

# Biosafety Evaluation and Quantitative Determination of Poly(hexamethylene biguanide) (PHMB) Coated on Cellulosic Fabrics by Kubelka-Munk Equation

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## Research Article

**Keywords:** Kubelka-Munk equation, colour strength, cationic finishing agents, cellulosic fabrics, quantitative determination, biosafety evaluation

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1 **Biosafety evaluation and quantitative**  
2 **determination of poly(hexamethylene**  
3 **biguanide) (PHMB) coated on cellulosic fabrics**  
4 **by Kubelka-Munk equation**

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11  
12 Abstract

13 It is a challenge to determine the quantity of cationic finishing agents on the surface  
14 of cellulosic fabrics. Herein, we report a direct and feasible method by Kubelka-  
15 Munk equation to quantify the cationic poly (hexamethylene biguanide)  
16 hydrochloride (PHMB) adsorbed onto cotton fabrics based on the principle of  
17 formation of a stable blue dye between PHMB and bromophenol blue sodium  
18 (BPB). The adsorption of PHMB onto cotton fabrics was first investigated and the  
19 maximum adsorption of PHMB was found to be around 8 mg per gram of cotton  
20 fabric. After being dyed with BPB, colour strength shows a positive correlation with  
21 PHMB at low concentrations (<2400 mg/L). A linear relationship with a high  
22 correlation ( $C(\text{PHMB}) = (K/S - 0.7411)/3.4517$ ,  $R^2=0.9983$ ) was thus established  
23 between colour strength and PHMB concentration. However, this equation should  
24 fulfill four requirements for quantifying PHMB: (1) the distribution of PHMB on  
25 the surface of cellulosic fabric should be in the form of a monolayer with the content  
26 less than 5.3 mg/g; (2) an excess of BPB dye bath should be applied; (3) the dyeing  
27 should come to equilibrium; and (4) the fabrics should be evenly dyed. Moreover,  
28 MTT assay results indicate that excess PHMB coated on cotton fabrics can cause  
29 cytotoxicity and the quantity of PHMB should not exceed 4.62 mg per gram of  
30 cotton fabrics for the purpose of biosafety. The sample can be considered non-  
31 cytotoxic if the  $K/S$  value is lower than 13.2 after dyeing with BPB.

32 *Keywords: Kubelka-Munk equation; colour strength; cationic finishing*  
33 *agents; cellulosic fabrics; quantitative determination; biosafety*  
34 *evaluation*

35  
36 Author contributions

37 Conceptualization: Wen-Yi Wang, Chi-Wai Kan; Methodology: Wen-Yi Wang, Jia-  
38 Chi Chiou, Wan-Xue Chen, Jia-Li Yu; Formal analysis and investigation: Wen-  
39 Yi Wang, Chi-Wai Kan; Writing- original draft preparation: Wen-Yi Wang;  
40 Writing- review and editing: Chi-Wai Kan; Funding acquisition: Chi-Wai Kan;  
41 Resources: Chi-Wai Kan, Jia-Chi Chiou; Supervision: Chi-Wai Kan.

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44

## 45 Introduction

46 Cationic surfactants are predominantly nitrogenous polymers such as  
47 guanidines and quaternary ammonium compounds (QACs) with positively charged  
48 surface-active moieties on the hydrophilic end. Cationic surfactants find  
49 wide applications in textile finishing primarily as fabric softeners,  
50 antistatic and antimicrobial agents (Sivaramakrishnan 2013; Kosswig 2000).  
51 Finishing agents used as fabric softeners can improve softness and wearability  
52 of the fabric, and reduce the static electricity built-up on the surface of  
53 fibres if used as antistatic agents (Beal et al. 1990; Agarwal et al. 2011).  
54 Antimicrobial agents are used in textile finishing not only to prevent  
55 deterioration of fibre strength and quality due to microbial colonization by  
56 insects, fungi and micro-organisms but also to circumvent cross-contamination  
57 and nosocomial infections (Gao et al. 2019, 2021). However, prolonged exposure  
58 to these chemicals, particularly in the case of overuse and even abuse of  
59 cytotoxic antibacterial agents, can cause skin irritation and damage because  
60 of the destroyed lipid membrane that serve as the external protective layer  
61 of the skin (Kosswig 2000). Therefore, determination of these cationic  
62 finishing agents coated on the fabrics is of high importance because that is  
63 necessary to ensure compliance with safety regulations and production  
64 standards in the public interest. To the best of our knowledge, only  
65 qualitative methods have been reported for identifying the existence of  
66 cationic surfactants in cellulosic textile fabrics (Fan 2005). A simple,  
67 direct, and efficient method for determining the quantity of cationic  
68 surfactants adsorbed onto the cellulosic fabrics does not seem to have been  
69 proposed hitherto, though it is highly imperative.

70 Cationic finishing agents are generally used in finishing of textile fabrics  
71 in the form of a multicomponent finishing solution or suspension mixed with  
72 inorganic salts, polymeric binders or other additives such as emulsifiers and  
73 dispersants by conventional exhaust, pad-dry-cure processes, spraying or foam  
74 finishing method (Yuan and Cranston 2008; Gargoubi et al. 2016). This makes  
75 it difficult to quantify the agents coated on cellulosic fabrics. Some surface  
76 analytical techniques such as scanning electron microscopy (SEM), X-ray  
77 photoelectron spectroscopy (XPS), Fourier Transform Infrared Spectroscopy  
78 (FTIR) and mass spectrometry (ToF-SIMS) are often used to qualitatively study  
79 the presence of cationic finishing agents on the fabrics (Brunon et al. 2011;  
80 Wang et al. 2020; Mitchell et al. 2005). Spectrophotometry provides an  
81 indirect method to quantify cationic finishing agents based on extraction of  
82 ion associates formed between cationic agents and anionic dyes such as  
83 disulfine blue and ethyl orange (Li and Zhao 2004; Motomizu and Gao 1994).  
84 Relatively more sophisticated chromatographic approaches such as gas  
85 chromatography (GC), gradient gel-permeation chromatography (GPC) and high-  
86 performance liquid chromatography (HPLC) are precise methods to quantify  
87 cationic finishing agents in fabrics (Lucas et al. 2009; Masadome et al.  
88 2018). Recently, various novel methods with high sensitivity such as glucose  
89 oxidase enzymatic reaction analytical method and Lactone-rhodamine B-based  
90 fluorescence optode technique have been introduced to quantify  
91 poly(hexamethylene biguanide) hydrochloride (PHMB) in the solution used for  
92 contact lens (Funaki et al. 2020; Masadome et al. 2018; Uematsu et al. 2019).  
93 However, these analytical methods are primarily suitable for trace analysis  
94 of cationic agents in aqueous, such as industrial wastewater and contact lens  
95 solution, and are incapable of quantifying cationic surfactants on the textile

96 fabrics.  
 97 Bromophenol blue (BPB) is a kind of triphenylmethane dye which has been  
 98 extensively used as laboratory acid-base indicator and biological stain for  
 99 proteins and nucleic acids (Li et al. 2019; Wang et al. 2017). Anionic BPB  
 100 can be complexed with cationic surfactants at room temperature ascribed to  
 101 electrostatic attraction, leading to formation of water-insoluble and stable  
 102 blue complex dyes (Sheiham and Pinfold 1969; Colichman 1947). A more recent  
 103 study on the application of BPB in dyeing of cotton fabric treated with  
 104 cationic finishing agents was reported by our group (Wang and Kan 2020), in  
 105 which the antibacterial agent PHMB was first coated on the fabric and then  
 106 the fabric was dyed with BPB solution at room temperature without use of any  
 107 inorganic salts. The dyed fabrics showed excellent colour levelness and  
 108 fastness to rubbing and washing, and the colour strength was found  
 109 controllable and highly dependent upon concentration of BPB dyebath and  
 110 quantities of coated PHMB.  
 111 Colour strength of dyed fabrics is customarily expressed as  $K/S$  value, where  
 112  $K$  and  $S$  are the absorption and scattering coefficients of the dyed sample,  
 113 respectively. Absorption coefficient  $K$  is primarily dependent on the dyestuff  
 114 whereas scattering coefficient  $S$  is determined by the substrate.  $K/S$  value  
 115 can be calculated from the reflectance value ( $R$ ) according to Kubelka-Munk  
 116 equation (Eq. (1)) (Becerir 2005; Tang et al. 2019).

