Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 67 (2007) 375–389
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

The effect of reactive dyes upon the uptake and antibacterial efficacy of poly(hexamethylene biguanide) on cotton. Part 3: Reduction in the antibacterial efficacy of poly(hexamethylene biguanide) on cotton, dyed with bis(monochlorotriazinyl) reactive dyes

Atsuko Kawabata, John A. Taylor *

Textiles and Paper, School of Materials, Faculty of Engineering and Physical Sciences, The University of Manchester, P.O. Box 88, Sackville Street, Manchester M60 1QD, UK

Received 6 February 2006; received in revised form 6 June 2006; accepted 6 June 2006
Available online 26 July 2006

Abstract

The antibacterial efficiency of poly(hexamethylene biguanide), PHMB, on un-dyed cotton has been compared with that of PHMB on cotton coloured with a range of reactive dyes. In each case, the presence of covalently bound dye resulted in a reduction in antibacterial efficiency. In the absence of dye, the cationic PHMB binds to the cotton via weak ion–ion linkages with the carboxylate groups present on cotton; these dissociate readily allowing release of free PHMB, the active antibacterial agent. In the presence of reactive dye, the cationic PHMB forms stronger ion–ion linkages with the strong sulphonic acid groups of the dyes; thus release of free PHMB, the active agent, is less facile and antibacterial efficiency reduced accordingly.

Keywords: Poly(hexamethylene biguanide); Antibacterial; Reactive dyes; Cellulose

1. Introduction

One of the major benefits of antibacterial treatments for fabrics is protection from mal-odour (Mao & Murphy, 2001). Bacteria are believed to be a major cause of skin eczema and clothing odour (Collier & Epps, 1999; Rajendran & Anand, 2001) which is caused by the production of amines and organic acids (Mao & Murphy, 2001). *Staphylococcus aureus* is a pathogenic Gram-positive bacterium (Tortora, Funke, & Case, 2002) which is frequently encountered in hospitals and homes (Bajaj & Sengupta, 1992) and which can survive on organic fibres (Rajendran & Anand, 2001; Vigo, 1994), such as cotton. The first industrial production of antibacterial textiles, in the late 1930's (Kourai, 1999), was for of German and US soldiers' uniforms, using quaternary ammonium salts to prevent infection and odour. Several different classes of antibacterial agent, such as salts of cadmium, copper, zinc, chromium and mercury, amine-formaldehyde condensates and tar were subsequently applied to textiles to confer antibacterial properties (Broughton, Worley, Unchin Cho, Lin, & Sun, 2003). Current commercial examples include chlorinated biphenyl ethers such as 2,4,4′-trichloro-2′-hydroxydiphenyl ether, triclosan (Mao & Murphy, 2001), siloxane derivatives which contain a cationic group quaternary ammonium group (Wallace, 2001) and poly(biguanides) (Payne, 1997). Other types that have been described include *N*-halo derivatives (Sun & Xu, 1998), peroxide-based reagents (Vigo, Parikh, & Danna, 2001) and chitosan derivatives (Hasebe, Kuwaraha, & Tokunaga, 2001). Cotton has been treated with many conventional antibacterial agents, including metal salts of Cu^{2+} and Zn^{2+} (Nakashima & Matsuo, 2001; Nakashima, Sakagami, & Matsuo, 2001; Nakashima, Matsuo, Bin, & Sakagami, 2003; Nakashima

* Corresponding author. Tel.: +44 0 161 306 4112; fax: +44 0 161 955 8150.
E-mail address: john.a.taylor@manchester.ac.uk (J.A. Taylor).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.06.022

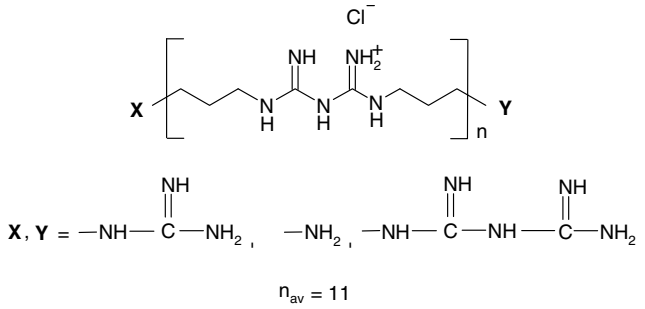


Fig. 1. Chemical structure of PHMB.

et al., 1991); also some direct dyes have been shown to possess weak antibacterial activity (Kobayashi, Sekiguchi, Nakanishi, & Komiyama, 2002).

Poly(hexamethylene biguanide), or PHMB, is marketed as a 20% solution of its hydrochloride under the name of Reputex 20. The biocide is a remarkably effective antimicrobial agent capable of controlling bacterial, yeast and fungal growth (Avecia, 2000; Cornish, McGeechan, & Yeates, 2002; Tan, Teng, & Omar, 2000). PHMB, and related types are repeating polymers containing 8–15 biguanide units, with an average of 11 units (Yates, 2004), as shown in Fig. 1, and exhibit much greater antibacterial activity than the corresponding monomeric or dimeric biguanides (Davies & Field, 1969). PHMB has been used as a swimming pool sanitizer, in cosmetics (Payne, 1997), antiseptics, mouth rinses for plaque control and preventing oral bacterial growth (Rosin et al., 2002) and also on viscose to provide antibacterial kitchen wipes (Avecia, 2004). The mode of action of PHMB on cellulosic fabrics is believed to involve temporary attachment of the cationic PHMB to anionic carboxylate groups present on the fibre, via weak ion–ion linkages (Avecia, 2000; Payne, Yates, Payne, Yates, & Payne, 2005). These allow subsequent slow release of the free antibacterial agent by dissociation. If this is a realistic representation of the mechanism which is operative, then it would be expected that the introduction of stronger acids, chemically attached to the cellulose, would allow the formation of stronger ion–ion linkages between fabric and PHMB, with consequential reduction in dissociation of the PHMB–dyed cotton salt complex. Hence less facile liberation of free PHMB and hence lower antibacterial efficiency would result.

Thus a study was initiate into the effect of sulphonate groups, fixed to cotton via chemically bound reactive dye, on the uptake and antibacterial efficacy of PHMB.

2. Experimental

2.1. Materials and apparatus

Desized and scoured 100% knitted, cotton fabric was supplied by Phoenix Calico Ltd., Cheshire. Procion H-E dyes were obtained from DyStar, Germany. Poly(hexamethylene biguanide) hydrochloride, nutrient agar (BM0540: E & O Laboratories, Scotland) and *Staphylococcus aureus* (National Collections of Industrial, Food and Marine Bacteria 9518) were also supplied by Avecia Ltd., Blackley, Manchester. All other chemicals were laboratory grade from Sigma–Aldrich, UK. UV/visible spectra were recorded using a Camspec M350 double beam ultraviolet–visible spectrophotometer.

2.2. Dyeing and fixation of dye on cotton

Cotton was dyed according to the manufacturer's recommendations, at 1%, 2%, 4%, 6% and 9% dye on mass of fabric (o.m.f.) as detailed previously (Kawabata & Taylor, 2004). Percentage dye fixation was determined by dissolution of dyed cotton in 70% sulphuric acid, followed by optical assessment of the amount of colour in the resulting solution (Kawabata & Taylor, 2004; Kawabata & Taylor, 2006; Murtagh & Taylor, 2004). A graph of dye concentration versus absorption gave a straight line, indicating adherence to Beer–Lambert's law (Christie, Mather, & Wardman, 2000).

Most of the commercial reactive dyes contain additional components such as de-dusting agents, salt and water (Knecht & Hibbert, 1925; Venkataraman, 1977). In order to determine the effective agent content of each commercial dye sample, these were titrated against a freshly standardised solution of titanium(III) chloride as described earlier (Kawabata & Taylor, 2004; Kawabata & Taylor, 2006; Murtagh & Taylor, 2004).

2.3. Uptake of PHMB by dyed and un-dyed cotton

In order to determine the uptake of PHMB by cotton, six aqueous solutions of PHMB, of different concentrations, were prepared and adjusted to pH 7.0 with sodium bicarbonate (Kawabata & Taylor, 2004). One gram pieces of cotton fabric, both dyed and un-dyed, were immersed in separate solutions of PHMB hydrochloride (100 g) at a liquor-to-goods ratio of 20:1 (Kawabata & Taylor, 2004). The percentage of PHMB, o.m.f., in each of the solutions are detailed in Table 1.

Table 1
Percentage of PHMB applied to cotton dyed with Procion H-E dyes

Sample	% of PHMB applied (o.m.f.) to seven pieces of cotton dyed at 1%, 2%, 4%, 6% and 9% o.m.f.				
	0%, 1%, 2% o.m.f.	4% o.m.f.[a]		6% o.m.f.	9% o.m.f.
(a)	0.00	0.00	(0.00)	0.00	0.00
(b)	0.08	0.14	(0.16)	0.17	0.21
(c)	0.16	0.28	(0.31)	0.34	0.38
(d)	0.31	0.56	(0.65)	0.67	0.76
(e)	0.65	1.13	(1.38)	1.39	1.66
(f)	1.44	2.25	(2.66)	2.56	3.36
(g)	2.60	4.49	(5.35)	4.91	6.18

[a] Values in parentheses are concentrations of PHMB applied to cotton dyed with Procion Navy H-ER.

2.4. Preparation of antibacterial testing plates

Four samples of individual colonies of *S. aureus* were taken from an isolation plate and mixed into 20 ml portions of nutrient broth. The broth was incubated at 37 °C for 24 h and then 1 ml was taken and diluted with 9 ml of sterile saline solution to furnish a concentration of approximately 10^7 colonies in 10 ml (Hyde, 2004). From this liquid, five parallel lines were streaked onto a nutrient agar plate, to provide gradient concentrations of organism.

Cotton samples (2 × 8 cm) were cut and autoclaved for 15 min at 121 °C and 15 psi, cooled to room temperature and then placed on an inoculated agar plate and pressed down carefully using a sterile plastic rod, in order to expel air from between the surface of plate and the cotton. The test plates were incubated at 37 °C for 24 h. Preparation of the plates is illustrated in Fig. 2.

