CELLULOSE CHEMISTRY AND TECHNOLOGY

QUANTITATION OF POLYHEXAMETHYLENE BIGUANIDE BIOCIDE ON COTTON FABRIC SURFACE

A. SHYICHUK, D. ZIÓŁKOWSKA and K. MROCZYŃSKA

Faculty of Chemical Technology and Engineering,
University of Technology and Life Sciences, Seminaryjna 3, 85-326 Bydgoszcz, Poland
✉ *Corresponding author: A. Shyichuk, szyjczuk@utp.edu.pl*

Received August 23, 2013

Two methods of determining the polyhexamethylene biguanide content on cotton fabric with polymer antimicrobial coating have been described. The indirect approach consists in binding of the Naphthol Blue Black dye, which is in excess in the equilibrium solution, by polyhexamethylene biguanide adsorbed onto cotton surface followed by determining the residual dye via spectrophotometry at 620 nm. This method assures high accuracy, however requires rather long time (24 h) until reaction equilibrium is attained. The direct determination of polyhexamethylene biguanide on cotton surface consists in acquiring the ATR-FTIR spectra (256 scans averaged) of the fabric sample, followed by integrating the absorbance values in the range of wavenumbers from 1490 to 1580 cm^{-1}. The peak area obtained in this way is proportional to the polyhexamethylene biguanide content. The ATR-FTIR method has the advantage of being very fast and non-destructive.

Keywords: PHMB, polyhexanide, oligobiguanidine, Amido Black 10B, Attenuated Total Reflectance

INTRODUCTION

Polyhexamethylene biguanide (PHMB, polyhexanide) is known as a powerful biocide, active against a wide variety of bacteria, fungi, algae and viruses.[1-4] Favorably, PHMB has low toxicity for humans and due to this it is used as biocide in swimming pools, as sanitizer in hospitals, as preservative in cosmetics and contact lens care solutions etc. Being positively charged, PHMB macromolecules have strong affinity to cellulose fibers, which possess low negative surface charge.[5] Due to good adsorption onto cellulose fibers, PHMB is used as germicide for wound dressings and hygiene wipes[4,6,7] and as an antimicrobial finishing agent for textiles as well.[4,8,9] Polyhexanide treated cotton fabrics have been reported to maintain bacteriocidal properties for 10 to 25 laundering cycles.[8,10] The textiles with durable antimicrobial coating are recommended for workwear used in health care and food handling institutions.[4,10,11] The current antimicrobial activity of such biocide textiles should be periodically assessed, but the standard test methods are rather time-consuming for 24-hour bacterial cultivation.[11,12] The content of PHMB on cotton surface may be also estimated by means of the sophisticated spectral techniques, such as X-ray photoelectron spectroscopy (XPS) and mass spectrometry (ToF-SIMS),[11,13] but both methods are time-consuming as well.

In this paper, two methods of determining the PHMB content on cotton fabric surface were examined. The first one consists in mass titration of the Naphthol Blue Black (NBB) dye solution with the fabric swatches. This procedure is based on the favorable properties of anionic dyes, which are well adsorbed onto polysaccharides bearing amine groups and can be easily measured by means of UV-Vis spectrophotometry.[14,15] The second method of PHMB quantitation consists in FTIR spectral measurements using Attenuated Total Reflectance mode. The ATR FTIR technique is a surface-sensitive one and was reported to be useful for qualitative confirmation of PHMB presence on wool fibers[16] and silver nanoparticles.[17]

EXPERIMENTAL

Reagents, materials and sample preparation

Polyhexamethylene biguanide hydrochloride (20% w/w solution, d=1.04 g/cm^3) from Acrylmed (Poland) and the NBB dye (C.I. 20470, also known as Amido

Black 10 B) from Sigma-Aldrich were used as received without further purification. Stock solutions of the reagents (2 mN in deionized water) were stored in the dark. The molar absorbance of the NBB dye at 620 nm was determined to be 50.8 $mM^{-1}cm^{-1}$.

The cotton samples, i.e. cosmetic pads, as well as white fabrics composed of 100% cotton, cotton and 20% polyester or cotton and 5% elastan, were purchased from a local market. The cotton pads were used as received, whereas the cotton fabrics were laundered five times before usage. The cotton fabric swatches (approximately 6 × 13 cm) and cotton pads (approximately 5 cm in diameter) were accurately weighed, impregnated with a known amount of the PHMB solution and dried under ambient conditions.

