A STUDY OF THE ANTIBACTERIAL ACTIVITY OF POLYHEXAMETHYLENE BIGUANIDE ON COTTON SUBSTRATE

U.R.AMRIT[1], R.HENDRIX[2], V.DUTSCHK[1], M.M.C.G. WARMOESKERKEN[1]
[1]*EFSM Group, CTW faculty, University of Twente, Drienerlolaan 5, 7522 NB Enschede, The Netherlands*
[2] *Lab microbiologie, Burg. Edo Bergsmalaan 1, 7512 AD Enschede, The Netherlands*
u.r.bhaskara@utwente.nl

ABSTRACT

Interest in antibacterial textiles has been increasing recently. Different kinds of antibacterial textiles with different effectivity are being sold in the market. The influence of time on the antibacterial activity of such textiles is not known generally. The goal of this research was to study the mechanism of commercially available antibacterial agent meant for cotton with regard to the influence of concentration and time. E coli bacterium was chosen as the test microganism and tests were conducted according to the JIS L 1902 standard. The tests indicated that the antibacterial activity increased with increasing concentration of the agent on the fabric while there was a mixed trend in regard to the relation between time of action and the concentration of the agent on the treated textile.

1. INTRODUCTION

Antibacterial agents have been used to functionalize textile surfaces for several decades [1-3]. These functionalized textiles find applications in hospitals, restaurants, hotels, etc [2, 4]. Antibacterial agents on the textiles not only kill microorganisms and prevent transmission of bacteria but such agents also prevent the degradation of the textile and prolong its lifetime. Several types of antibacterial agents are currently in use for functionalizing textiles, such as quaternary ammonium compounds, metal and metal salts, agents based on biopolymers such as chitosan, etc [1]. Our goal was to study the antibacterial activity of a commercially available antibacterial agent meant for textile surfaces particularly cotton. Polyhexamethylene biguanide (PHMB) was selected as such an agent mainly due to its easy application onto to cotton substrates. This agent also finds ubiquitous use in other industries such as food equipment industry, surgerical dressings and cleaning of swimming pools, etc [5-7]. Polyhexamethylene biguanides are similar to quaternary ammonium compounds as in that they both are cationic membrane active agents, meaning they proceed to mainly attack the bacterial cell membranes. However, polyhexamethylene biguanides differ from quaternary ammonium compounds in their higher molecular weight and precise mechanism in their actions against the dissolution of the cell membranes. The hydrophobic tails in the structures of quaternary ammonium compounds (QACs) are known to enter the cell envelope during the solubilization of cell membrane while in case of PHMBs, the hydrophobic hexamethylene groups are inflexible and cannot enter the core of the cell membrane. This renders PHMBs less likely in causing resistant microrganisms [8] and this makes it safer to use than quaternary ammonium compounds, silver based agents, triclosan or other metal salts.

2. EXPERIMENTAL

2.1. Materials

Cotton fabric of 250 g/m^2 fabric density was procured from Tencate (The Netherlands). PHMB was procured from Lonza group (UK), E coli ATCC 11229 was obtained from LGC standard (USA) and Triton X100 was bought from Sigma Aldrich.

2.2. Methods

A cotton textile material was functionalized with polyhexamethylene biguanide at varying concentrations of from an aqueous solution through exhaustion process. Textile samples were immersed in PHMB solutions at 40°C at pH 7 for 30 min. The liquor to cloth ratio was 20:1. The samples were treated with PHMB to obtain 0.4%, 0.8%, 1.2%, 1.6%, and 2.0% with regard to the weight of fabric or on weight of the fabric (referred to as o.w.f). After the treatment, the samples were rinsed in cold water once and then air dried. The treated samples were tested for antibacterial activity according to the Japanese standard JIS L 1902 ‘s absorption

testing method [9]. This testing method is considered to be a quantitative method as compared to the commonly used agar diffusion testing method which is one of qualitative type.

For the antibacterial tests, 0.5 g of cotton material was taken for the inoculation with 200 µl of 1-3 x 10^6 CFU (Colony Forming Unit)/ml of E coli bacterium. This concentration was obtained by the measurement of the optical density of inoculum with a WPACO 8000 Biowave personal cell density meter (Biochrom, UK) at 600 nm. The required optical density was obtained after diluting the bacterium grown until 10^8 CFU/ml with Luria Broth medium. This testing method requires 24 hours of incubation, it was, however, modified in our studies by testing also at 12 hours of incubation time.