$$117 \quad K/S = \frac{(1-R)^2}{2R} \quad (1)$$

118  $K/S$  value has been commonly used to quantitatively evaluate the quantity of  
 119 dye on the fabric at the maximum absorption wavelength.  $K/S$  value increases  
 120 with dye content of the fabric increasing. The peak height in the  $K/S$  spectrum  
 121 (plotted by Eq. (1)) is proportional to the quantity of dye in the substrate,  
 122 and the functional relationship is as given in Eq. (2) (Ahmed et al. 2006;  
 123 Khatri et al. 2014).

$$124 \quad K/S = A \cdot C \quad (2)$$

125 Where  $A$  is a constant equal to the gradient of the plot of  $K/S$  versus dyestuff  
 126 quantity in the substrate which depends on the absorption coefficient of the  
 127 dyestuff, dyeing conditions and textile substrates;  $C$  is the dyestuff quantity  
 128 in the textile substrate. Theoretically,  $A$  is independent of concentration  
 129  $C$ . Therefore, the dye quantity can be obtained from Eq. (2), which equals to  
 130 the quantity of cationic surfactants coated in the fabric substrate due to  
 131 stoichiometric reaction (Eq. (3)).

$$132 \quad C(\text{dye}) = C(\text{PHMB}) = (K/S)/A \quad (3)$$

133 Here we are inspired to quantify the cationic finishing agents on the  
 134 cellulosic fabrics by measuring the colour strength after dyeing with BPB.  
 135 On the other hand, biosafety properties such as skin irritation and  
 136 cytotoxicity of treated fabrics are closely associated with the quantity of  
 137 cationic agents on the fabrics, which may also be manifested by colour  
 138 strength. In the present study, PHMB is exemplified to validate the assumption  
 139 that quantifies the cationic agents on the fabrics using Kubelka-Munk equation.  
 140 The correlation between PHMB content on the fabrics and colour strength dyed  
 141 with BPB dyebath, as well as biosafety properties, was investigated in detail.

## 142 Experimental

### 143 Materials

144 Plain weave 100 % cotton fabric (40 ends per inch in warp and weft; fabric  
145 weight 175 g/m<sup>2</sup>) was used. The fabric was initially cleaned in an aqueous  
146 solution with 2 g/L sodium carbonate (obtained from Sigma-Aldrich, St. Louis,  
147 MO, USA) and 2 g/L non-ionic detergent (C13-oxoalcohol ethoxylates (7EO),  
148 supplied by SDC Enterprises Limited, Holmfirth, UK) at 60 °C for 30 minutes  
149 to remove impurities. Afterwards, the fabric was thoroughly rinsed with tap  
150 water and dried at room temperature.

151 Dulbecco modified eagle medium (DMEM) without phenol red, DMEM, newborn calf  
152 serum (NBS), 0.5% Trypsin-EDTA and phosphate buffer saline (PBS) were  
153 purchased from Thermo Fisher (Waltham, Massachusetts, USA).  
154 Poly(hexamethylene biguanide) (PHMB, 20% w/v aqueous solution), the model  
155 cationic antibacterial agent, was supplied by Breakthrough Textiles Co., Ltd  
156 (Taipei, Taiwan). Bromophenol blue (BPB) sodium salt was obtained from Sigma-  
157 Aldrich (St. Louis, MO, USA).

### 158 PHMB coating on cotton fabrics

159 Fabric samples (10×20 cm) were immersed in PHMB solution of different  
160 concentrations overnight for complete exhaustion with a liquor ratio of 30:1.  
161 The samples were then withdrawn and cured at 150 °C for 3 minutes. Afterwards,  
162 the liquor was retained for concentration determination by UV-vis  
163 spectrophotometry. Fabric samples treated with different concentrations of  
164 PHMB were labelled as P1~P15, respectively, as shown in Table 1.

165 **Table 1** Sample list for cotton fabrics coated with different concentrations of  
166 PHMB

Sample no. :	PHMB conc. (mg/L)	Sample no. :	PHMB conc. (mg/L)	Sample no. :	PHMB conc. (mg/L)
P1	100	P6	1600	P11	4000
P2	200	P7	2000	P12	5000
P3	400	P8	2400	P13	6000
P4	800	P9	3000	P14	8000
P5	1200	P10	3600	P15	10000

### 167 Adsorption equilibrium studies

168 Concentration of exhausted PHMB solution was measured using a UV-2401PC UV-  
169 visible spectrophotometer at  $\lambda_{\max}$  of PHMB by the calibration curve. The  
170 amount of adsorption at equilibrium  $q_e$  (mg/g) was calculated by Eq. (4).

$$171 \quad q_e = \frac{(C_i - C_e)V}{m} \quad (4)$$

172 Where  $C_i$  and  $C_e$  are the initial and equilibrium concentration in mg/L of PHMB  
173 before and after exhaustion;  $V$  is the volume of PHMB solution (L);  $m$  is the  
174 mass of applied cotton fabric (g).

### 175 Dyeing procedure

176 BPB solution (500 mg/L) was initially prepared and stored in an amber  
177 laboratory bottle prior to dyeing. The dyeing was performed by immersing the

178 fabric samples (3 g) treated with PHMB solution in 100 mL of BPB solution in  
179 a sealed beaker, withdrawn after 30 minutes and then rinsed with tap water  
180 to remove unfixed dyes. Finally, the dyed samples were air-dried in the fume  
181 hood.

## 182 **Colour strength measurement**

183 After dyeing, colour strength ( $K/S$ ) of BPB-dyed samples was measured for  
184 visible wavelength of 400 to 700 nm by using a spectrophotometer GretagMacbeth  
185 Color Eye 7000A spectrophotometer. Parameters were small aperture and D65  
186 daylight with a 10° standard observer. Each sample was folded twice to provide  
187 a total of four layers of material. Three measurements at different locations  
188 for each specimen were randomly conducted. The colour strength ( $K/S$ ) was  
189 calculated according to the Kubelka-Monk equation (Eq. (1)).