Dye-binding method

In the preliminary kinetic experiments, accurately weighed (0.1 g) cotton fabric samples (two pristine and two impregnated with 4.5 mg/g PHMB) were placed in 25 and 50 cm^3 portions of 60 μN NBB solution, and magnetically stirred in 100 cm^3 conical flasks. Low volumes of the solution (approximately 2.5 cm^3) were collected at predetermined intervals of time, measured for absorbance at 620 nm, using deionized water as reference and immediately returned to the flask. The concentrations of the NBB dye solutions were measured by means of a Spectroquant Pharo 300 spectrophotometer (Merck), using a quartz cuvette with 1 cm optical path.

During the essential part, PHMB bearing cotton samples were accurately weighed (0.05 to 0.5 g), placed in 50 cm^3 portions of 60 μN NBB solution and stirred for 24 h at ambient temperature. Afterwards, the absorbance of the NBB dye solution was measured, as described above.

ATR-FTIR method

Attenuated total reflectance spectra of the PHMB impregnated cotton samples were recorded by means of an Alpha-P Fourier-transform infrared spectrometer (Bruker) in the ATR mode on a diamond window with 256 scans at a 6 cm^{-1} resolution. Each spectrum was an average of six ones recorded at different points of a studied cotton sample. The baselines of the obtained spectra were corrected via the Rubberband procedure (with 64 points) using the Opus 6.5 software.

RESULTS AND DISCUSSION

Dye-binding method

Preliminary experiments revealed that the NBB dye is well adsorbed from aqueous solutions onto cotton fabric bearing PHMB (Fig. 1, dark symbols), whereas the control pristine cotton fabric adsorbs the dye to a very limited extent (Fig. 1, light symbols). The NBB concentrations became equilibrated after 24 hours (Fig. 1). One can infer that exhaustion of the NBB dye by the PHMB bearing fabric proceeds rather slowly. Apparently, the high porosity of the fabric results in diffusion-limited kinetics. It is known that cotton fabric contains both inter-fiber pores (of mean effective radius 22÷55 μm) and intra-fiber pores (as small as 1÷22 μm).[18] Having in mind the results of kinetic measurements (Fig. 1), the contact time of the PHMB bearing cotton samples with the NBB solution has been established to be 24 hours for the analytical measurements.

Fortunately, for the analytical purpose, the final solution absorbance is linearly related with the cotton sample mass (Fig. 2 a) and the corresponding slope coefficients are linearly related with the PHMB content (Fig. 2 b).

As a result, the following formula for analytical calculations was derived:

$$q = \frac{A_o - A_e}{1.315 \cdot m} + 0.08$$

where q denotes the PHMB content (mg/g), A_o and A_e denote initial and equilibrium absorbances of the NBB solution, respectively, and m stands for the mass (g) of the cotton sample examined. When rewritten in terms of mmoles, the obtained formula implies that one mmole of PHMB mer is bonded with 0.163 mmoles of the NBB dye. This stoichiometry ratio is inconsistent with the theoretical one (1:0.5) resulting from single positive charge of PHMB mer and double negative charge of the NBB dye. The most probable cause is that the positive charges of PHMB macromolecules are partly neutralized by the anionic sites of the cotton surface.

In order to assure maximum accuracy of spectrophotometric measurements, optimal absorbance values should decrease within the range from 0.6 to 1.2. For this reason, the cotton sample mass should be properly chosen keeping in mind the expected range of the PHMB content. The recommended loadings of cotton swatches are presented in Table 1. When the conditions above are met, the method is characterized with quite high accuracy.

For instance, ten repeated measurements with the cotton sample bearing PHMB (2 mg/g) resulted in mean recovery equal to 99.9%. On the other hand, the measured values were rather scattered with standard deviation equal to 0.34 mg/g (RSD = 17%). Therefore, to obtain reliable results, it is recommended to carry out 8-10 repeated measurements and average the results.