2.5. Assessment of antibacterial efficacy

The effect of fixed reactive dye on the antibacterial efficacy of PHMB adsorbed onto dyed cotton was assessed using the AATCC 147 test procedure (AATCC, 2000). This method is simple, visual and suitable for qualitative analysis of bacterial growth on incubated plates thus antibacterial activity can be assessed (AATCC, 2000). *S. aureus* inoculum was clear when streaked onto the agar plate, but, after the plate had been incubated for 24 h, bacterial growth appeared. In the more highly concentrated regions, the growth was observed as a yellowish line; in the regions of lower concentration bacterial growth was observed as individual circular colonies on the surface of the cotton and on the agar plate.

Each incubated agar plate containing a sample of dyed cotton, (seven different concentrations of applied PHMB for each of five depths of shade *i.e.,* 35 plates) was placed onto a coloured background (black or light blue, depending on the colour of fabric). Additionally, agar plates, each containing a sample of un-dyed PHMB treated cotton, or simply un-dyed cotton were also included.

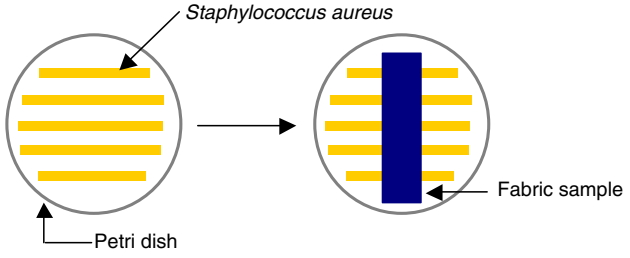


Fig. 2. Representation of parallel streaked Petridish. For convenience, the five parallel streaks are illustrated by lines of the same width. However, the top streaked line was the highest concentration of *S. aureus* and the concentration was gradually reduced, and the bottom line represents the lowest bacterial concentration.

2.6. Concentration of carboxylate groups on un-dyed cotton

The concentration of carboxylate groups on cotton depends on the source of the cotton and the chemical treatments used in its preparation, and usually is within the range of 10 and 50 mequiv of carboxylate group per kg cotton (milliequivalents of COO^-/kg cotton) (Bae, Motomura, & Morita, 1997; Klemm, Philipp, Heinze, Heinze, & Wagenknecht, 1998; Lawson, 2001; McGregor, 1972; McGregor & Ezuddin, 1974; Payne et al., 2005). The concentration of carboxylate groups on cotton used in this project was found to be 42.8 mequiv COO^-/kg by titration against Methylene Blue adsorption (Davidson, 1948; Klemm et al., 1998) as described in previously (Kawabata & Taylor, 2004).

3. Results and discussion

3.1. Determination of effective agent content of dyes

Each commercial dye was titrated against titanium(III) chloride (Giles & Grezek, 1962; Knecht & Hibbert, 1925; Venkataraman, 1952) in order to determine its effective agent content. Because titanium(III) chloride is readily oxidised by air, it was standardised immediately prior to use, by titration against a dye of known strength. With a knowledge of chemical structure, percentage fixation (%*F*) and effective agent content (%*EA*) of each dye, it was possible to determine the molar concentration of "pure" dye fixed to the cotton (Kawabata & Taylor, 2004); details are listed in Table 2.

The three Procion dyes are large disazo polysulphonated derivatives. Procion Red H-E3B and Procion Navy H-ER each contain six sulphonic acid groups per molecule and Procion Yellow H-E4R is an octasulphonated dye, see

Table 2
Percentage fixation and concentration of anionic residues on dyed cotton

Dye	% Applied[a]	[Fixed pure dye][b]	[SO_3Na][c]
Procion Yellow H-E4R	1	1.5	12.1
	2	2.7	21.8
	4	5.1	41.0
	6	7.1	56.9
	9	9.2	74.0
Procion Red H-E3B	1	2.0	12.2
	2	3.7	22.3
	4	7.1	42.4
	6	9.1	54.4
	9	11.5	69.0
Procion Navy H-ER	1	3.0	18.1
	2	5.8	34.9
	4	10.5	63.2
	6	13.5	80.8
	9	17.5	105.2

[a] Dye applied, g/100 g cotton.
[b] Dye fixed to cotton (mmol/kg cotton).
[c] Concentration of fixed sulphonic acid groups (milliequivalents of SO_3^-/kg cotton).

reference (Kawabata & Taylor, 2004) for the chemical structures. The concentrations of fixed sulphonate groups, in milliequivalents of sulphonate per kg cotton (milliequivalents of SO_3^-/kg cotton), are listed in Table 2. For example, the fixation of Procion Yellow H-E4R was 47.2% (Kawabata & Taylor, 2004). This means 10 g of dye applied per kg cotton (for 1% dye o.m.f. dyeing) equates to 4.72 g dye fixed per kg cotton. Because the effective agent content of this dye was 58.9% and the molecular weight is 1840, this equates to 1.5 millimoles of fixed dye per kg cotton, or 12.1 mequiv SO_3^-/kg cotton. Similarly, the concentration of fixed sulphonate is 41.0 mequiv SO_3^-/kg cotton at 4% dye o.m.f., and 73.9 mequiv SO_3^-/kg cotton at 9% dye o.m.f. for Procion Yellow H-E4R.

Increasing the concentration of fixed dye was accompanied by a corresponding increase in fixed sulphonate group concentration. Therefore, PHMB uptake would be expected to increase in line with increasing concentration of fixed dye, if attachment of PHMB is by ion–ion bonding to the sulphonate groups of fixed dye.

3.2. Attachment of PHMB to dyed and un-dyed cotton fabrics

Pieces of un-dyed cotton were immersed in different strengths of aqueous solutions of PHMB hydrochloride (100 g) to give a liquor-to-goods ratio of 20:1 (Kawabata & Taylor, 2004). The absorption of PHMB on cotton was assessed spectrophotometrically by measuring the optical absorbance of each PHMB solution before and after addition of cotton. The optical density of each PHMB hydrochloride solution was recorded at 236 nm (λ_{max}). From the difference in absorbance before (Abs_i) and after (Abs_f) addition of cotton, it was possible to calculate the percentage uptake of PHMB onto cotton (%S), Eq. (1).

$$\%S = \frac{(\mathrm{Abs}_i - \mathrm{Abs}_f)}{\mathrm{Abs}_i} \times 100 \quad (1)$$

Uptake of PHMB on un-dyed cotton is the result of ion–ion interaction between cationic PHMB and anionic carboxylate groups on cotton (Avecia, 2000; Payne et al., 2005). Cotton takes up PHMB readily until all the available anionic carboxylate sites are occupied by cationic PHMB, after which little further uptake of PHMB is observed.

It is reasonable to expect that, in the case of cotton dyed with reactive dyes, cationic PHMB would be adsorbed onto the anionic sulphonate sites of covalently fixed reactive dyes, as well as onto the carboxylate groups present on cotton as depicted in Fig. 3.

Table 3
Percentage uptake of PHMB by un-dyed cotton against various concentrations of PHMB hydrochloride

Sample	Concentration of PHMB applied (% PHMB o.m.f.)	% Uptake of PHMB
(a)	0.00	0.0
(b)	0.08	100.0
(c)	0.16	100.0
(d)	0.31	98.5
(e)	0.65	93.4
(f)	1.44	45.3
(g)	2.60	29.7

Several other workers have used a similar methodology to study the uptake of solute on different fibres (Balmforth, Bowers, & Guion, 1964; Chang & Juang, 2004; Chairat, Rattanaphani, Bremner, & Rattanaphani, 2005; Xin, Zhu, Hua, & Shen, 2002). It was possible to describe the adsorption of PHMB by cotton mathematically, for example using the Langmuir adsorption isotherm model which allowed the calculation of the saturation value of PHMB on dyed and un-dyed cotton (AATCC, 2000; Bae et al., 1997; Balmforth et al., 1964; Burdett, 1989; Chang & Juang, 2004; Chairat et al., 2005; Christie et al., 2000; Davidson, 1948; Giles & Grezek, 1962; Hyde, 2004; Kawabata & Taylor, 2006; Knecht & Hibbert, 1925; Klemm et al., 1998; Lawson, 2001; McGregor, 1972; McGregor & Ezuddin, 1974; Venkataraman, 1952; Venkataraman, 1977; Xin et al., 2002). These values were compared with the saturation values obtained by simple manual extrapolation.

Table 3 lists PHMB adsorption on un-dyed cotton as determined by immersing pieces of cotton into separate aqueous solutions of PHMB hydrochloride (see Table 1). At low concentrations (0.08% and 0.16% PHMB o.m.f.), PHMB was cotton (100% uptake); thus the Langmuir isotherm equation was evaluated using only data from stronger solutions, in which some PHMB remained in solution.

The saturation value (of PHMB by cotton) was thus determined as *ca.* 7.2 g PHMB/kg cotton, which was in good agreement with that of manual extrapolation, 7.7 g of PHMB/kg cotton, as shown in Fig. 4. For expediency, manual extrapolation was used for all subsequent determinations. This equates to 41.8 ($7.7/184 \times 1000$) mequiv of monomeric biguanide/kg cotton. Additionally, knowing that the concentration of carboxylic acid groups was 42.8 mequiv COO^-/kg cotton (see (Kawabata & Taylor, 2004)), it was possible to deduce that each carboxylate unit

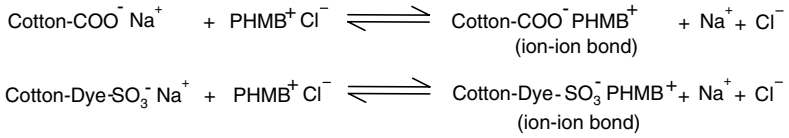


Fig. 3. Uptake of PHMB by un-dyed and dyed cotton.