## 190 ***In vitro* cytotoxicity evaluation**

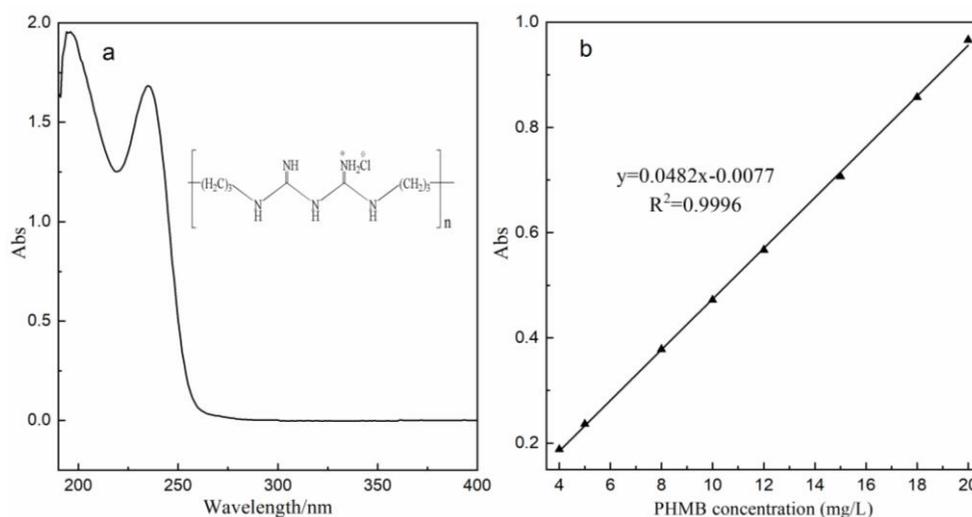
191 *In vitro* cytotoxicity of PHMB coated cotton fabrics was evaluated by MTT  
192 assay in accordance with the standard ISO 10993-5:2009(E) using commercially  
193 available fibroblast (NIH3T3, ATCC CRL-1658; the American Type Culture  
194 Collection, Manassas, VA, USA). Samples of PHMB-coated cotton fabrics were  
195 cut into square shape (4×4 mm), sterilized by UV radiation for 3 hours on  
196 both sides in a biological safety cabinet, and then extracted with the culture  
197 medium of Dulbecco's Modified Eagle's Medium (DMEM, 1 mL) at 37°C for 24  
198 hours. NIH3T3 was cultured in 12 mL DMEM with Newborn Calf Serum (NBS, 10%)  
199 at 37°C and 5% carbon dioxide for 72 hours. The culture medium was replaced  
200 with fresh medium every 24 hours. Then, the cells were transferred into a  
201 96-well plate with the seeding number of 30000 per well and allowed to grow  
202 in fresh medium for 24 hours. Afterwards, the medium was removed, and the  
203 cells were rinsed by PBS. The extraction (100µL) was added into the cells  
204 and incubated at 37°C and 5% carbon dioxide for 24 hours. The DMEM (without  
205 phenol red) and 2% Triton (in DMEM) were used as negative and positive control,  
206 respectively. Next, the cells were centrifuged at 1500 rpm for 5 minutes with  
207 the S2096 rotor (Beckman Coulter, Indianapolis, IN, USA). Then, the  
208 supernatant was removed and 50µL MTT assay reagent (MTT dissolved in DMEM at  
209 1 mg/mL and filtered to sterilize) was added. After 2 hours, MTT reagent was  
210 removed and isopropanol (100µL) was added into the cells for formazan  
211 extraction for 10 minutes. Then the spectrophotometric analysis of extracted  
212 formazan was conducted by a microplate reader (CLARIOstar, BMGLabtech,  
213 Offenburg, Germany) at 570 nm. Cytotoxicity was expressed as the ratio of the  
214 cell viability of each treatment to the maximum cell viability of the negative  
215 control.

## 216 **Results and discussion**

### 217 **Calibration curve of PHMB solution**

218 PHMB has high solubility (>40% w/w) in water in the form of micelle. Fig.  
219 1(a) shows absorbance of diluted PHMB solution as a function of wavelength  
220 over the ultraviolet-visible spectrum. Clearly, PHMB shows no absorption in  
221 the visible range, but strong characteristic band at 235 nm ascribed to  $\pi$ -  
222  $\pi^*$  transition of -C=N- groups in the biguanide and band at 194 nm due to  
223  $\pi$ - $\pi^*$  transition of -C-NH- groups (De Paula et al. 2011). In this study,

224 the characteristic peak at 235 nm was used to establish a calibration curve  
225 based on the Beer-Lambert law (Fig. 1(b)). A high correlation ( $R^2=0.9996$ )  
226 between the absorbance and PHMB concentration ranging from 4 to 20 mg/L was  
227 obtained.

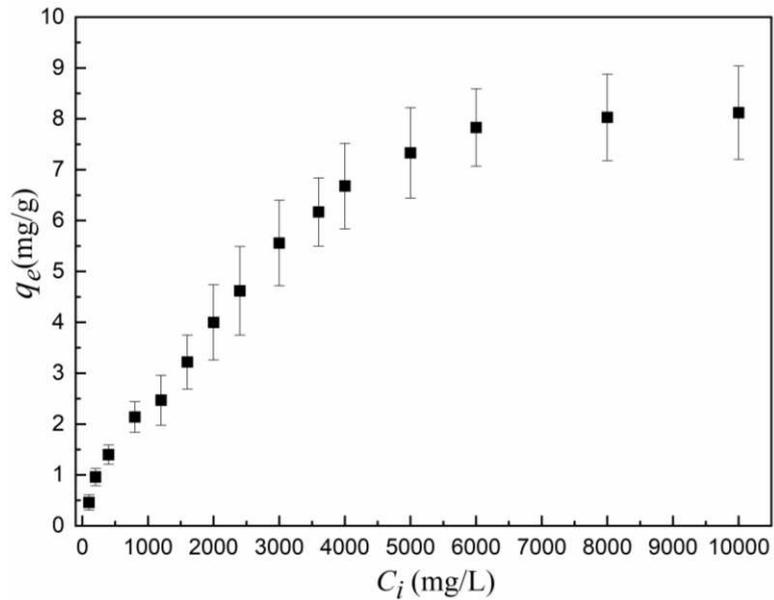


228

229 **Fig. 1** The maximum absorbance of PHMB solution over the UV-vis spectrum (a); and  
230 the calibration curve of PHMB solution established at 235 nm (b)

### 231 **Adsorption of PHMB on cotton fabric**

232 A previous study has demonstrated that adsorption of PHMB on cellulosic fibres  
233 is concentration-dependent and has characteristics of both Langmuir and  
234 Freundlich isotherms attributed to a combination of electrostatic attraction  
235 and hydrogen-bonding forces between PHMB and the carboxylic acid groups of  
236 cellulose (Blackburn RS et al. 2006; Wågberg et al. 1988). This explains the  
237 good affinity and durability of PHMB-coated antibacterial cotton fabrics. The  
238 present study first investigated the adsorption of PHMB on cellulosic fabrics  
239 to establish the relationship between PHMB content adsorbed on cotton fabrics  
240 and the colour strength after dyeing in BPB dye bath. Fig. 2 displays the  
241 quantity of PHMB adsorbed by cotton fabrics based on UV-vis spectrophotometric  
242 analysis. As can be clearly seen, the higher the initial concentration of  
243 PHMB applied, the higher is the amount of PHMB adsorbed by cotton fabrics.  
244 The amount of adsorbed PHMB first increases sharply from 100 mg/L to 5000  
245 mg/L, and then it has a flattened region up to 10000 mg/L, as the initial  
246 concentration of PHMB increases. This suggests that a higher initial  
247 concentration of PHMB solution (>5000 mg/L) fails to enhance the adsorption  
248 of PHMB under the fixed exhaustion duration. The maximum quantity of PHMB at  
249 equilibrium adsorption is around 8 mg per gram of cotton fabric.

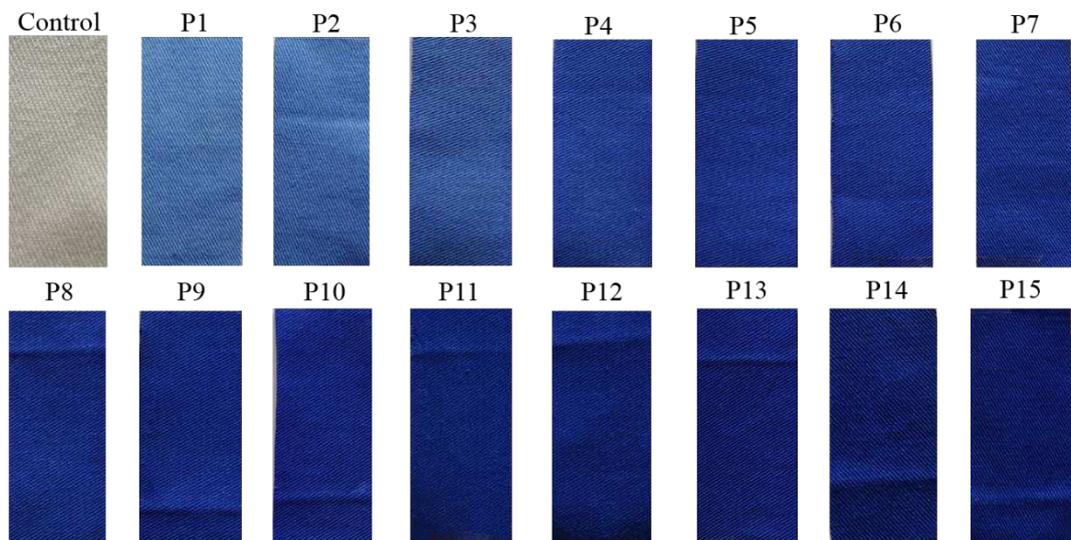


250

251 **Fig. 2** Adsorption of PHMB onto the cotton fabric with concentration ranging from  
 252 100 mg/L to 10000mg/L

