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A new approach to evaluate antibacterial activity of textile materials using image processing technique

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A new method has been developed for evaluating the antibacterial activity of sample based on the area instead of the length of zone of inhibition, using image processing technique. The suggested method has been carried out on 51 Ag coated antibacterial fabrics and the results are compared with those of the standard test method. It is observed that, the results obtained from the present study follow the same trend as in standard method, in almost all samples with correlation coefficient of 0.96; this shows the reliability of described method.

Keywords: Antibacterial activity, Image processing technique, Parameter Z, Zone of inhibition

1 Introduction

It has long been recognized that the microorganisms can grow on natural fibre such as cotton due to their porous hydrophilic structures which provide a perfect environment for their growth. These microorganisms can cause fibre degradation by feeding on reactive sites[1]. Microbial infestation has unpleasant consequences such as stains, odors, and loss of functional properties, tensile strength and elasticity, along with disruption of textile manufacturing processes like dyeing, printing and finishing operations[2].

Antimicrobial finishing, on the other hand, kills bacteria or interferes with the multiplication, growth or activity of bacteria. In general, antimicrobial properties of textile materials can be achieved by incorporating functional agents such as zinc[3], silver[4,5] and titanium[6,7] onto fabrics, by either chemical[8,9] or physical finishing[10,11] or by modification of cotton using biopolymers[12,13] or minerals[14]. As a substitute, textile materials with antibacterial activity can be obtained with dyeing of textile fabrics with some natural dyes with inherent antimicrobial activity[15,16]. The antibacterial activity of fabrics can be examined both qualitatively, in which the major principal is zone diffusion assay, and quantitatively, which is based on cell suspension intimate contact test[17]. Quantitative procedure for evaluating the degree of antibacterial activity of textile materials refers to AATCC Method 100, in which the antibacterial activity of fabrics was tested by the reduction of colony forming units (CFU) of desired microbe. The percentage reduction of CFU was calculated using the following relationship:

$$\text{Reduction in CFU (\%)} = \frac{C-A}{C} \times 100 \qquad \dots (1)$$

where C and A are the bacterial colonies of the untreated and the treated cotton fabrics respectively. In a valid test, the number of bacteria, detected from the inoculated untreated control specimen and incubated for the specified contact time, increases significantly respect to the inoculated untreated specimen swatches at zero contact time[18].

For qualitative evaluation, AATCC Method 147 provided relatively quick and easily executed method to demonstrate bacteriostatic activity by the diffusion of the antibacterial agent through agar. As a preliminary test to detect the diffusive antimicrobial finish, agar diffusion tests are simple to perform and are most suitable when a large number of samples are to be screened for the presence of antimicrobial activity. The Parallel Streak Method has proven effective in providing evidence of antibacterial activity against both Gram positive and Gram negative bacteria[19].

In these tests, bacterial cells are inoculated on nutrient agar plates over which textile samples are

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laid for close contact. The plates are then incubated at 37°C for 18–24 h and examined for growth of bacteria directly underneath the fabrics and immediately around the edges of the fabrics [zone of inhibition (ZOI)]. The zone of inhibition should not be expected if the antimicrobial agent is attached to the textile covalently which prevents its diffusion into the agar. If the antimicrobial agent can diffuse into the agar, a zone of inhibition becomes apparent and its mean size provides some indication of the potency of the antimicrobial activity[20].

One of the computerized technologies for advanced quality and structure inspection methods is the application of digital image processing. Digital image processing methods have been developed to utilize in textile industry in a variety of applied fields such as yarns[21] and fabric defect detection[22,23], analysis of drape behavior of woven fabrics[24] quality control[25,26] and so on.

The aim of the present study is to implement an alternative procedure to evaluate the antibacterial performance of treated fabrics using area of ZOI instead of the mean size of ZOI. To accomplish this, cotton fabrics coated with silver nitrate have been chosen as antibacterial specimen and antibacterial performance of specimens is investigated against Gram positive Staphylococcus aureus and based on AATCC method 147–1998. In order to calculate area of ZOI, image processing technique has been used through an in-house MATLAB Algorithm. After the area of ZOI is calculated, obtained results are compared with corresponding mean size of ZOI.