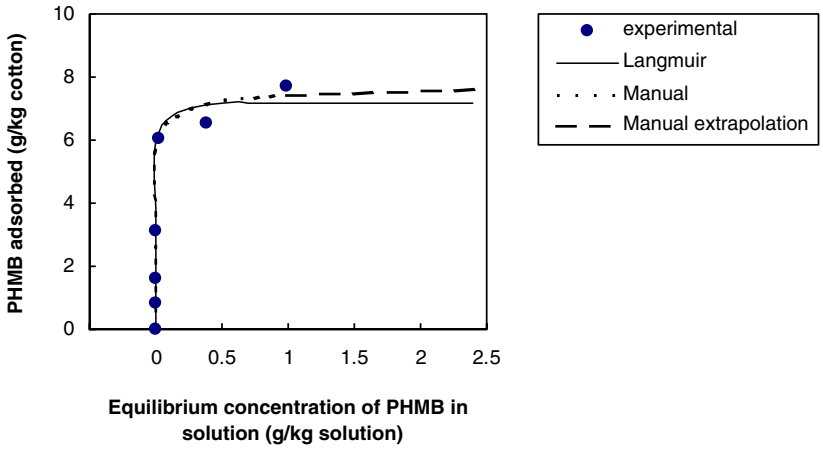


Fig. 4. Comparison of the adsorption of PHMB on cotton by manually extrapolated value and Langmuir isotherm equation together with the experimental values.

was associated with 0.99 (41.8/42.8) equivalents of biguanide unit, or essentially, 0.09 (0.99/11) equivalents of polymeric PHMB, per carboxylate group on cotton.

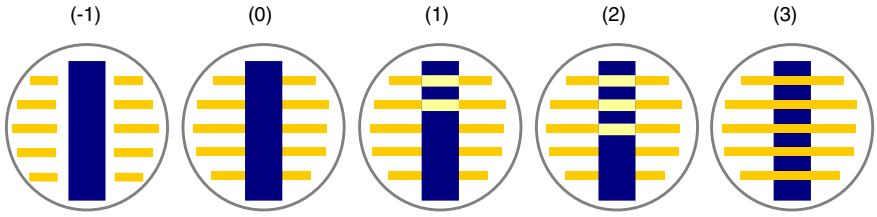
3.3. Assessment of bacterial growth

Initially, the bacterial growth on each of three samples of cotton, which had neither been dyed nor treated with PHMB, was confirmed. The growth of *S. aureus* on cotton was categorised depending on whether a "*zone of inhibition*" of bacteria was observed on the agar gel or whether "*growth*" was observed only on cotton. The visual bacterial growth levels on the agar plates are illustrated in Fig. 5. A "*zone of inhibition*" of bacteria on the agar slope indicated that biocide had been eluted from the cotton into the agar, Fig. 5(−1).

The bacterial growth on cotton was further subdivided into four subgroups, by strength of the growth. These were "*no growth*", Fig. 5(0), "*low growth*", Fig. 5(1), "*moderate growth*", Fig. 5(2) and "*heavy growth*", Fig. 5(3) as assessed visually.

Ideally no bacterial growth should be observed on the fabric (Hyde, 2004). Thus a "*no growth*" category represents "ideal" antibacterial efficacy; that is, no bacteriocide elutes into the bulk of the agar but no bacterial growth is observed on the cotton.

Fig. 6 depicts the magnitude of bacterial growth, this could be conveniently displayed in a bar chart. The scale of the bacterial growth was recorded as a numerical value, −1, 0, 1, 2 or 3, representing the extent of bacterial growth. A value of 0 (zero) indicated "*no growth*" of bacteria, corresponding to "ideal" antibacterial performance. The numerical values 1, 2 and 3 corresponded to "*low growth*", "*moderate growth*" and "*heavy growth*". Although the size of a "*zone of inhibition*" of bacteria varied, all such results were rated at "−1" without further sub-categorisation.



(-1) "***zone of Inhibition***" of bacteria, PHMB leached from fabric and zone of inhibition observed on agar plate.

(0) "***no growth***" of bacteria, PHMB does not leach from fabric and bacterial growth observed only on the agar, not on cotton.

(1) "***low growth***" of bacteria, weak bacterial growth observed on cotton only at sites lowest concentration of streaked inoculum (first and second streaks).

(2) "***moderate growth***" of bacteria, slightly stronger bacterial growth on cotton (first, second and third streake of inoculum).

(3) "***heavy growth***" of bacteria, bacterial growth observed on cotton at every site of inoculum.

Fig. 5. Determination of bacterial growth.

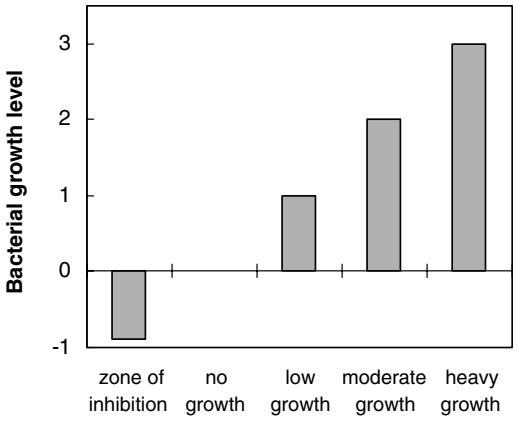


Fig. 6. Graphical representation of bacterial growth.

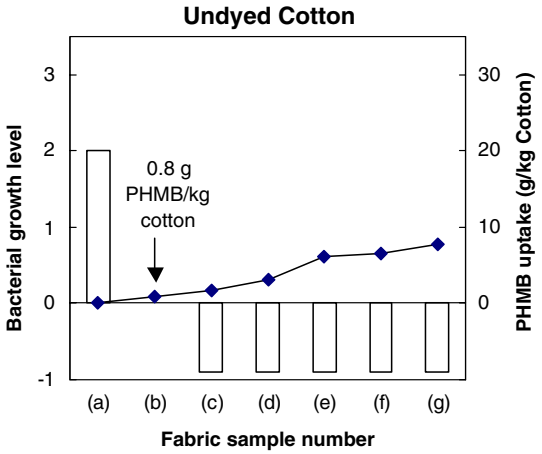


Fig. 7. Uptake and antibacterial activity of PHMB on un-dyed cotton. The solid lines represent the concentration of PHMB and the bars represent the level of bacterial growth; −1, zone of inhibition; 0, no growth; 1, low growth; 2, moderate growth and 3, heavy growth of bacteria.

Because of the lack of colour contrast between cotton and the growth of *S. aureus* some bacterial growth on dyed cotton plates proved difficult to assess. In addition, it is possible that, because of the opacity of the cotton, weak bacterial growth occurred on the side of cotton that was in contact with the agar without appearing on the topside of the cotton. Thus, some cotton samples were carefully removed from the agar plates using tweezers, and the bacterial growth assessed on the surface of cotton which had been in contact with the agar. Moreover, if assignment of a rating for bacterial growth proved difficult, for example, between "*no growth*" and "*low growth*", the plates were kept at room temperature overnight and assessed again. By prolonging the period that the cotton was in contact with the agar the growth became much clearer and visual assessment was facilitated.

4. Determination of antibacterial activity of PHMB on un-dyed cotton

Seven pieces of un-dyed cotton which had been treated with increasing concentrations of PHMB were assigned the letters (a) to (g), Fig. 7, with sample (a) possessing no PHMB. Having chosen a simple system for designating each piece of cotton, the variations in antibacterial efficacy, with increasing concentration of adsorbed PHMB, were depicted graphically as shown in Fig. 7.

By plotting bacterial growth against concentration of PHMB it was possible to determine the approximate concentration of PHMB required for "ideal" activity; that is, complete inhibition of bacterial growth on cotton, but with no elution of PHMB into the agar (to form a zone of inhibition): this was 0.8 g of PHMB/kg of un-dyed fabric (b). The "ideal" antibacterial activity is thought to have been achieved as a result of PHMB release, by dissociation of the weak ion–ion links between cationic PHMB and anionic carboxylate groups on un-dyed cotton (Avecia, 2000).

Knowing the molecular weight of each biguanide unit and the concentration of PHMB required for "ideal" antibacterial activity it was possible to determine the molar concentration of PHMB required per mole of carboxylic acid group present on cotton. The "ideal" performance of un-dyed cotton was recorded at 0.11 equivalent of biguanide per carboxylate group. Increasing the amount of PHMB adsorbed onto un-dyed cotton resulted in migration of PHMB from the fabric and a "*zone of inhibition*" of bacterial growth was observed in the agar (assigned rating: −1) for each of samples (c) to (g).

5. Antibacterial efficacy of PHMB on dyed cotton

It has been demonstrated earlier (Table 2) that the presence of covalently bound bis(monochlorotriazinyl) dye on cotton increases dramatically the capacity of the cotton to take up PHMB. This was attributed to the fact that the dyed cotton effectively possesses more anionic (sulphonate) groups than un-dyed cotton, and these are capable of forming additional ion–ion linkages with the cationic PHMB.

Having demonstrated that the presence of reactive dyes influences the magnitude of the uptake of PHMB by cotton, an attempt was made to determine whether the presence of covalently bound dye also influences the antibacterial efficacy of the bound PHMB. This might be expected because the sulphonate groups would form stronger ion–ion linkages, with the PHMB, than would the carboxylate groups present in cotton (March, 1992). Cationic PHMB is capable of forming ion–ion linkages with the anionic sulphonate groups of reactive dyes, which are covalently bonded with cotton, as well as with carboxylate groups present on cotton. It would be expected that stronger ion–ion bonding of PHMB, with sulphonate groups of fixed dye, might lead to reduced dissociation of PHMB and thus reduced antibacterial efficacy of PHMB, if dissociation of bound PHMB is indeed a pre-requisite for effective antibacterial activity.

Therefore, the antibacterial activity of PHMB treated cotton, which had been dyed with Procion Yellow H-E4R, Procion Red H-E3B or Procion Navy H-ER, was assessed. A similar protocol to that used for un-dyed cotton was employed.