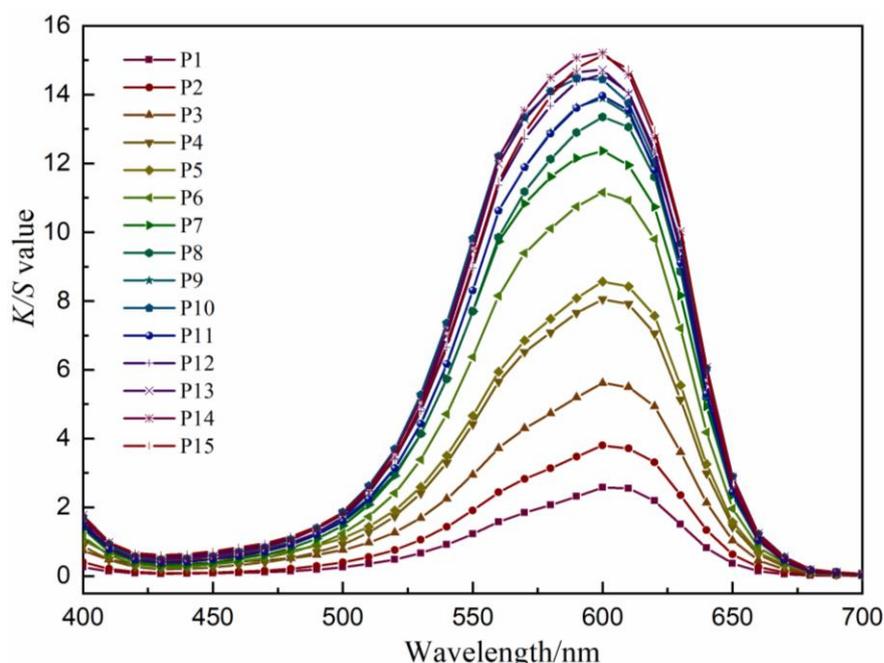
253 **Colour strength analysis**

254 Fig. 3 presents the appearance of PHMB-treated cotton fabrics after dyeing  
 255 with excess BPB solution (500 mg/L). Clearly, the blue shade is achieved and  
 256 colour depth shows gradient from light to dark as PHMB concentration increases.  
 257 All samples exhibited a similar pattern and there was no shift in the peak  
 258 of colour strength curves (Fig. 4). The maximum band observed in the visible  
 259 range was at 600 nm. Colour strength is basically proportional to the  
 260 initially applied PHMB concentrations, particularly for lower concentrations  
 261 of PHMB. A distinct increase was seen in samples treated with less than 2400  
 262 mg/L of PHMB (P1-P8), and with PHMB concentration rising to 10000 mg/L, colour  
 263 strength shows a slow growth and overlaps with each other in the maximum peak  
 264 of curves. This suggests that colour strength is highly dependent on the  
 265 initial PHMB concentration, to be exact, depends on the quantity of PHMB  
 266 adsorbed by the fabrics. Next, the relationship of colour strength with the  
 267 adsorbed PHMB on the fabrics is discussed as below.



268

269 **Fig. 3** Colour shades of PHMB-treated cotton fabrics after dyed with BPB dyebath



270

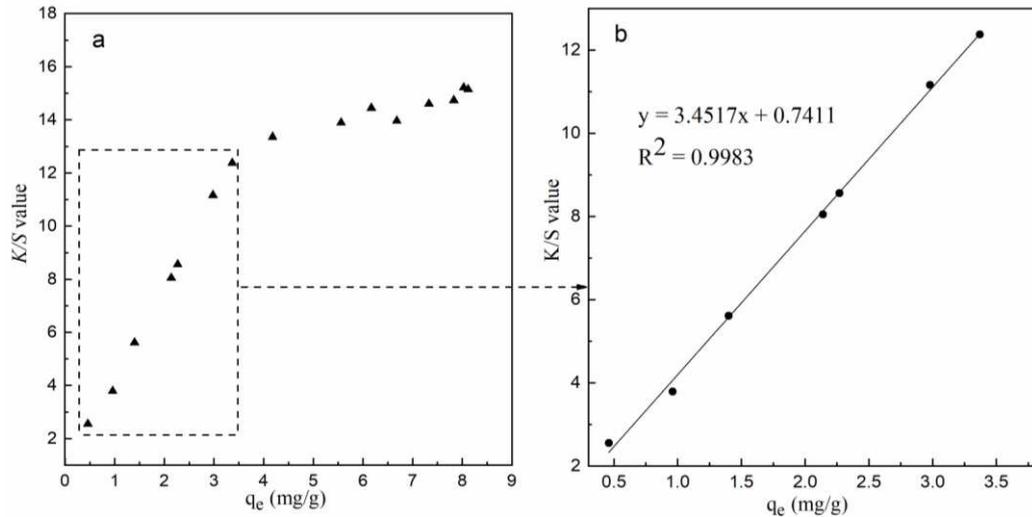
271 **Fig. 4** Colour strength curves of cotton fabrics treated with different  
 272 concentrations of PHMB solutions after dyeing with BPB dyebath