Three PHMB treated samples were inoculated for each measurement. For the control, a untreated cotton fabric of similar dimensions and weight was taken and inoculated in the same way. After the inoculation, the samples were placed on the inside of a petri dish lid and then covered with an inverted agar filled petri dish bottom and sealed with paraffin tape. This was done to prevent drying of the sample during the incubation. The samples were then placed in an incubator; operating at 37°C for the required duration (12 and 24 hours).

The amount of bacteria eluted from untreated/control and treated samples at time 0 (CFU^*_0 and CFU_0) was 1-3 x 10^6 CFU/ml. This was determined by eluting the bacteria immediately from the samples after inoculation by the above described procedure, however, without incubation step. After the incubation period, the samples were taken out and shaken in 20 ml of physiological saline containing 2 g/l of nonionic surfactant (Triton X100) as prescribed by the standard. 1 ml of this shake out solution was then serial diluted in 9 ml of physiological saline for the required number of dilutions and then 0.1 ml was plated. The number of colonies grown on the plate was counted the next day to calculate the bacteria eluted from each specimen (through plate count method). The resolution of the plate count method was 6 x 10^3 CFU per specimen below which the CFUs obtained were considered 0. The reason for this being that plates with colonies only 30-300 were considered and plates with colonies below 30 were considered 0 CFUs. The antibacterial activity was also estimated using the bacteriostatic formula in JIS L 1902 standard as shown below.

$$\text{Antibacterial activity} = (\log CFU^*_t - \log CFU^*_0) - (\log CFU_t - \log CFU_0) \quad (1)$$

where CFU^*_0 and CFU^*_t are the Colony Forming Units obtained on the control sample at time 0 and time t. CFU_0 and CFU_t are the Colony Forming Units obtained on the treated sample at time 0 and time t.

3. RESULTS AND DISCUSSIONS

As mentioned, at first the bacterium was eluted from the textile samples treated at different concentrations of the agent on the weight of the fabric. Figure 1 shows the amount of bacteria in Log CFU (colony forming units) recovered from antibacterial agent treated textile after a certain incubation time against concentrations of polyhexamethylene bigaunide on the weight of the fabric.