Fig. 8 illustrates the antibacterial efficacy of PHMB on un-dyed cotton and cotton dyed with Procion Yellow H-E4R, at 1%, 2%, 4%, 6% and 9% dye o.m.f. Bacterial growth on dyed cotton, untreated with PHMB (fabric sample (a) in each graph), was observed to have "*low growth*" of bacteria at 1% and 2% dye o.m.f., "*moderate growth*" of bacteria at 4% dye o.m.f., and "*heavy growth*" of bacteria at 6% and 9% dye o.m.f. This indicated that fixed Procion Yellow H-E4R itself possessed no antibacterial activity.

As increasing quantities of PHMB were applied to dyed cotton, antibacterial activity was observed and the amount

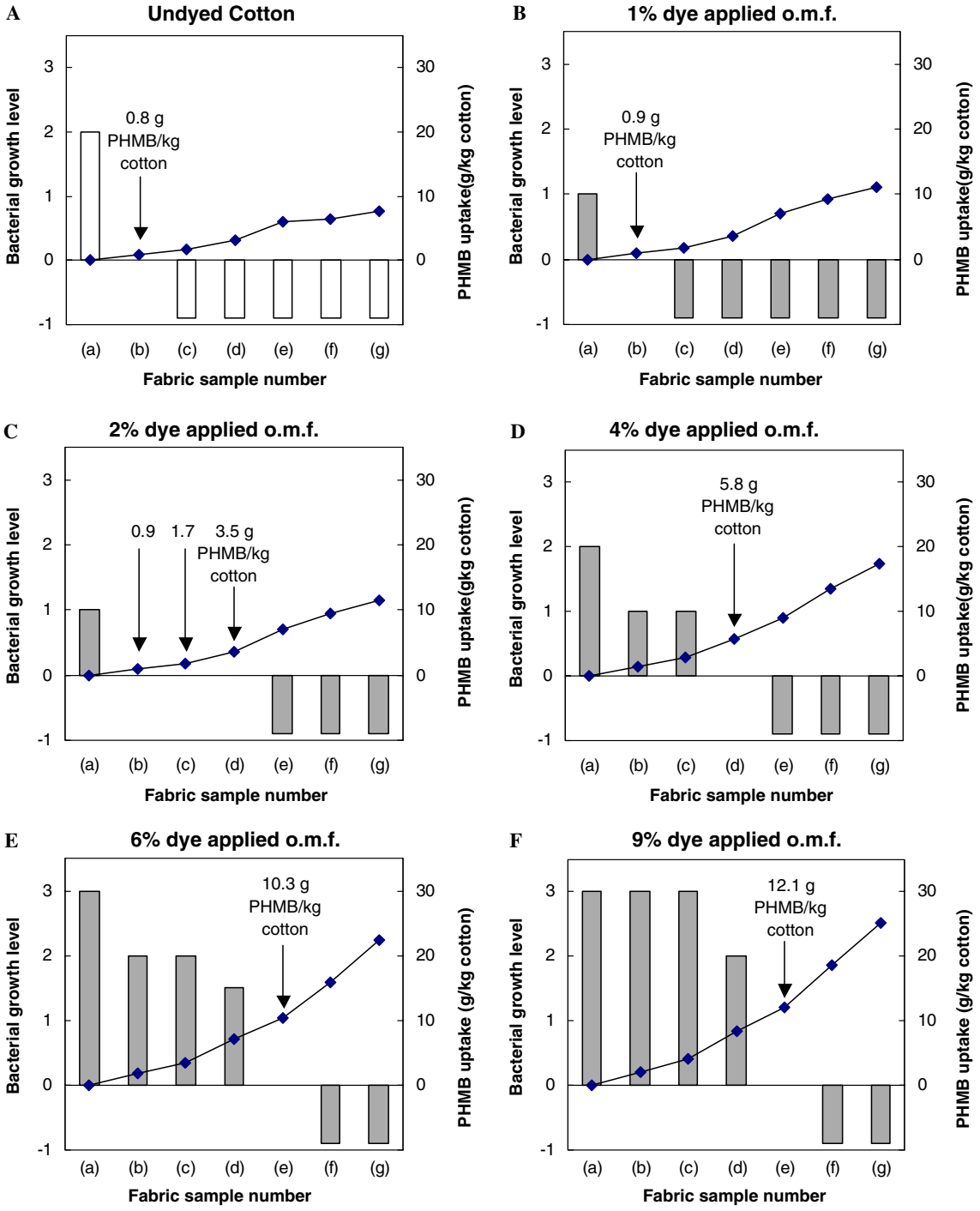


Fig. 8. Uptake and antibacterial activity of PHMB on cotton dyed with Procion Yellow H-E4R at 1%, 2%, 4%, 6% and 9% dye o.m.f. The solid lines represent the concentration of PHMB and the bars represent the level of bacterial growth; −1, zone of inhibition; 0, no growth; 1, low growth; 2, moderate growth and 3, heavy growth of bacteria.

of PHMB adsorbed increased with increasing dye concentration. At 1% dye o.m.f., "*no growth*" of bacteria (rated 0 value on the bar chart) was observed at 0.9 g of PHMB/kg cotton on fabric sample (b), Fig. 8B. In the case of 2% Procion Yellow H-E4R o.m.f., "ideal" antibacterial activity was observed at each of three concentrations of absorbed PHMB, at 0.9, 1.7 and 3.5 g of PHMB/kg cotton Fig. 8C. It is interesting to note that at 2% dye o.m.f., "*no growth*" of bacteria was observed in the agar even at a loading of 3.5 g of PHMB/kg cotton. That is, the fixed dye appeared to prevent leaching of PHMB into the agar. Similarly, the "ideal" antibacterial activity on cotton dyed with 4% dye o.m.f. was increased to 5.8 g of PHMB/kg cotton. "Ideal" activities were observed at 10.3 and 12.1 g of PHMB/kg cotton dyed with 6% and 9% Procion Yellow H-E4R o.m.f., respectively. Increasing the concentration of PHMB on the dyed cotton above the "ideal" level, at each concentration of applied dye, resulted in leaching of PHMB into the agar and a "*zone of inhibition*" of bacterial growth.

Un-dyed cotton showed "ideal" antibacterial activity at 0.8 g of PHMB/kg cotton. That is, the antibacterial efficacy of PHMB was greater on un-dyed cotton than on dyed cotton. From inspection of Fig. 8, it can be seen that the "ideal" concentration of PHMB increases with increasing concentration of fixed dye. This is consistent with PHMB forming ion–ion linkages with fixed sulphonate groups. Thus at 0%, 1%, 2%, 4%, 6% and 9% dye o.m.f. the "ideal" concentration of PHMB were 0.8, 0.9, 0.9–3.5, 5.8, 10.3 and 12.1 g of PHMB/kg cotton, respectively. This observation is consistent with fixed dye impeding the release of free PHMB, the active antibacterial agent. Thus, the concentration of adsorbed PHMB for "ideal" antibacterial activity on dyed cotton increased with increasing concentration of fixed dye.

Fig. 9 depicts the antibacterial efficacy of PHMB on cotton dyed with Procion Red H-E3B. Again, in absence of PHMB [fabric sample (a)], all displayed moderate to heavy bacterial growth, indicating that Procion Red H-E3B itself exhibits little or no antibacterial action. When insufficient amounts of PHMB were adsorbed on the dyed fabrics, this resulted in reduced bacterial growth. If the dyed cotton was overloaded with PHMB, undesirable leaching of PHMB from the cotton, took place and a "*zone of inhibition*" of bacteria was observed on the agar plate. At the lowest levels of added PHMB [fabric sample (b)], "ideal" antibacterial activity was observed on cotton dyed with 1% dye o.m.f., (see Fig. 9B). Increasing the dye concentration resulted in more PHMB being required for "ideal" performance. Increasing the concentration of PHMB beyond this, on any sample, resulted in a "*zone of inhibition*" of bacterial growth in the agar.

In the case of cotton dyed at 2% dye o.m.f., "ideal" antibacterial performance, corresponding to "*no growth*" of bacteria with a numerical value of zero on the bar chart, was not observed with any of the six pieces of PHMB treated cotton. The bacterial growth of the fabric sample (d) was rated at "*low growth*" at 3.5 g of PHMB/kg cotton and fabric sample (e) showed a "*zone of inhibition*" at 6.8 g of PHMB/kg cotton. Thus, the "ideal" concentration of PHMB for 2% of Procion Red H-E3B o.m.f was taken as the average of 5.1 g of PHMB/kg cotton.

Increasing the concentration of fixed dye on cotton resulted in lower antibacterial activity of PHMB. This was indicated by an increasing concentration of PHMB being required to produce "ideal" antibacterial activity. Thus, the concentrations of PHMB required for "ideal" activity on un-dyed cotton, and cotton dyed with 1%, 2%, 4%, 6% and 9% dye o.m.f., were 0.9–1.7, 3.5–6.8, 8.7, 10.7 and 8.5–11.5 g of PHMB/kg cotton, respectively. That is, as was the case with Procion Yellow H-E4R, increasing amounts of fixed dye reduced the antibacterial efficacy of PHMB on cotton.

As with cotton which had been dyed with Procion Yellow H-E4R (Fig. 8) or Procion Red H-E3B (Fig. 9), the inhibition of bacterial growth by PHMB was reduced on cotton dyed with Procion Navy H-ER, relative to un-dyed cotton (Fig. 10). Generally, the greater the concentration of fixed dye, the greater was the concentration of adsorbed PHMB required for "ideal" antibacterial activity. As with Procion Yellow H-E4R and Procion Red H-E3B dyed cotton, the amount of PHMB required for "ideal" activity increased with increasing concentration of fixed dye. Thus, "ideal" activity was observed at 0.8, 4.5, 6.8, 11.5, 14.7 and 12.7–20.4 g of PHMB/kg on un-dyed cotton and cotton dyed with 0%, 1%, 2%, 4%, 6% and 9% dye o.m.f., respectively.