273

#### 274 **Establishment of linear relationship between colour strength and PHMB contents**

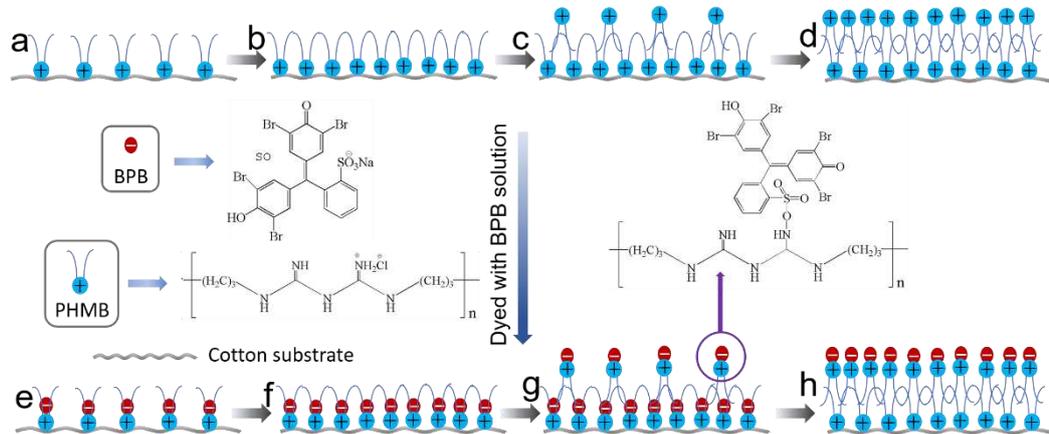
275

276 Fig. 5 presents the increasing  $K/S$  value associated with BPB dyeing of PHMB-  
 277 treated cotton fabrics as the amount of adsorbed PHMB increases. A similar  
 278 curve with adsorption isotherm of PHMB (Fig. 2) was observed, as shown in  
 279 Fig. 5(a). The higher the quantity of PHMB adsorbed by cotton fabrics, the  
 280 higher is the peak height of the  $K/S$  spectrum achieved after being dyed with  
 281 BPB dyebath. A drastic increase of the  $K/S$  value is clearly observed with  
 282 PHMB adsorption increasing from approximately 0.5 mg/g to 5 mg/g, and then  
 283 it levels up to around 15. This demonstrates that the colour strength ( $K/S$   
 284 value) is proportional to the quantity of PHMB adsorbed on the fabrics at  
 285 low concentrations, whereas at higher concentrations, the  $K/S$  value shows a  
 286 slow and fluctuating growth. The reason may be related to the conformation  
 287 and arrangement of PHMB molecule on the surface of cotton fabrics (Fig. 6).  
 288 At lower concentrations, PHMB molecules transport and adsorb from solution  
 289 to the surface of cellulosic fibres in the form of monolayer loose  
 290 distribution (Fig. 6a), and with the increasing concentrations, the loosely-  
 291 arranged PHMB aggregates form a densely-packed monolayer (Fig. 6b) (Blackburn  
 292 RS et al. 2006). Afterwards, a multilayer stacking of PHMB molecules via  
 293 reconfirmation on the surface of cellulosic fibres is formed with the outer  
 294 layer changing from loosely-arranged to densely-packed, as the concentration  
 295 of PHMB further increases (Fig. 6(c, d)). The dyeing primarily occurs between  
 296 BPB and the loosely distributed PHMB molecules (Fig. 6(e, f, g)), and due to  
 297 the steric hindrance effect, BPB molecules cannot bond with inner layer of  
 298 PHMB molecules (Fig. 6h). Therefore, there is no significant increase in the  
 299 colour strength ( $K/S$  value) as PHMB concentration increases beyond the  
 300 critical monolayer capacity. From Fig. 5(a), the critical monolayer capacity  
 301 of PHMB adsorption onto cellulosic fabrics is approximately 4.5 mg/g, which  
 302 is pretty close to the theoretical monolayer capacity 5.3 mg/g, as reported  
 by Blackburn *et al.* (2006).



303

304 **Fig. 5** Plot of  $K/S$  values of BPB dyed cotton fabrics versus the amount of adsorbed  
 305 PHMB (a); and (b) plot of  $K/S$  values versus samples with low quantity of PHMB  
 306 adsorption (P1-P7)



307

308 **Fig. 6** Schematic diagram to show the arrangement of PHMB molecules adsorbed on  
 309 the surface of cellulosic fibres and the dyeing behavior: (a) loose distribution;  
 310 (b) dense distribution; (c) loose distribution of outer layer; (d) dense  
 311 distribution of outer layer; (e) loosely-distributed PHMB dyed with BPB; (f)  
 312 densely-distributed PHMB dyed with BPB; (g) loosely-distributed multilayer PHMB  
 313 dyed with BPB; and (h) densely-distributed multilayer PHMB dyed with BPB

314 Fig. 5(b) shows a linear relationship between  $K/S$  value and the quantity of  
 315 PHMB adsorbed on the fabric at low concentrations. The high correlation  
 316 ( $R^2=0.9983$ ) suggests that it is a promising and efficient way to quantify the  
 317 PHMB coated on the fabrics by measuring the colour strength after being dyed  
 318 with excess BPB dyebath. According to Eq. (2), the formula in Fig. 5(b) can  
 319 be written as Eq. 5:

320

$$K/S = 3.4517 \times C(\text{PHMB}) + 0.7411 \quad (5)$$

321

So, the quantity of PHMB adsorbed onto the cotton fabric substrate is given  
 322 in Eq. (6):

323

$$C(\text{PHMB}) = \frac{\left(\frac{K}{S} - 0.7411\right)}{3.4517} \quad (6)$$

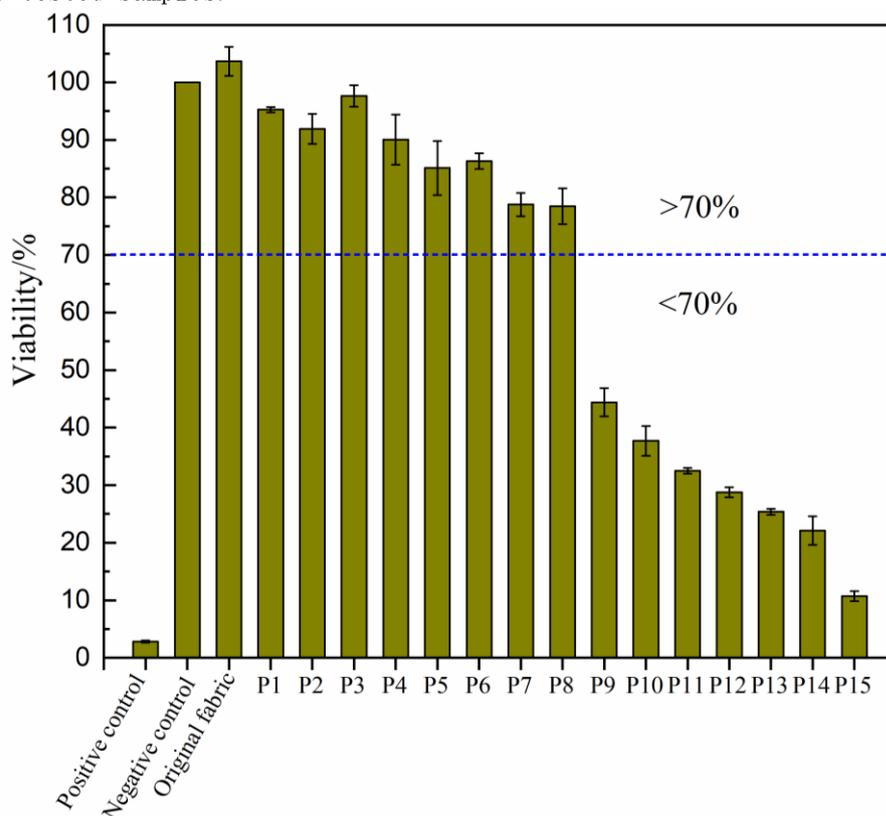
324

This equation should fulfill four requirements: (1) distribution of PHMB on  
 325 the surface of cotton fabric should be in the form of monolayer with content  
 326 less than 5.3 mg/g; (2) an excess of BPB dyebath should be applied; (3) dyeing

327 should come to equilibrium; and (4) fabrics should be evenly dyed.

### 328 ***In vitro* biosafety evaluation**

329 Biosafety is an important property for textiles which is imparted by proper  
330 finishing agents used for performance improvement such as antibacterial  
331 activity. Long-term exposure to textiles coated with excess of cytotoxic  
332 antibacterial agents may cause skin irritation and inflammation, particularly  
333 in case of the elderly and young children, as well as people with sensitive  
334 skin. In the present study, cytotoxicity of PHMB-coated cotton fabrics was  
335 evaluated by MTT assay according to ISO 10993-5:2009(E). MTT, a yellow water-  
336 soluble tetrazolium salt, can be reduced in viable cells to a blue-violet  
337 insoluble formazan. The number of viable cells correlates to the colour  
338 intensity which can be photometrically determined after the formazan is  
339 dissolved in alcohol. Therefore, MTT assay is commonly used for biosafety  
340 evaluation by measuring the cell viability. Fig. 7 shows the cell viability  
341 of cotton fabrics coated with different quantities of PHMB. The higher the  
342 cell viability, the lower is the cytotoxicity and the better is the biosafety  
343 of the tested samples.



344

345 **Fig. 7** *In vitro* cytotoxicity of coated cotton fabrics with different quantities  
346 of PHMB evaluated by MTT assay

347 As shown in Fig. 7, the positive control has a strong cytotoxicity with 2.8%  
348 viability of cells, whereas the sample of negative control is completely non-  
349 toxic, with 100% cell viability, as expected. Likewise, the untreated original  
350 cotton fabric is also non-toxic. However, it is worth noting that the average  
351 cell viability of the untreated original fabric is over 100%, which means  
352 cells are reproduced after being cultured. This could explain why the cotton  
353 fabric is highly susceptible to microbial colonization and leads to  
354 deterioration of fibre strength and quality (Chauhan et al. 2019; Rong et al.