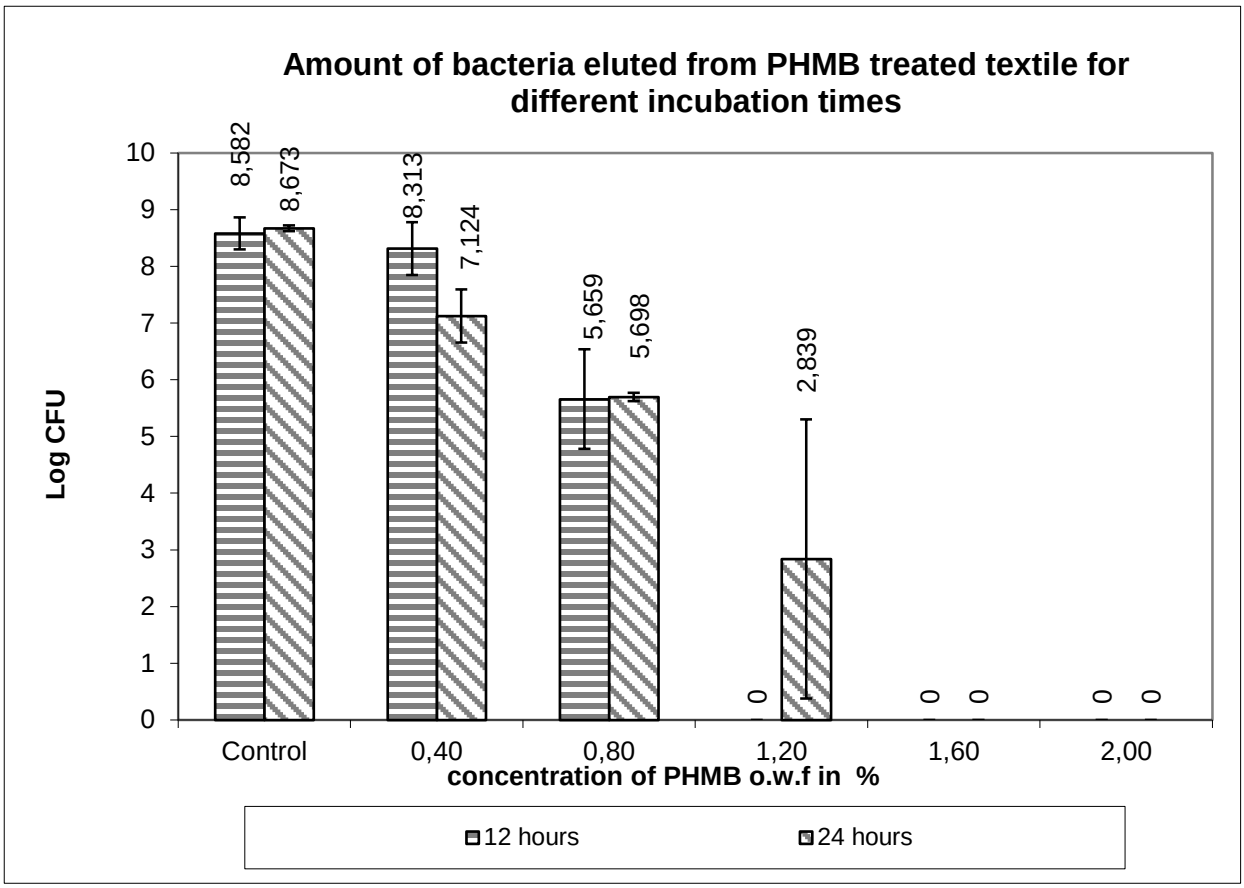

Figure 1. Amount of bacteria eluted against concentration of PHMB o.w.f in % for different incubation times

The figure shows that there is no substantial growth from 12 to 24 hours on the control sample indicating that the bacteria on the control had reached a stable state after a growth of around 100 times from time 0 to 12 hours of incubation time. The results also show that, as expected, the antibacterial activity increases with increasing concentration of polyhexamethylene biguanide on the fabric. The figure also shows that at 1.2% of PHMB o.w.f, the amount of bacteria eluted from the textile showed a high amount of standard deviation.