2 Materials and Methods

2.1 Materials

Silver nitrate ($AgNO_3$) and D-(+)-glucose monohydrate ($C_6H_{12}O_6 \cdot H_2O$) were purchased from Merck, Thailand, and then used without any further purification or treatment. Deionized water was used throughout the study. Simultaneous synthesis and finishing processes were used by means of a mechanical stirrer. The antibacterial tests were carried out under biological incubation.

The antibacterial activity of nano $AgNO_3$ coated cotton fabrics was investigated against Gram positive *Staphylococcus aureus* (AATCCTM 147–1998: Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method).

2.2 MATLAB Operation

At the first step, antibacterial performance of $AgNO_3$ coated fabrics was evaluated by measuring the mean ZOI size grown around the sample specimen, under the same condition of the size of fabrics and equal distance between camera and sample. Images were captured using a high definition camera in mentioned time intervals. Therefore, proper image with high resolution, good quality and correct illumination for image processing technique, is of critical importance. ZOI size in several points of the sample specimen was measured using the distance tool (imtool), which displays the Euclidean distance between the two endpoints of a line in pixel unit which is then converted to millimeter unit. After that, the average of acquired numbers was reported as mean ZOI size.

In the next step, parameter Z was calculated utilizing image processing technique. Image processing operations performed using the MATLAB Algorithm, are described below:

(i) Reading and displaying the acquired Red Green Black (RGB) image (imread)

(ii) Carrying out image binarization (im2bw). In fact, for a RGB image to change into binary format, all pixel values with lightness of less than a certain value will be reduced to 0, which will be the "off pixels"; and all pixel values with lightness of more than a certain value, will be increased to 1, which will be "on pixels".

(iii) Edge detection of the sample specimen using a gradient edge detection function (edge canny). This code is used for outline of each sample specimen (Q), as well as for the outline of ZOI (W).

(iv) Filling the holes using a flood-fill operation on binary images (imfill). For binary images, imfill changes the connected background pixels to foreground pixels, stopping when it reaches object boundaries.

(v) ZOI was obtained by subtracting W from Q, after conducting the stage 4. After that, the area of the ZOI (Z) was measured (bwarea). Z is a scalar value which corresponds roughly to the total number of on pixels in the image.

(vi) Finally, parameter Z was measured as follow:

$$\text{Parameter } Z = \frac{\text{Area of ZOI}}{\text{Area of sample}} = \frac{Z}{Q} \quad \dots (2)$$

3 Results and Discussion

It is well known that silver has the potential to be an excellent antibacterial agent. Thus, silver nanoparticles are selected as antibacterial agent, and the antibacterial activity of silver nanoparticle coated cotton fabrics is determined in the terms of zone of inhibition (ZOI) on agar medium. Figures 1(a) and (b) show antibacterial activity of control and treated cotton fabrics against Gram positive *Staphylococcus aureus*. Control specimen does not show any antibacterial activity but a distinct inhibition zone is formed around the silver coated cotton samples. The size of ZOI is measured using image processing tool box of MATLAB program. After that, the pixel unit is converted to millimeter unit.

Figure 1(b) shows that ZOI has different sizes in different positions around the sample specimen. Therefore, the size of ZOI for each sample should be measured in several points. After that, the mean of measured data should be calculated and reported as ZOI size. The mean, standard deviation and the difference between maximum and minimum values (Diff) of the corresponding ZOI for each sample are calculated and the results for all samples are summarized in Fig 2. The mean value for ZOI size varies in 0-1.58 range. In addition, standard deviation, that measures the amount of dispersion from the average is in 0-0.67 range; and Diff, a representative of the range of the data, fluctuates between 0 and 2.51 distance. Among the acquired data, Diff has the highest value, which indicates that ZOI do not grow homogeneously around the sample, which is obvious from Fig. 1(b). Having ZOI with heterogeneous size, makes it hard to compare antibacterial activity of different specimens. Obtained results are sufficient for evaluating a specimen for its antibacterial activity, but a more accurate procedure is required for comparative studies where the extent of antibacterial activity is necessary.