2019). For fabric samples treated with antibacterial agent PHMB (P1-P15), a clear decrease in the number of viable cells was observed. The cell viability is basically inversely proportional to the PHMB content. The higher is the PHMB content, the lower the cell viability of the sample is. Moreover, there is a sharp decline in cell viability for sample P9, as compared to P8. As per the standard ISO 10993-5:2009(E), the sample should be considered non-cytotoxic if the relative cell viability is greater than 70%. Therefore, samples of P9-P15 can be considered cytotoxic, whereas the non-cytotoxic samples of P1-P8 are acceptable for safe use. On the other hand, the  $K/S$  values of P8-P15 range from 13.2 to 15.2. Therefore, it was concluded that the sample can be considered cytotoxic if the  $K/S$  value exceeds 13.2. Accordingly, the PHMB content for sample P8 (4.62 mg/g) can be regarded as the critical value for safe use, suggesting that the quantity of PHMB coated on cotton fabrics should not exceed 4.62 mg/g which falls within the range of the actual value (4.5 mg/g) and theoretical value (5.3 mg/g) of monolayer capacity for PHMB adsorbed onto cellulosic fibres. This could be explained by the fact that a multilayer stacking of PHMB molecules via reconfirmation could be formed on the surface of cellulosic fibres if the PHMB content exceeds the critical value 4.62 mg/g (Fig. 6(C, D)) (Einarson et al. 1991). PHMB molecules of the outer layer are more liable to dissociate from the PHMB molecules in the inner layer because Van der Waals' force between the hydrophobic moieties of PHMB molecules is weaker than the electrostatic attraction between PHMB molecules of inner layer and cellulosic molecules. The dissociated PHMB molecules are responsible for the cytotoxicity and antibacterial activity (Yuan and Cranston 2008; Simoncic and Tomsic 2010). Therefore, it is a reasonable conclusion that the quantity of PHMB coated on cotton fabrics should not exceed 4.62 mg/g, and the sample can be considered cytotoxic if the  $K/S$  value is greater than 13.2.

## 383 **Conclusions**

384 Determination of quantity of cationic finishing agents on the surface of  
385 cotton fabrics is a challenge in textile industry. The present study employs  
386 PHMB as a model cationic agent to validate the assumption that quantifies  
387 cationic agents on the cotton fabrics, using Kubelka-Munk equation. The  
388 adsorption of PHMB onto cotton fabrics was first investigated and found to  
389 be concentration-dependent which increases with increase in the concentration  
390 of PHMB applied. The maximum adsorption of PHMB is around 8 mg per gram of  
391 cotton fabric. After being dyed with BPB dye bath, a positive correlation was  
392 found between colour strength and the initial PHMB concentrations,  
393 particularly for the low concentration (<2400 mg/L). At high concentration  
394 (>2400 mg/L), colour strength increases slowly. A linear relationship with a  
395 high correlation ( $R^2=0.9983$ ) between  $K/S$  value and the adsorbed quantity of  
396 PHMB on the fabrics at low concentrations was established, as shown in Eq.  
397 (6), which could be used for determination of quantity of PHMB coated on the  
398 cotton fabrics. However, this equation should fulfill four requirements: (1)  
399 distribution of PHMB on the surface of cotton fabric should be in the form  
400 of monolayer with the content less than 5.3 mg/g; (2) an excess of BPB dye bath  
401 should be applied; (3) the dyeing should come to equilibrium; and (4) the  
402 fabrics should be evenly dyed. Furthermore, MTT assay demonstrates that the  
403 quantity of PHMB coated on cotton fabrics should not exceed 4.62 mg/g, and  
404 the sample is considered non-cytotoxic if the  $K/S$  value is lower than 13.2.

405 In summary, the present study reports a direct and feasible method to  
406 determine the quantity of PHMB on cellulosic textile fabrics by Kubelka-Munk  
407 equation, which may provide an enlightening guidance for a quantitative assay  
408 of cationic finishing agents on cellulosic fabrics.

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413 Conflicts of interest

414 The authors declare they have no financial interests.

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541

542 **Fig. 1** The maximum absorbance of PHMB solution over the UV-vis spectrum (A); and  
543 the calibration curve of PHMB solution established at 235 nm (B)

544 **Fig. 2** Adsorption of PHMB onto the cotton fabric with concentration ranging from  
545 100 mg/L to 10000mg/L

546 **Fig. 3** Colour shades of PHMB-treated cotton fabrics after dyed with BPB dye bath

547 **Fig. 4** Colour strength curves of cotton fabrics treated with different  
548 concentrations of PHMB solutions after dyeing with BPB dye bath

549 **Fig. 5** Plot of  $K/S$  values of BPB dyed cotton fabrics versus the amount of adsorbed  
550 PHMB (A); and (B) plot of  $K/S$  values versus samples with low quantity of PHMB  
551 adsorption (P1-P7)

552 **Fig. 6** Schematic diagram to show the arrangement of PHMB molecules adsorbed on  
553 the surface of cellulosic fibres and the dyeing behavior: (A) loose distribution;  
554 (B) dense distribution; (C) loose distribution of outer layer; (D) dense  
555 distribution of outer layer; (E) loosely-distributed PHMB dyed with BPB; (F)  
556 densely-distributed PHMB dyed with BPB; (G) loosely-distributed multilayer PHMB  
557 dyed with BPB; and (H) densely-distributed multilayer PHMB dyed with BPB

558 **Fig. 7** *In vitro* cytotoxicity of coated cotton fabrics with different quantities  
559 of PHMB evaluated by MTT assay

560 **Table 1** Sample list for cotton fabrics coated with different concentrations of  
561 PHMB  
562

# Figures

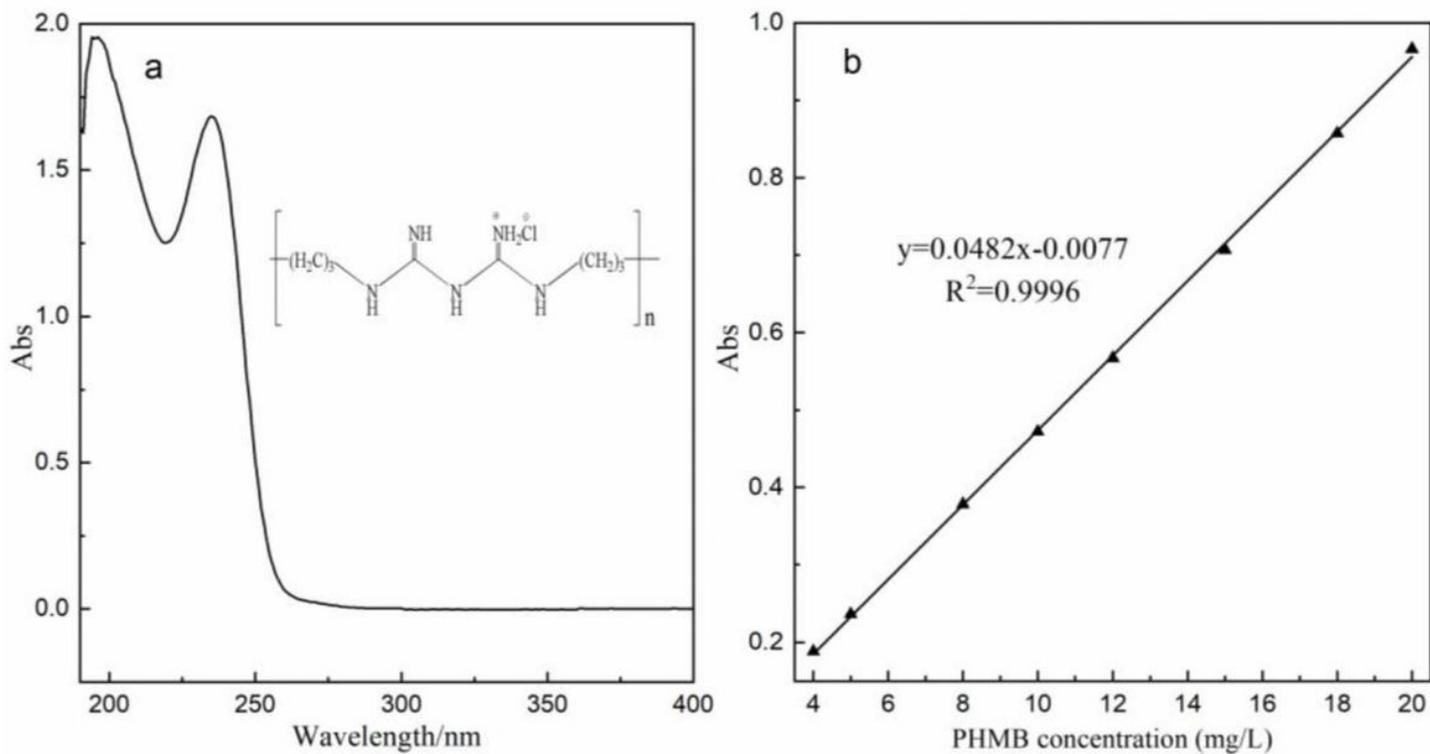


Figure 1

The maximum absorbance of PHMB solution over the UV-vis spectrum (a); and the calibration curve of PHMB solution established at 235 nm (b)