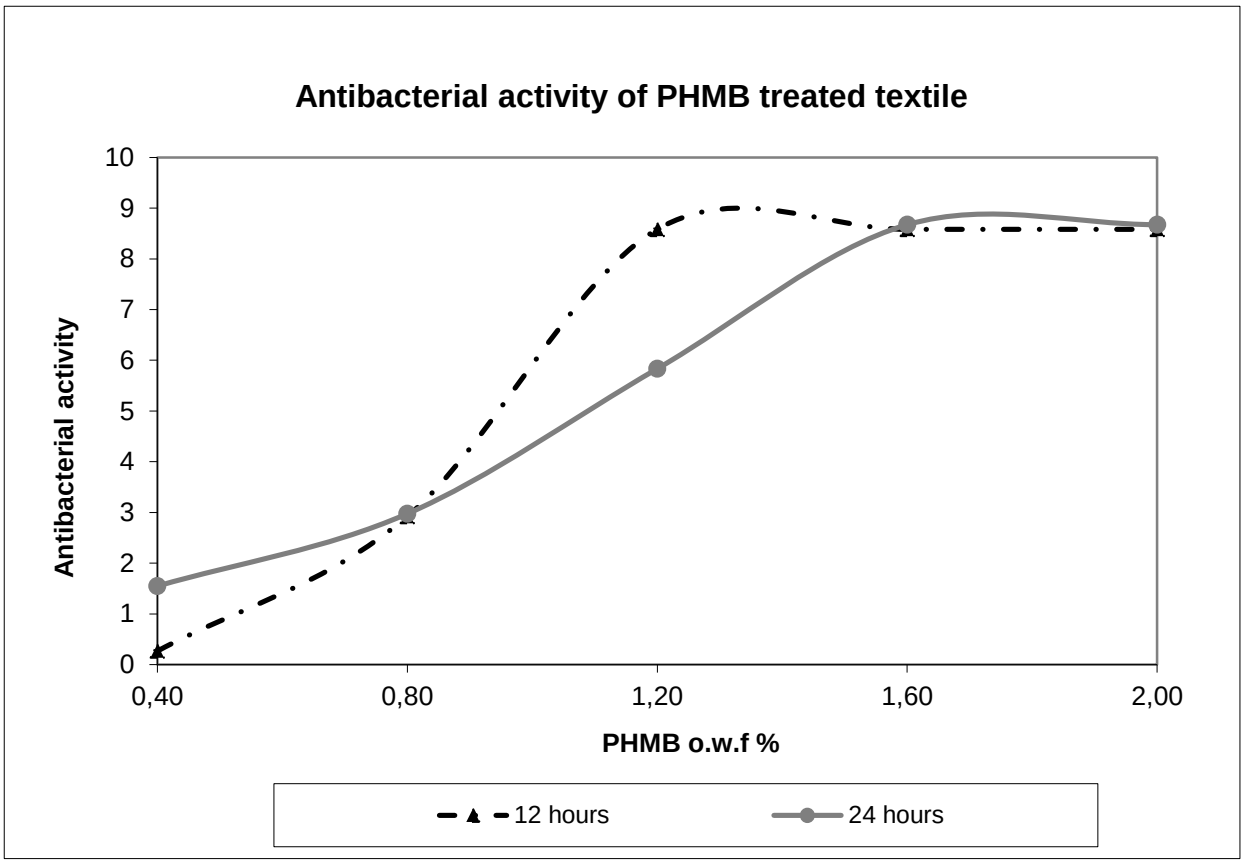

Figure 2. Antibacterial activity against the PHMB concentration o.w.f. in %

Figure 2 shows the antibacterial activity value obtained according to equation (1), for different concentrations of the agent o.w.f for the two incubation times. The antibacterial activity is dependent not only on the concentration of the agent but can also be dependent on the time. The antibacterial activity increases with increase in concentration, however, the trend of the antibacterial activity curve here is not clear while comparing the two incubation times as seen in Figure 2. It is normally expected that with increased incubation time, the antibacterial activity at all concentrations of the agent on the textile would result in higher antibacterial activity since increased time is also a type of stress just as increased concentration. However, this singular trend is not seen. At a certain concentration, the antibacterial activity is higher at the shorter incubation time and at certain other concentration, it is reverse while in some, they are the same. There could be several reasons for this; the nature of bacterial growth, regrowth of bacteria at longer incubation time (regrowth refers to the recovery and subsequent multiplication of mildy injured bacteria), mechanism of the antibacterial agent as well as the degeneration of the agent itself, etc. According to the JIS L 1902 standard, an antibacterial activity of 2 when calculated by equation (1) is required for an antibacterial textile to be considered antibacterial.

In Figure 2, the antibacterial activity curve can be divided into two zones as the curve moves from lower concentrations to higher concentrations of the antibacterial agent o.w.f in %. These zones are namely; bacteriostatic zone and bactericidal zone. It is not always that a sharp distinction is seen between these zones. It is known that at lower concentrations, bacteriostatic action is normally expected where specific sites within the bacterium are targeted and therefore, in a given population of bacteria, some have the opportunity to recover and grow with time when the agent is below the lethal concentration (known as the minimum inhibitory concentration in homogenous solutions). However, at higher concentrations of the agent, multiple sites within the bacterium are targeted, therefore recovery is less likely leading to the increased number of killed numbers [10].