Accordingly, a new method has been suggested for evaluation of antibacterial activity of sample specimens based on the area instead of the size of ZOI. In order to do this, the area of ZOI was measured using image processing program. Figure 3 shows the step by step image processing operations performed using the MATLAB algorithm, for a typical sample. The area of sample specimen and ZOI were measured with counting the "on pixels" in binary image, and parameter Z was calculated and reported in Fig 4. This figure shows ZOI for FSN, Mix-SN-G and FG series of antibacterial specimens, with different coating time, together with

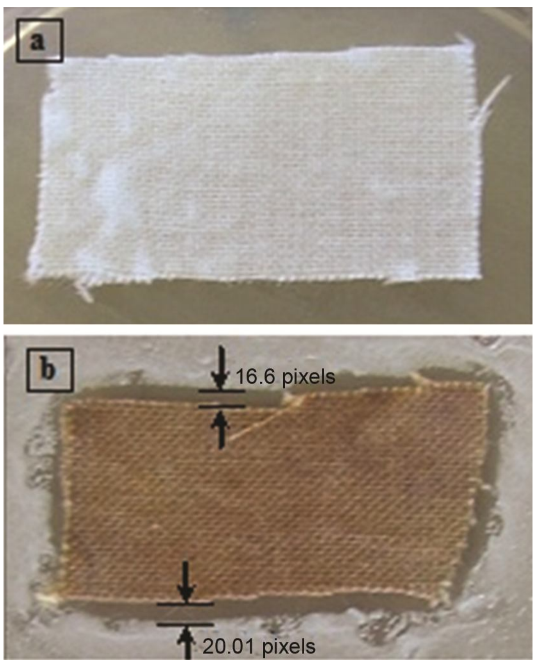

Fig. 1 — Antibacterial activity of (a) control and (b) treated -Mix-SN-G4- sample