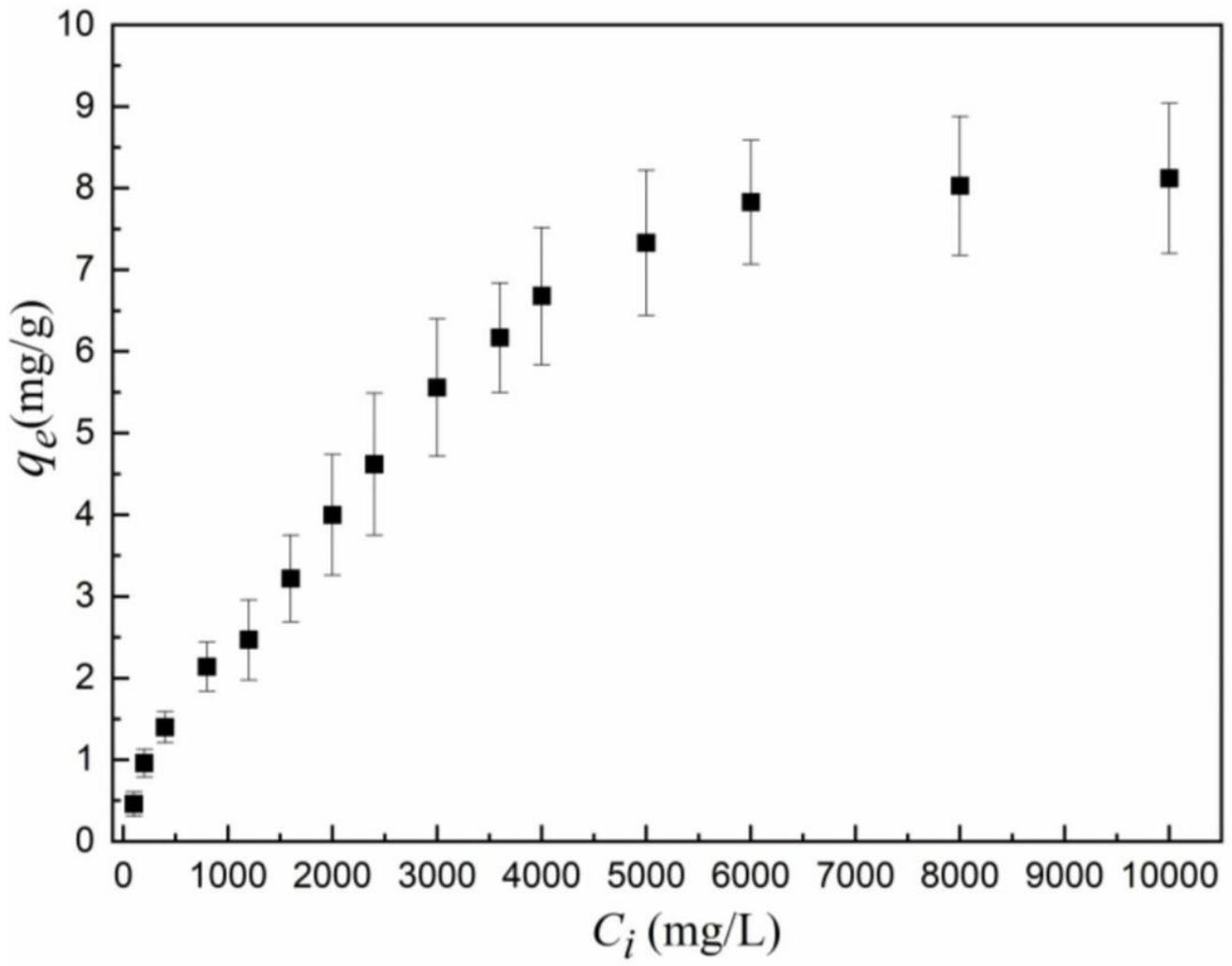
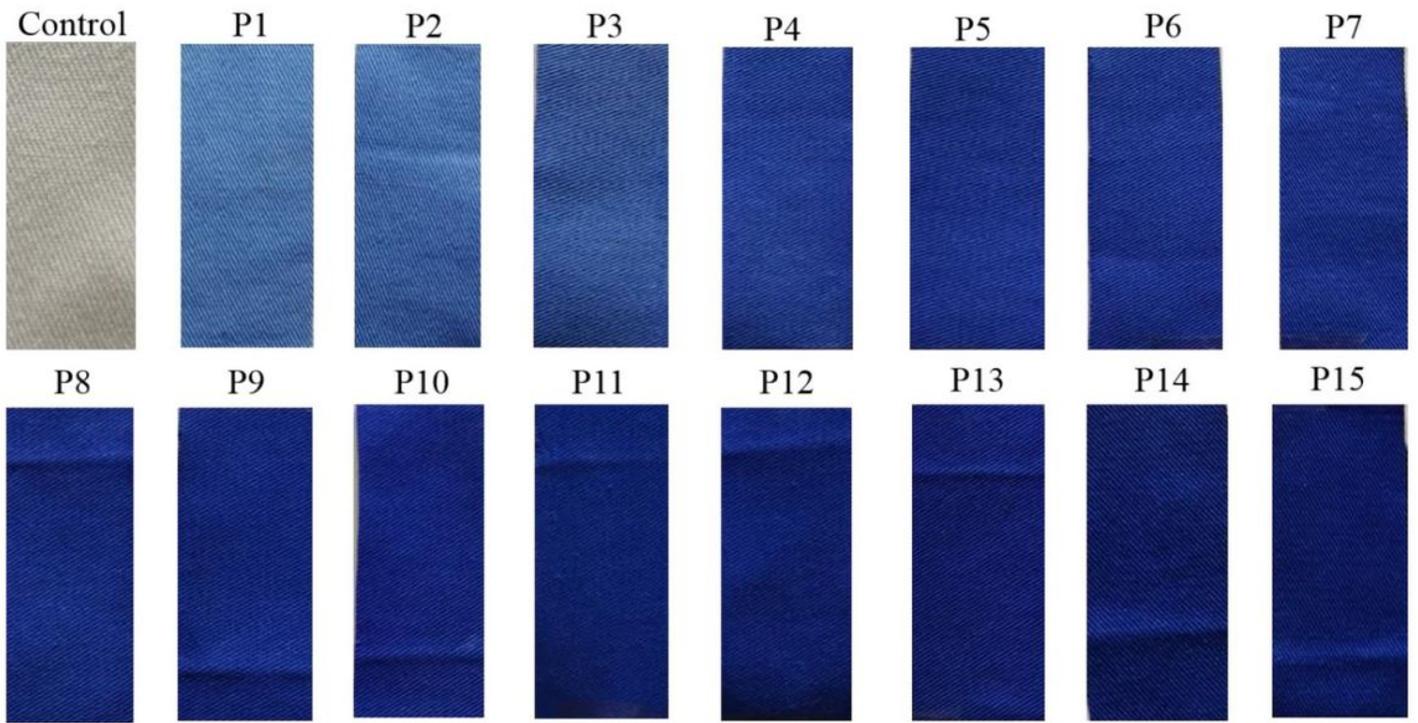