"Ideal" antibacterial performance of Procion Navy H-ER at 1%, 2% and 4% dye o.m.f. was not observed on any of the six pieces of PHMB treated cotton. Taking the average value of the PHMB concentration between adjusted +1 ("*low growth*" of bacteria) and −1 ("*zone of inhibition*" of bacteria) for antibacterial effect furnished an approximate value of the "ideal" concentration of adsorbed PHMB in these cases. Thus, in the case of Procion Navy H-ER at 1% dye o.m.f., the "ideal" concentration of PHMB was taken as the average of 3.1 and 5.8 g of PHMB/kg cotton, that is 4.5 g of PHMB/kg cotton. Similarly, for 2% and 4% dye o.m.f., the corresponding values were determined as 6.8 and 11.5 g of PHMB/kg cotton, respectively. "Ideal" antibacterial activity was found to be 14.7 g of PHMB/kg cotton at 6% dye o.m.f. and in the range 12.7 and 20.4 g of PHMB/kg cotton at 9% dye o.m.f. dyeing. As before, increasing the concentration of fixed dye on cotton resulted in reduced antibacterial activity of PHMB. Thus, more PHMB was required for "ideal" antibacterial efficacy on dyed cotton; this increased with the concentration of fixed dye on cotton.

Having determined that the variation in the concentration of PHMB required for "ideal" antibacterial activity on dyed cotton was consistent with chemical attachment of PHMB to the sulphonate groups present, in covalently bound dyes, attention was directed to a comparison of

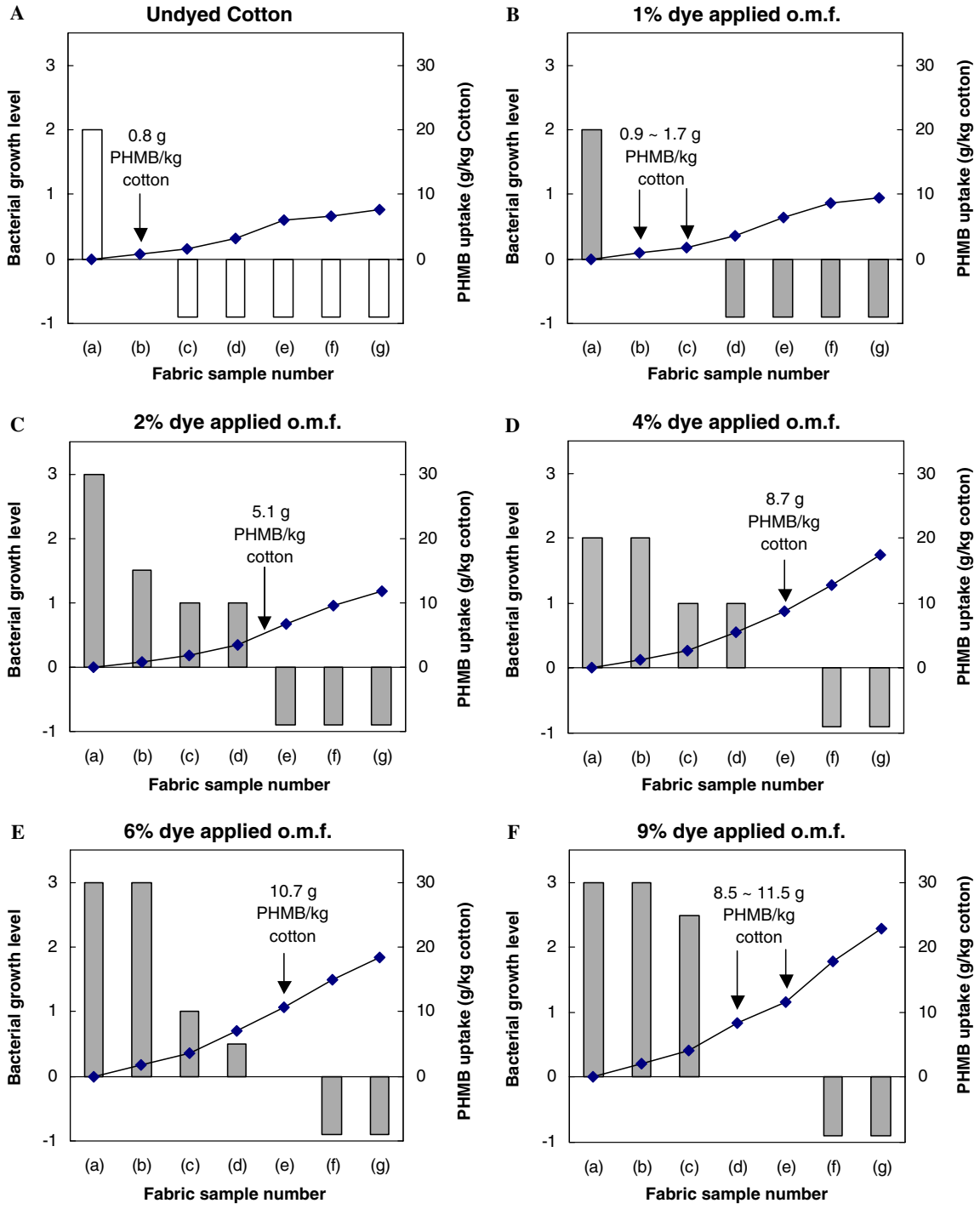


Fig. 9. Uptake and antibacterial activity of PHMB on cotton dyed with Procion Red H-E3B at 1%, 2%, 4%, 6% and 9% o.m.f. The solid lines represent the concentration of PHMB and the bars represent the level of bacterial growth; −1, zone of inhibition; 0, no growth; 1, low growth; 2, moderate growth and 3, heavy growth of bacteria.

the effect, on antibacterial activity of PHMB, of varying the dye. Due to ion–ion bonding between the anionic sulphonate groups and cationic PHMB, it is reasonable to expect that the concentration of PHMB for "ideal" antibacterial activity on dyed cotton would be related to the concentration of a given fixed dye. In this section, the variation in "ideal" antibacterial activity of PHMB against the concentration of fixed dye, and thereby sulphonate group concentration, is discussed.

A comparison of the PHMB concentration required for "ideal" antibacterial activity plotted against concentration of fixed Procion Yellow H-E4R is displayed in Fig. 11A. This indicated, broadly, that the concentration of PHMB required for "ideal" antibacterial activity

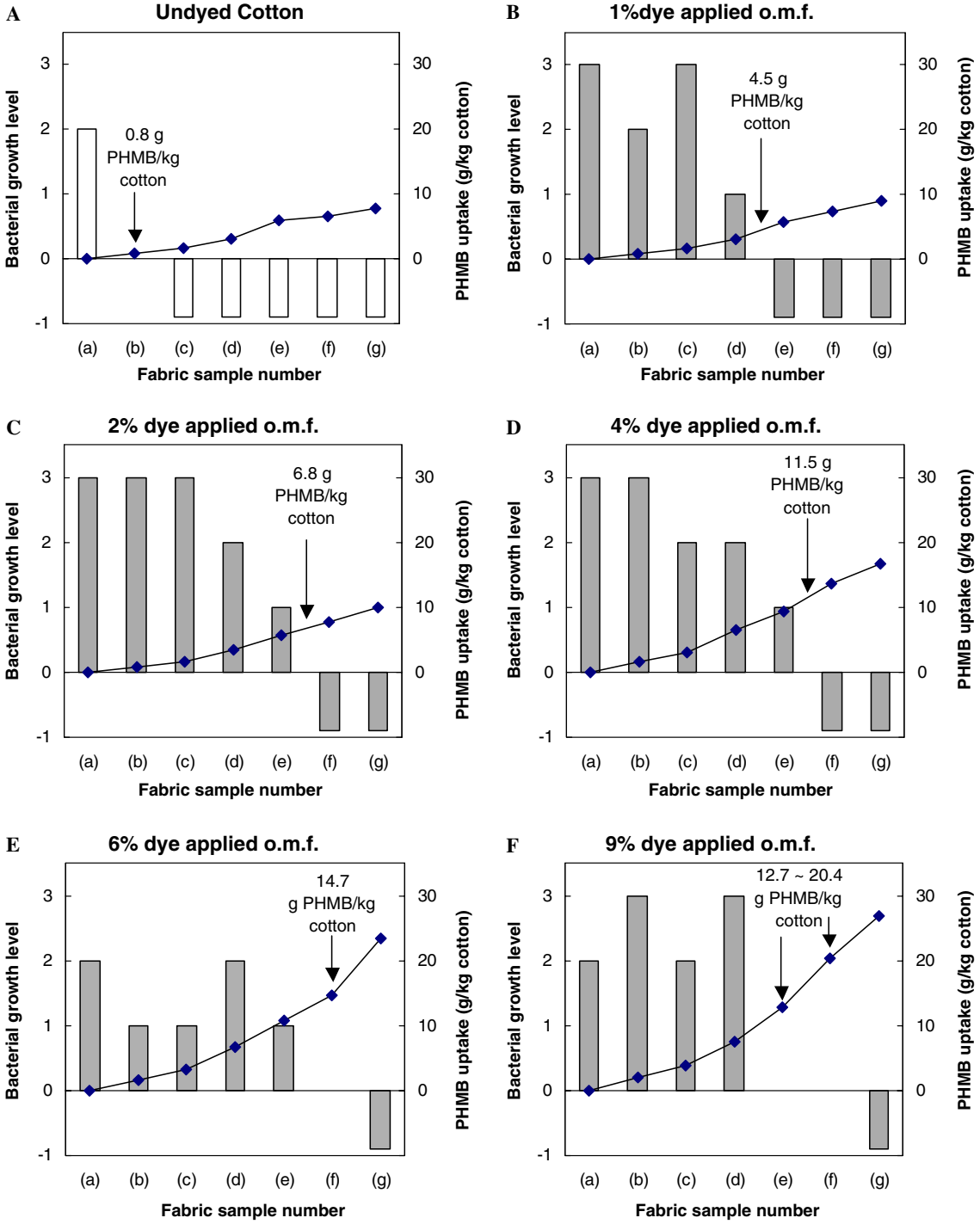


Fig. 10. Uptake and antibacterial activity of PHMB on cotton dyed with Procion Navy H-ER at 1%, 2%, 4%, 6% and 9% o.m.f. The solid lines represent the concentration of PHMB and the bars represent the level of bacterial growth; −1, zone of inhibition; 0, no growth; 1, low growth; 2, moderate growth and 3, heavy growth of bacteria.

increased with increasing concentration of fixed dye on cotton. This was attributed to the increasing concentration of sulphonate groups present which effectively reduced the antibacterial efficacy of PHMB, because of strong ion–ion bonding between anionic sulphonate groups and cationic PHMB, impeding the release of PHMB.

