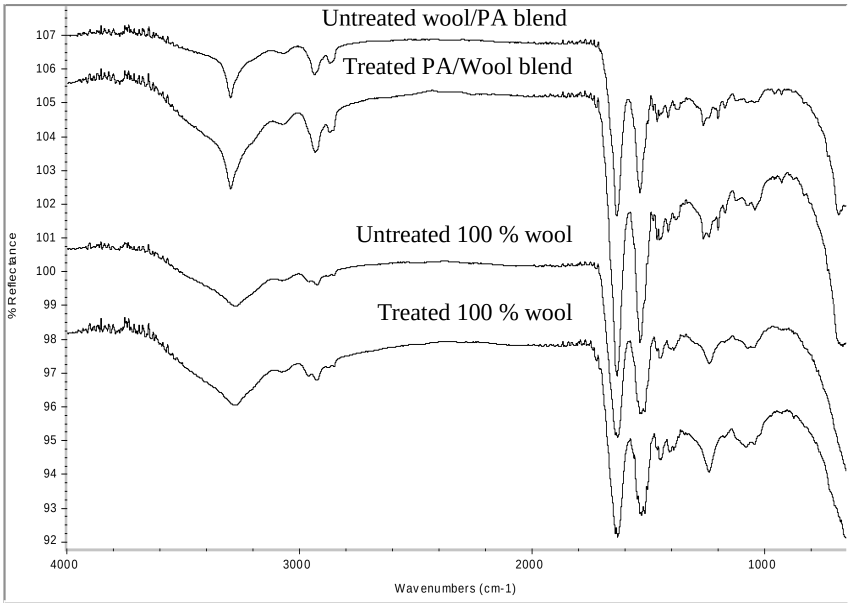


Figure 12 .FTIR spectra of treated and untreated fabrics
Treatment condition: treatment temperature 85 ^{o}C, treatment time: 4 hr, pH 6.5, Material: liquor ratio 1:60

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