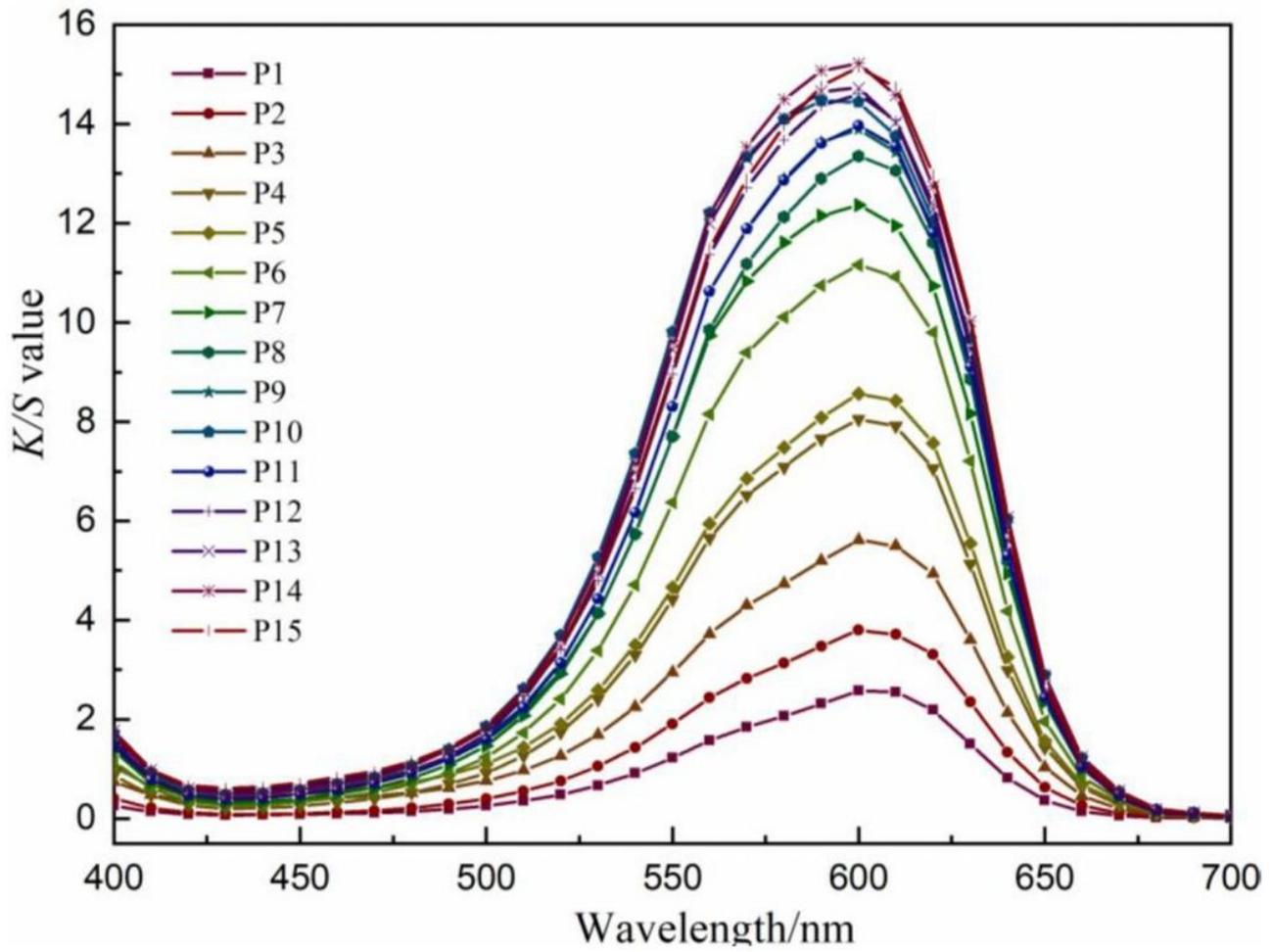
Figure 2

Adsorption of PHMB onto the cotton fabric with concentration ranging from 100 mg/L to 10000mg/L



**Figure 3**

Colour shades of PHMB-treated cotton fabrics after dyed with BPB dye bath



**Figure 4**

Colour strength curves of cotton fabrics treated with different concentrations of PHMB solutions after dyeing with BPB dyebath



distributed PHMB dyed with BPB; (g) loosely-distributed multilayer PHMB dyed with BPB; and (h) densely-distributed multilayer PHMB dyed with BPB

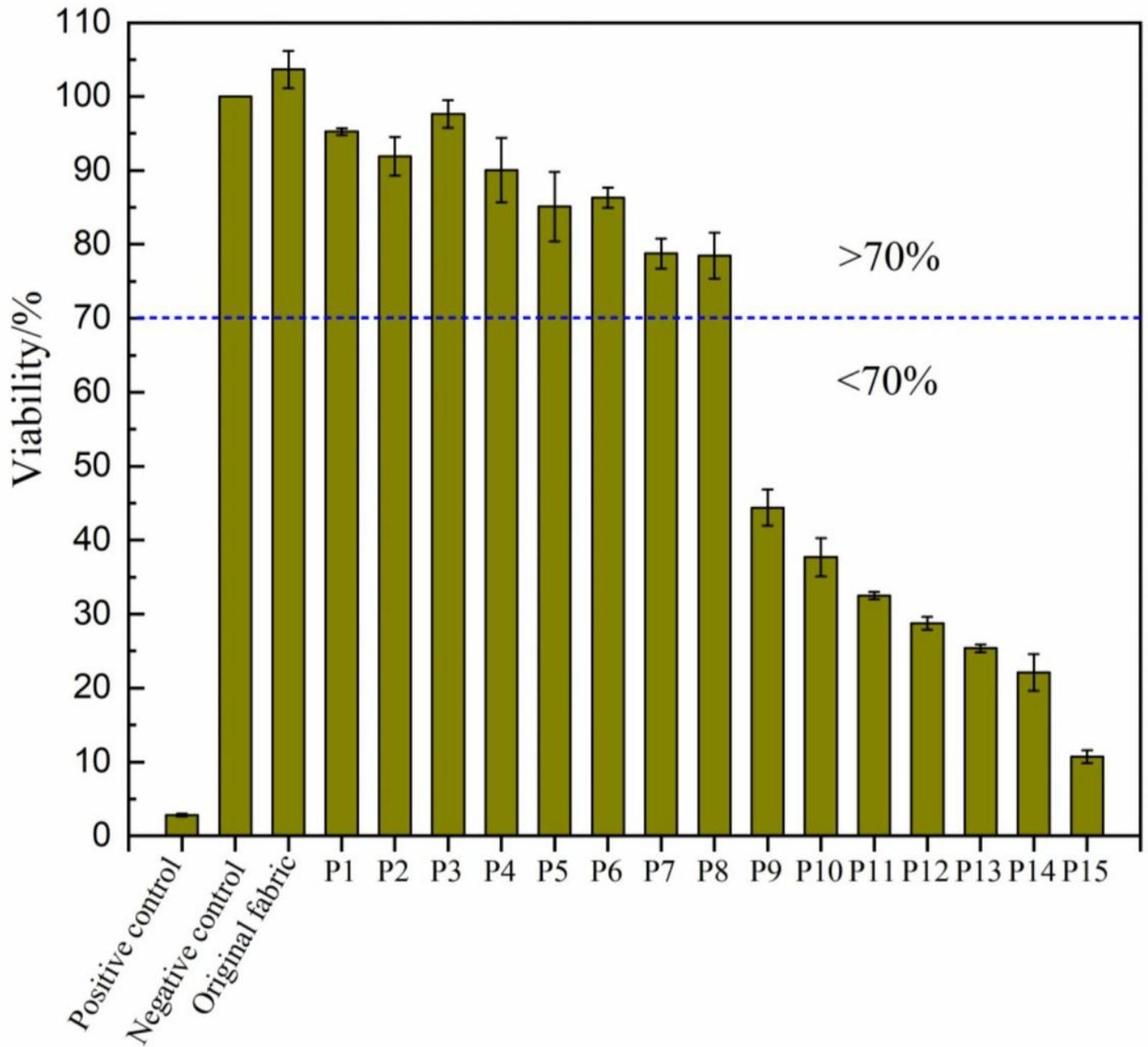


Figure 7

In vitro cytotoxicity of coated cotton fabrics with different quantities of PHMB evaluated by MTT assay