A similar trend was noted for the other two dyes, Procion Red H-E3B (Fig. 11B) and Procion Navy H-ER (Fig. 11C). In each case, the presence of increasing quantities of fixed dye required more PHMB for "ideal" antibacterial activity. It can be seen in Figs. 11A–C, that almost linear relationships exact between "ideal" PHMB concentration and sulphonate groups concentration with

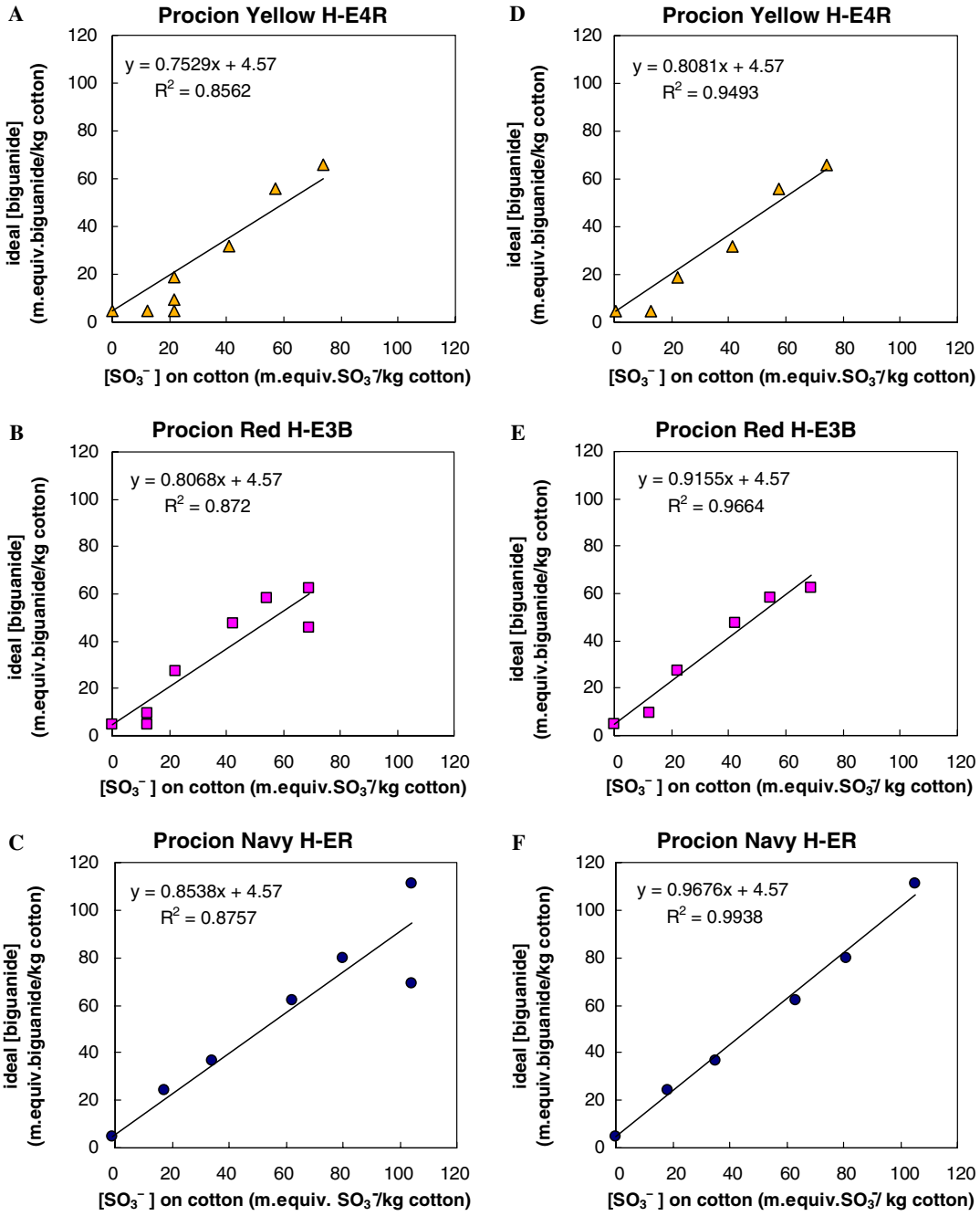


Fig. 11. Relationship between concentration of fixed sulphonic acid in bis(monochlorotriazinyl) reactive dyes and PHMB required for "ideal" antibacterial activity. In (A–C), all points at which "ideal" activity was observed have been included. When "ideal" activity was observed at more than one concentration of PHMB a much better straight line relationship was observed if a single higher value was included (D–F).

a correlation coefficients, R^2, values of 0.86, 0.87 and 0.88, respectively.

However, it can be seen from Fig. 11A, that for 2% Procion Yellow H-E4R the "ideal" PHMB activity was observed at three different concentrations 4.8, 9.3 and 19.1 mequiv of monomeric biguanide units/kg cotton. If the first and second of these values are discarded the resulting R^2 value increases to 0.95, (Fig. 11D), indicating an almost straight line relationship between PHMB concentration for "ideal" activity and concentration of bound dye, or sulphonate group.

A similar situation obtained in the cases of cotton dyed with Procion Red H-E3B and Procion Navy H-ER. In the case of the former, a good straight line relationship was observed between the concentration of PHMB for "ideal" activity and the concentration of fixed sulphonate groups, Fig. 11E, with an R^2 value of 0.97. In the case of Procion Navy H-ER if appropriate single points are selected for "ideal" PHMB concentration, R^2 rises to 0.99, Fig. 11F. Thus, in the case of each of the three Procion H-E dyes there is a clear linear relationship between the concentration of PHMB required for "ideal" antibacterial activity

and the concentration of fixed sulphonate groups. There is however, a difference in the slope of each of these lines, suggesting the concentration of sulphonate groups is not the sole determinant of antibacterial activity.

In order to understand the above results it is necessary also to consider the mode of antibacterial activity of poly(hexamethylene biguanide) hydrochloride, PHMB. This is a broad spectrum cationic antibacterial agent which is effective against both Gram-positive and Gram-negative bacteria (Payne & Kudner, 1996). It can readily be applied to cotton by several application methods such as padding, spraying and exhaustion (Avecia, 2000).

The outermost layer of bacterial cells invariably has a net negative charge, which is often stabilised by the presence of divalent cations, *e.g.*, Mg^{2+} and Ca^{2+} (Gilbert & Moore, 2005). The teichoic acid and polysaccharide elements of Gram-positive bacteria, the lipopolysaccharide of Gram-negative bacteria, and the cytoplasmic membrane are associated with these cations (Gilbert & Moore, 2005). PHMB is attracted to these anionic sites via electrostatic interaction, for example with lipoteichoic acid, which is linked to the peptidoglycan layer and membrane, Fig. 12.

It has been reported (Davies & Field, 1969; Davies, Bentley, & Field, 1968; Rosin et al., 2002) that the target site for PHMB antibacterial activity is the cytoplasmic membrane of microbes. The cytoplasmic membranes are composed primarily of protein, such as neutral and acidic phosphatidylglycerol (PG) (Gilbert & Moore, 2005). They are embedded within a lipid matrix approximating to a bilayer and stabilized by divalent cations such as Ca^{2+} (Gilbert & Moore, 2005). Cationic antimicrobials interact initially with the membrane by displacing the divalent cations (Gilbert & Moore, 2005). The interaction of PHMB with phospholipids in the cytoplasm membrane of bacteria has been studied by several workers (Davies & Field, 1969; Davies et al., 1968; Gilbert, Pemberton, & Wilkinson, 1990; Gilbert, Das, Jones, & Allison, 2001; Ikeda, Tazuke, & Watanabe, 1983; Rosin et al., 2002). Additionally PHMB has been shown to interact strongly with nucleic acids (Allen, Morby, & White, 2004). In both cases, cationic PHMB forms ion–ion linkages with anionic monophosphate groups present in the bacterial cell wall or in nucleic acids (Allen et al., 2004). Ikeda *et al.* (Ikeda, Ledwith, Bamford, & Hann, 1984) found that derivatives such as cationic PHMB are capable of interacting strongly with anionic surfaces, while the hydrophobic segments in their structure interact with the membrane interior.

In the case of *S. aureus*, acidic phosphatidylglycerol (PG) constitutes 37% of total lipids (Rosin et al., 2002) and, thus, interaction of PHMB with this can damage the cell wall leading eventually to its cleavage; this is depicted in Fig. 13.

The interaction of PHMB with a bacterial cytoplasmic membrane is illustrated in Fig. 14. The cytoplasmic membrane is stabilised by the presence of divalent cations, *e.g.*, Ca^{2+} (Fig. 14a). Polycationic PHMB can displace the surface cations (Fig. 14b), inducing changes to the structure of the phospholipids phase (Fig. 14c), the surface charge of the phospholipids phase is neutralised (Avecia, 2002) and the cytoplasmic membrane is disrupted (Maillard, 2002) (Fig. 14d). It has been reported that such electrostatic attraction of PHMB is rapid, reaching stable equilibrium within five minutes (Avecia, 2002).

The mode of the action of PHMB thus depends on the relative abundance of acidic phospholipids in the cytoplasmic membrane (Avecia, 2002), inducing a phospholipid phase separation in the cytoplasmic membrane (Fig. 4c). This separation affects the concentrations of proteins essential for the cell composition. This causes an increase of the permeability of the membrane with an efflux of the potassium ions (K^+), which surround the protein. As the result, the enzyme function of bacteria is lost (Avecia, 2002).