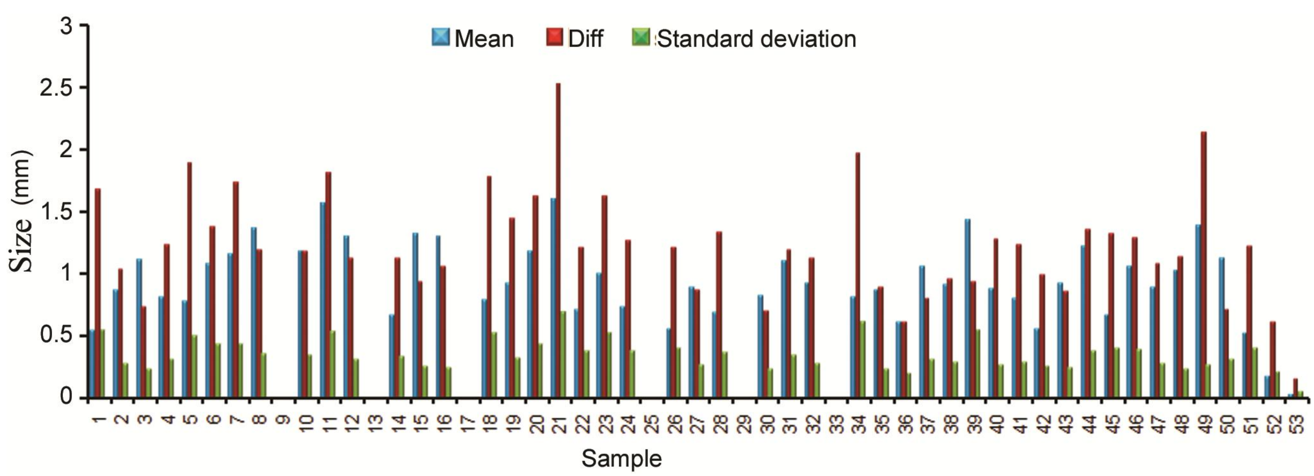

Fig. 2 — Mean, diff and standard deviation for ZOI size

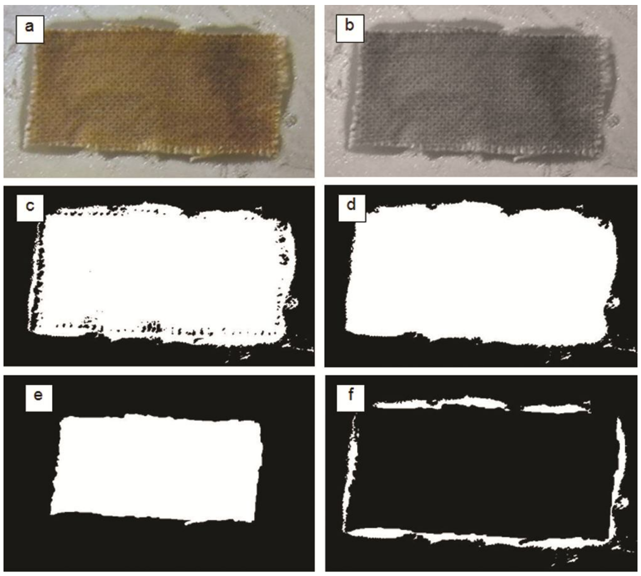

Fig. 3 — Image processing operations (a) experimental image, (b) gray scale image, (c) binary image, (d) areas of the sample specimen with associated ZOI, (e) area of sample specimen, and (f) zone of inhibition

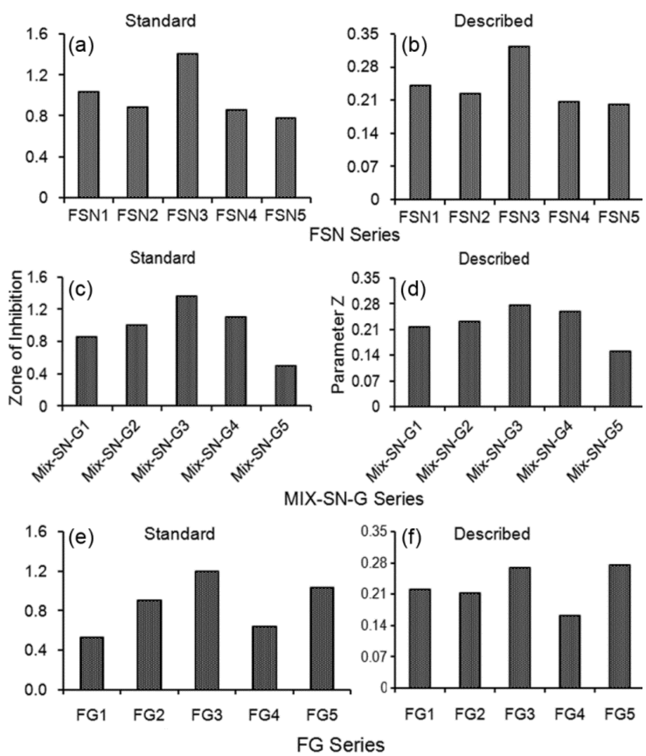

Fig. 4 — Antibacterial activity of Ag coated cotton fabrics for (a) FSN series (SN=0.15M and G=0.0.0669M) for different coating time and (b) the corresponding parameter Z; (c) Mix-SN-G series(SN=0.15M and G=0.117M) for different coating time and (d) the corresponding parameter Z; and (e) FG series (SN=0.06M and G=0.117M) for different coating time and (f) the corresponding parameter

corresponding parameter Z. It is observed that up to a certain time, the antibacterial activity of Ag coated fabrics increases with increasing the coating time. Further increase in coating time results in decrease of antibacterial activity due to formation of agglomeration. As can be seen from the charts, parameter Z in almost all samples follows the same trend as in ZOI size.

The antibacterial activity of the FSN, Mix-SN-G and FG series with different pretreatment procedures are represented in Fig. 5. According to Fig. 5(a), the ZOI size for the sample, in which *p*H has been adjusted using NaOH, shows maximum value, followed by Ca_2CO_3 and NH_3, which has roughly the same effect on antibacterial activity. Turning to Fig 5(b), it is clear that the identical outcomes are acquired from parameter Z.