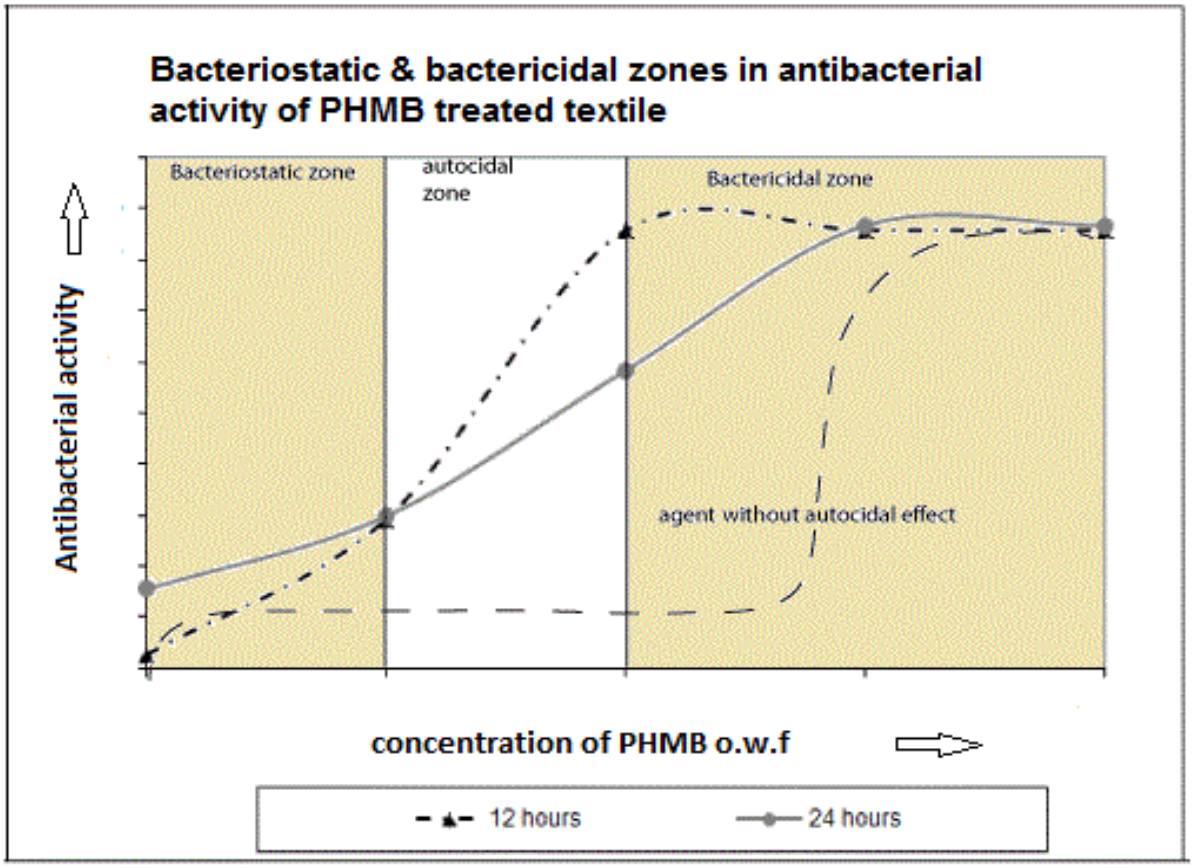

Figure 3. Bacteriostatic and bactericidal zone in the action of PHMB

The transition from bacteriostatic activity of the agent to bactericidal activity in the case of PHMB treated textile can be seen as shown in Figure 3 which is adapted from Denyer and Stewart's paper [10]. According to the just referred authors, this type of transition from bacteriostasis to bactericidal effect in some membrane active agents could be due to the contribution of the autocidal effect that can take place as a result of free radical generation in the bacteria due to metabolic imbalance and ion homeostasis (this however, was in reference to disinfectants). Antibacterial agents which do not have the latter effects do not show such autocidal response and thereby do not have this advantageous effect in their antibacterial activity.

This autocidal zone or the transition zone for PHMB treated textile lay between 0.8% to 1.2 % o.w.f. This observation helps in understanding that the correlation between antibacterial activity and concentration of the agent is very agent dependent and specific agents can be chosen in which an minimum agent concentration is enough to trigger the autocidal effect leading to the compounded antibacterial activity.

4. CONCLUSION

A cotton textile was treated with a commercially available antibacterial agent at various concentrations with regard to the weight on fabric. Antibacterial tests were conducted using JIS L 1902 testing method. Antibacterial tests showed that at lower concentrations, bacteriostatic effect occurred while at higher concentrations, bactericidal effect was noted. Tests further indicated a mixed trend in regard to influence of incubation time on the antibacterial activity of the textiles. Further tests have to be conducted to study the corelation between time and agent concentration on the antibacterial activity of such textiles.

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