When up to 40% of loss of K^+ has occurred, PHMB is categorised as bacteriostatic, because bacterial cells can still

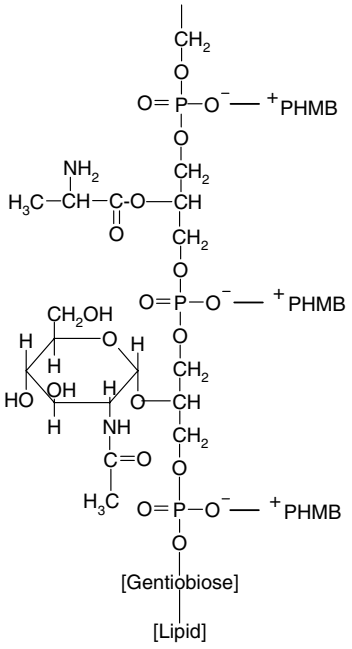


Fig. 12. Chemical attraction between lipoteichoic acid and PHMB (Mathews et al., 2000).

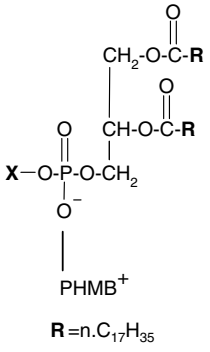


Fig. 13. Chemical attraction of PHMB to acidic phosphatidylglycerol (PG) (Tortora et al., 2002).

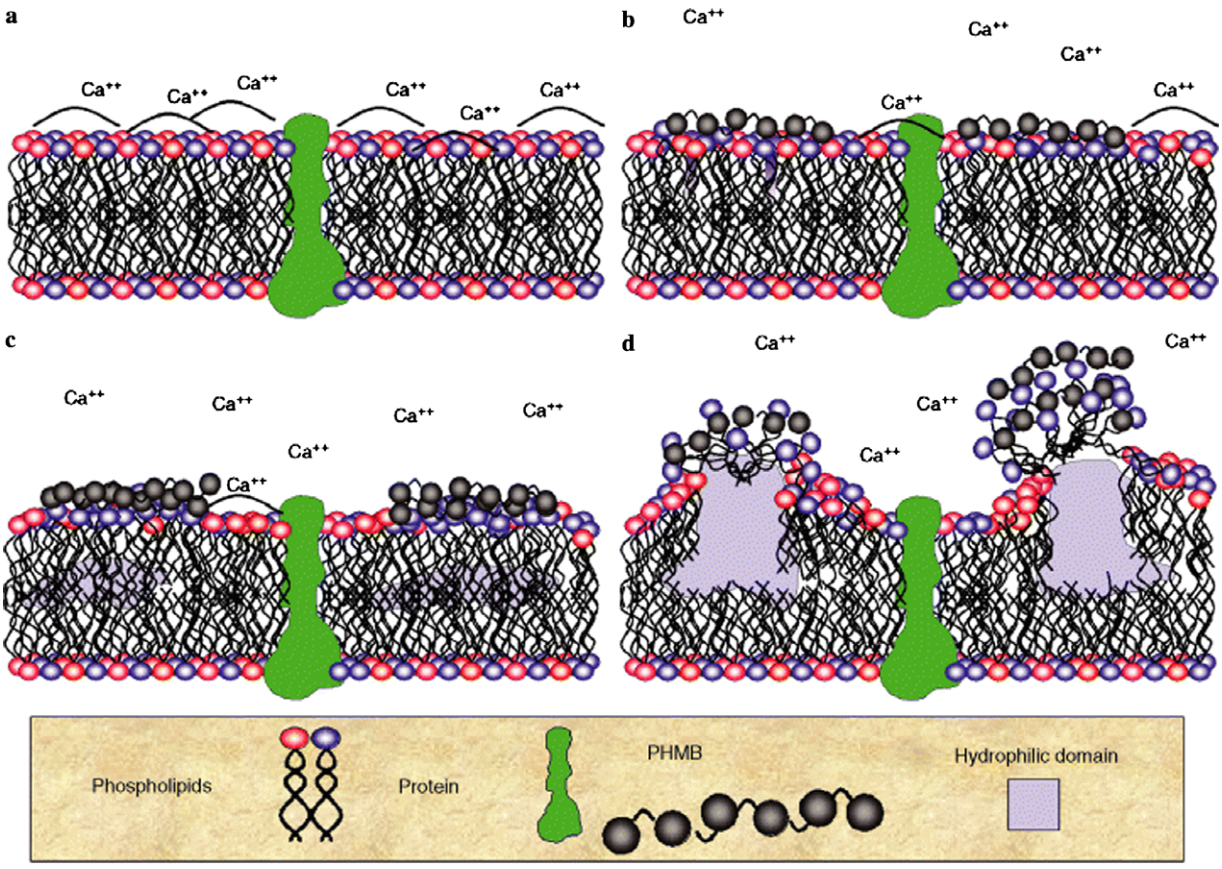


Fig. 14. Mechanism of antibacterial action of PHMB on a bacterial cytoplasmic membrane. The segments show progressive adsorption of PHMB. (a) Bacterial cytoplasmic membrane is stabilised by calcium ions and phospholipids mixture and distribution. (b) PHMB displaces the surface of cations to bind to acidic phospholipids (blue head colour of phospholipids). (c) PHMB induces a phospholipids phase separation. (d) Destabilised zones aggregate and leading to a loss of the membrane function (diagram was adapted from Gilbert and Moore (2005)).

survive such loss (Avecia, 2002). At higher percentage loss of K^+, PHMB is categorised as bacteriocidal. Also the extent of membrane disruption is related to the increasing level of polymerisation of biguanide (Broxton, Woodcock, & Gilbert, 1983); increasing the degree of polymerisation of PHMB increases its bactericidal efficacy (Broxton et al., 1983).

Although Gram-positive *S. aureus* was employed in this study, the crucial step in antibacterial action, involving electrostatic interaction of the cationic PHMB with the anionic cytoplasmic membrane, is common to both Gram-positive and Gram-negative bacteria (Gilbert & Moore, 2005; Paulus, 1993).

The biocidal action of PHMB is believed to involve interaction with anionic phosphate groups via ion–ion linkages (Gilbert & Moore, 2005). Thus PHMB bound to cotton, via ion–ion linkages with carboxylate groups, would be expected to undergo partial dissociation in an aqueous medium followed by rapid ion–ion bond formation with monophosphate groups present in the cytoplasmic membrane. For PHMB on un-dyed cotton such an equilibrium would be expected to favour ion–ion binding, of the cationic PHMB to the anion of the stronger, monophosphate acid [p*K*a *ca.* 1.2–1.7 (Williams, 2005)] over ion–ion bonding to weaker carboxylic acid groups, p*K*a 4–5 (March, 1992), on un-dyed cotton. In contrast, on cotton dyed with reactive dyes such an equilibrium would be expected to lie in favour of PHMB binding more strongly to the sulphonate groups (p*K*a Ar-SO_3H is *ca.* −6.5) (March, 1992), thus reducing its antibacterial efficacy.

6. Conclusions

The antibacterial efficacy of PHMB on cotton dyed with a range of bis(monochlorotriazinyl) reactive dyes was determined by the AATCC 147 test method, using *S. aureus* (AATCC, 2000).

As increasing quantities of PHMB were applied to cotton, dyed or un-dyed, bacterial growth on the cotton reduced until, at the "ideal" concentration of applied PHMB, no growth was observed on the cotton and no inhibition of growth was detected in the adjacent agar. At even heavier loading of PHMB leaching of agent into the agar was observed.

The "ideal" concentration of PHMB increased linearly with the concentration of a fixed dye, and thereby of sulphonate groups present. More PHMB was needed to confer "ideal" activity on cotton dyed with Procion Yellow

H-E4R, an octa-sulphonate, than on cotton possessing equimolar concentration of fixed Procion Red H-E3B or Procion Navy H-ER, both hexa-sulphonates.

These observations are consistent with ion–ion bond formation between cationic PHMB, and anionic carboxylate on un-dyed cotton, and anionic sulphonate and/or carboxylate on dyed cotton with antibacterial action dependent on dissociation to release free PHMB, followed by interaction with the bacterial cell wall.

References

American Association of Textile Chemists and Colorists (2000). AATCC Test Method 147 antibacterial activity assessment of textile materials: Parallel streak method. *AATCC Technical Manual 2000*, 75, pp. 260–261.

Allen, M. J., Morby, A. P., & White, G. F. (2004). Cooperativity in the binding of the cationic biocide polyhexamethylene biguanide to nucleic acids. *Biochemical and Biophysical Research Communications, 318*(2), 397–404.

Avecia. (2000). *Reputex 20; Application Information*. Manchester: Avecia Protection and Hygiene Avecia Ltd.

Avecia. (2002). Vantocil technical brief: mode of action of poly (hexamethylene biguanide) hydrochloride.

Avecia. (2004). Vantocil TG application information antimicrobial for wet wipes. In A4.2.1/0303/E ed. Avecia: Avecia Protection & Hygiene.

Bae, S.-H., Motomura, H., & Morita, Z. (1997). Adsorption behavior of reactive dyes on cellulose. *Dyes and Pigments, 34*(1), 37–55.

Bajaj, P., & Sengupta, A. K. (1992). Protective clothing: A critical appreciation of recent developments. In P. W. Harrison (Ed.), *Textile progress* (pp. 68). Manchester: The Textile Institute.

Balmforth, D., Bowers, C. A., & Guion, H. (1964). Acrylic-polymer composition and its relation to basic-dye substantivity. *Journal of the Society of Dyers and Colourists, 80*, 577–583.

Broughton, R. M., Worley, S. D., Unchin Cho, S. D., Lin, J., & Sun, G. (2003). Incorporating antimicrobial materials in fabric. *Textile Technology International, 56*, 56–59.