Figure 6 shows the correlation between the parameter Z and ZOI size obtained from the standard test method. The correlation is evaluated by calculating the correlation coefficient (r) or the Pearson product moment correlation coefficient. The correlation coefficient between the ZOI size and the parameter Z is given by the following equation:

$$\Delta Z = \sqrt{1 - \frac{\sum_{i=1}^{N}\left(ZOI_{(i)} - Z_{(i)}\right)^2}{\sum_{i=1}^{N}\left(ZOI_{(i)} - \overline{ZOI}\right)^2}} \quad \ldots (3)$$

where $\overline{ZOI}$ is the mean of ZOI size; and N, the size of available dataset[27]. With respect to Fig 5, correlation coefficient is more than 0.96, indicating the highly correlation among variables and this confirms preceding results.

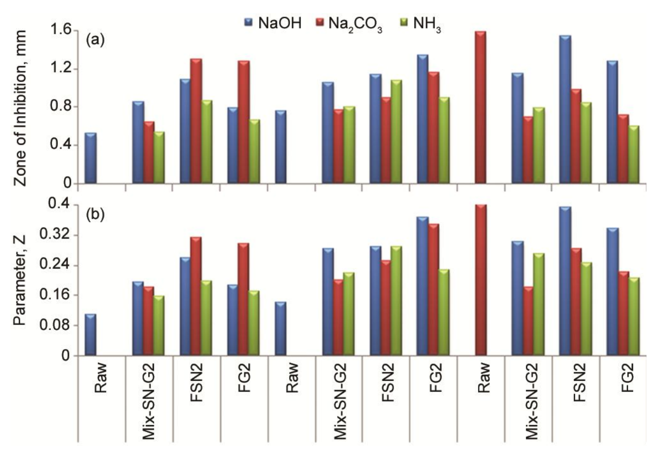

Fig. 5 — (a) Antibacterial activity of Ag coated cotton fabrics for the FSN, Mix-SN-G and FG series (coating time=2 min) with different pretreatments and (b) the corresponding parameter Z

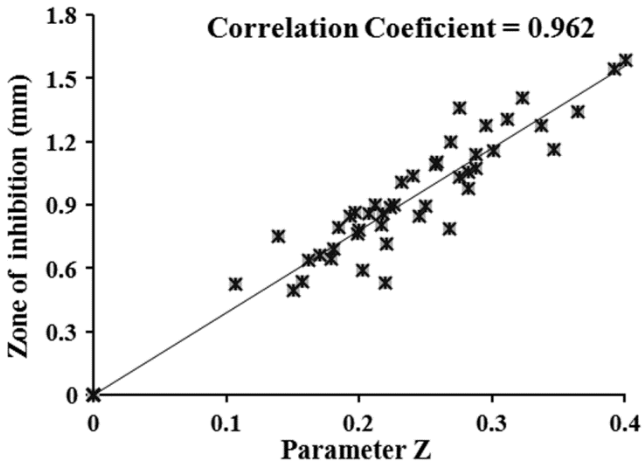

Fig. 6 — Correlation between parameter Z and ZOI size obtained from the standard test method

4 Conclusion

Heterogeneous size of ZOI in antibacterial test of textile fabrics, makes it hard to compare antibacterial activity of different sample specimens. Accordingly, image processing technique has been utilized to evaluate the antibacterial performance of antibacterial fabrics through a newly introduced procedure based on area of ZOI (parameter Z) instead of the mean size of ZOI.

In order to do that, antibacterial test has been carried out on 51 $AgNO_3$ coated fabrics against Gram positive *Staphylococcus aureus* and based on AATCC method 147–1998. According to acquired results, parameter Z follows the same trend as in ZOI size for all samples. In addition, the correlation coefficient is found more than 0.96, which indicates that the ZOI size and parameter Z can be considered as highly correlated. The described algorithm is in its earliest step and further study needs to be done for more flexible and more reliable results.

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