Broxton, P., Woodcock, P. M., & Gilbert, P. (1983). A study of the antibacterial activity of some polyhexamethylene biguanides towards *Escherichia coli* ATCC 8739. *Journal of Applied Bacteriology, 54*, 345–353.

Burdett, B. C. (1989). Physical chemistry essential to dyeing theory. In A. Johnson (Ed.), *The theory of coloration of textiles* (2nd ed., pp. 54). Bradford: Springer.

Chairat, M., Rattanaphani, S., Bremner, J. B., & Rattanaphani, V. (2005). An adsorption and kinetic study of lac dyeing on silk. *Dyes and Pigments, 64*(3), 231–241.

Chang, M.-Y., & Juang, R.-S. (2004). Adsorption of tannic acid, humic acid, and dyes from water using the composite of chitosan and activated clay. *Journal of Colloid and Interface Science, 278*(1), 18–25.

Christie, R. M., Mather, R. R., & Wardman, R. H. (2000). Chapter 8: Introduction to the physical basis and measurement of colour application of dyes. In *The chemistry of colour application* (pp. 183). Oxford: Blackwell Science.

Collier, B. J., & Epps, H. H. (1999). *Textile testing and analysis*. Upper Saddle River, NJ: Prentice Hall, p. 238.

Cornish, A., McGeechan, P., & Yeates, T. (2002). *COSMOCIL™ CQ for the delivery of gentle preservation and antimicrobial effects in personal care products*. Manchester: Avecia Protection & Hygiene.

Davidson, G. F. (1948). The acidic properties of cotton cellulose and derived oxycelluloses. Part 2. The absorption of methylene blue. *Journal of Textiles Institute, 39*, T65–T86.

Davies, A., Bentley, M., & Field, B. S. (1968). Comparison of the action of vantocil, cetrimide and chlorhexidine on *Escherichia coli* and its spheroplasts and the protoplasts of gram positive bacteria. *Journal of Applied Bacteriology, 31*, 448–461.

Davies, A., & Field, B. S. (1969). Action of biguanides, phenols and detergents on *Escherichia coli* and its sphereoplasts. *Journal of Applied Bacteriology, 32*(2), 233–243.

Gilbert, P., Das, J. R., Jones, M. V., & Allison, D. G. (2001). Assessment of resistance towards biocides following the attachment of micro-organisms to, and growth on, surfaces. *Journal of Applied Microbiology, 91*(2), 248–254.

Gilbert, P., & Moore, L. E. (2005). Cationic antiseptics: diversity of action under a common epithet. *Journal of Applied Microbiology, 99*(4), 703–715.

Gilbert, P., Pemberton, D., & Wilkinson, D. E. (1990). Barrier properties of the gram-negative cell envelope towards high molecular weight polyhexamethylene biguanides. *Journal of Applied Bacteriology, 69*, 585–592.

Giles, C. H., & Grezek, J. (1962). A review of methods of purifying and analyzing water-soluble dyes. *Textile Research Journal, 7*, 506–515.

Hasebe, Y., Kuwaraha, H., & Tokunaga, S. (2001). Chitosan hybrid deodrant agent for finishing textiles. *AATCC Review, 11*, 23.

Hyde, H. (2004). Personal communication.

Ikeda, T., Ledwith, A., Bamford, C. H., & Hann, R. A. (1984). Interaction of a polymeric biguanide biocide with phospholipid membranes. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 769*(1), 57–66.

Ikeda, T., Tazuke, S., & Watanabe, M. (1983). Interaction of biologically active molecules with phospholipid membranes: I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 735*(3), 380–386.

Kawabata, A., & Taylor, J. A. (2004). Effect of reactive dyes upon the uptake and antibacterial action of poly(hexamethylene biguanide) on cotton. Part 1: Effect of bis(monochlorotriazinyl) dyes. *Coloration Technology, 120*(5), 213–219.

Kawabata, A., & Taylor, J. A. (2006). The effect of reactive dyes upon the uptake and antibacterial action of poly(hexamethylene biguanide) on cotton. Part 2: Uptake of poly(hexamethylene biguanide) on cotton dyed with b-sylphatoethylsulphonyl reactive dyes. *Dyes and pigments, 68*, 197–204.

Klemm, D., Philipp, B., Heinze, T., Heinze, U., & Wagenknecht, W. (1998). *Comprehensive cellulose chemistry. Volume 1 Fundamental and analytical methods*. Weinheim: Wiley-VCH, p. 175.

Knecht, E., & Hibbert, E. (1925). *New reduction methods in volumetric analysis* (2nd ed.). London: Longmans Green & Co, p. 42.

Kobayashi, Y., Sekiguchi, A., Nakanishi, T., & Komiyama, J. (2002). Deodorant properties of cotton fabrics dyed with direct dyes and a copper salt. *Textile Research Journal, 72*(12), 1088–1092.

Kourai, H. (1999). Current situation and future of inorganic anti microbial agents. *Inorganic Materials, 6*, 428–436.

Lawson, J. A. (2001). Personal communication.

Maillard, J.-Y. (2002). Bacterial target sites for biocide action. *Journal of Applied Microbiology, 92*(s1), 16s–27s.

Mao, J., & Murphy, L. (2001). Durable freshness for textiles. *AATCC Review, 11*, 28.

March, J. (1992). *Advanced organic chemistry* (4th ed.). New York: John Wiley and Sons, pp. 250–251.

Mathews, C. K., van Holde, K. E., & Ahern, K. G. (2000). *Biochemistry*. San Francisco: Addison Wesley Longman, p. 307.

McGregor, R. (1972). Ionizable groups in fibers and their role in dyeing. *Textile Research Journal, 68*, 536–552.

McGregor, R., & Ezuddin, K. H. (1974). The distribution of dye and inorganic ions between cellulose films and aqueous solutions. *Journal of Applied Polymer Science, 18*, 629–632.

Murtagh, V., & Taylor, J. A. (2004). A simple titrimetric method for the estimation of reactive dye fixation on cellulosic fabrics. *Dyes and Pigments, 63*(1), 17–22.

Nakashima, T., & Matsuo, M. (2001). Relationship between antimicrobial activity and deodrant efficacy for cotton socks treated with metal. *Biocontrol Science, 6*(1), 1–8.

Nakashima, T., Matsuo, M., Bin, Y., & Sakagami, Y. (2003). The antimicrobial activity of cotton socks treated with metal salts as

affected by dirty materials and microorganisms adhering to them. *Biocontrol Science, 8*(1), 31–36.

Nakashima, T., Sakagami, Y., & Matsuo, M. (2001). Antibacterial efficacy of cotton fabrics chemically modified by metal salts. *Biocontrol Science, 6*(1), 9–15.

Nakashima, T., Takeuchi, Y., Yoshioka, M., Kamimura, Y., Fuse, G., & Enoki, A. (1991). Study on antimicrobial and deodorant finish of textiles (Part 1): Antimicrobial activity of wool-socks treated with heavy metal. *Journal of Antibacterial and Antifungal Agents, 19*(9), 451–458.

Paulus, W. (1993). *Microbicides for the protection of materials: A handbook*. London: Chapman and Hall, p. 8.

Payne, J. D. (1997). From medical textiles to smell-free socks. *Journal of the Society of Dyers and Colourists, 113*(2), 48–50.

Payne, J. D., Yates, J. E., Payne, J. D., Yates, J. E., Payne, J. D., Yates J. E. S. (2005). Arch UK Biocides Limited, assignee. Figres treated with antimicrobial agents. UK patent GB 2 408 516.

Payne, J. D., & Kudner, D. W. (1996). A new durable antiodor finish for cotton textiles. *Textile Chemist and Colorist, 28*(5), 28–30.

Rajendran, S., Anand, S. C. (2001). Development of a versatile antimicrobial finish for textile materials for healthcare and hygiene application. In S. C. Anand (Ed.), *Medical textiles – Proceeding of the International Conference 24 and 25 August 1999* (pp. 107–116). Cambridge: Woodhead.

Rosin, M., Welk, A., Kocher, T., Majic-Todt, A., Kramer, A., & Pitten, F. A. (2002). The effect of a polyhexamethylene biguanide mouthrinse compared to an essential oil rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque regrowth. *Journal of Clinical Periodontology, 29*(5), 392–399.

Sun, G., & Xu, X. (1998). Durable and regenerable antibacterial finishing of fabrics: biocidal properties. *Textile Chemist and Colorist, 30*(6), 26–30.

Tan, B. H., Teng, T. T., & Omar, A. K. M. (2000). Removal of dyes and industrial dye wastes by magnesium chloride. *Water Research, 34*(2), 597–601.

Tortora, G. J., Funke, B. R., & Case, C. L. (2002). *Microbiology: An introduction* (7th ed.). San Francisco: Benjamin Cummings, p. 582.

Tortora, G. J., Funke, B. R., & Case, C. L. (2002). *Microbiology: An introduction* (7th ed.). San Francisco: Benjamin Cummings, p. 44.

Venkataraman, K. (1952). *The chemistry of synthetic dyes* (Vol. II). New York: Academic Press Inc. p. 1345.

Venkataraman, K. (1977). *The analytical chemistry of synthetic dyes*. New York: John Wiley and sons, pp. 11–12.

Vigo, T. L. (1994). Protective clothing effective against biohazards. In M. Raheel (Ed.), *Protective clothing systems and materials* (pp. 230). New York: Marcel Dekker.

Vigo, T. L., Parikh, D. V., & Danna, G. F. (2001). Improved durability of peroxide-based antibacterial agents on fabrics. *AATCC Review, 11*, 40.

Wallace, M. L. (2001). Testing the efficacy of PHMB as an antimicrobial treatment for cotton fabrics. *AATCC Review, 11*, 18.

Williams R. (2005). p*K*a data compiled. Available from <http://research.chem.psu.edu/brpgroup/pKa_compilation.pdf/>, accessed in 22nd December 2005.

Xin, J. H., Zhu, R., Hua, J., & Shen, J. (2002). Surface modification and low temperature dyeing properties of wool treated by UV radiation. *Coloration Technology, 118*(4), 169–173.

Yates, J. (2004). Personal communication.