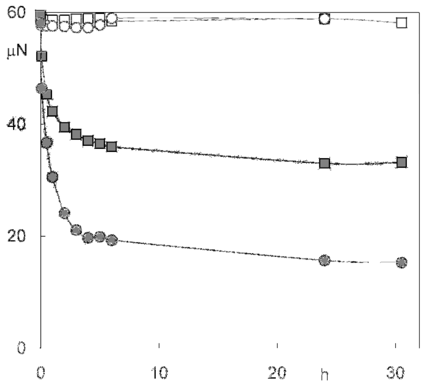

Figure 1: Dependences of NBB dye concentration on time (pristine (○□) and PHMB impregnated (●■) cotton fabric samples were added to 25 (○●) and 50 (□■) cm^3 of the solutions)

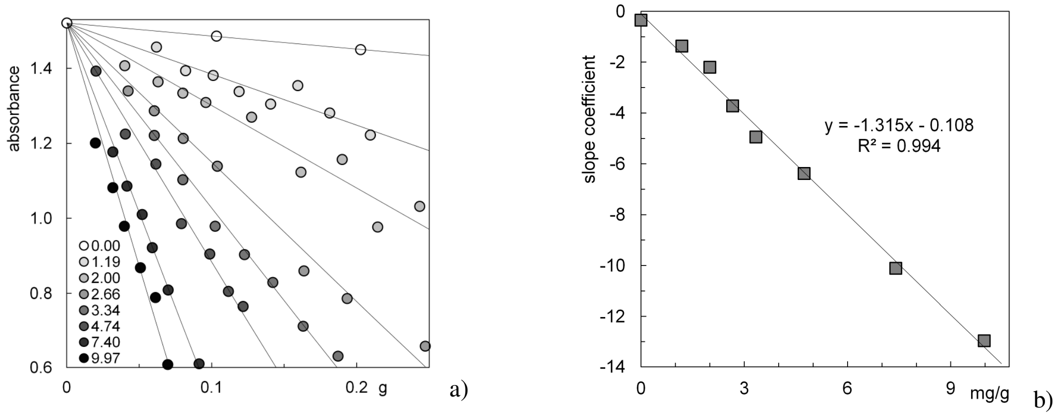

Figure 2: (a) Absorbance of the dye solution at equilibrium vs. mass of cotton sample with indicated PHMB content (mg/g) and (b) the corresponding plot slopes vs. PHMB content

Table 1
Recommended loadings of PHMB bearing cotton swatches for 50 cm^3 volume of the dye solution

Expected PHMB content, mg/g	Cotton sample mass, g
1 ÷ 2	0.1 ÷ 0.5
2 ÷ 4	0.06 ÷ 0.3
4 ÷ 7	0.03 ÷ 0.14
7 ÷ 10	0.02 ÷ 0.08

ATR-FTIR method

The broad-range ATR-FTIR spectra of cotton fabric and solid PHMB are presented in Fig. 3. The infrared frequencies characteristic of pure cellulose are well-known and ascribed to the corresponding vibrations of chemical bonds.[19,20] The obtained ATR-FTIR spectrum of the PHMB oligomer is very similar to those described in the literature.[16,17] The PHMB spectrum (Fig. 3) contains a series of intensive bands at wavenumbers from 1600 to 1300 cm^{-1} with the most intensive one at 1539 cm^{-1}, characteristic of imine group.[17] It is the spectral band that has the least overlap with the spectrum of cellulose and for that reason is the most suitable for analytical purposes. Favorably, the spectra of the studied cotton samples proved to have minor differences in the wavenumber range from 1650 to 1500 cm^{-1} (Fig. 4).

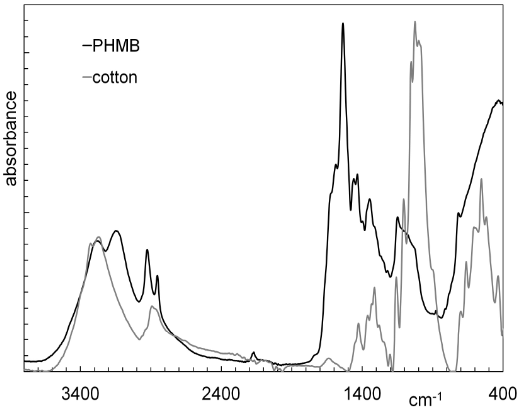

Figure 3: Normalized ATR-FTIR spectra of solid PHMB and cotton fabric

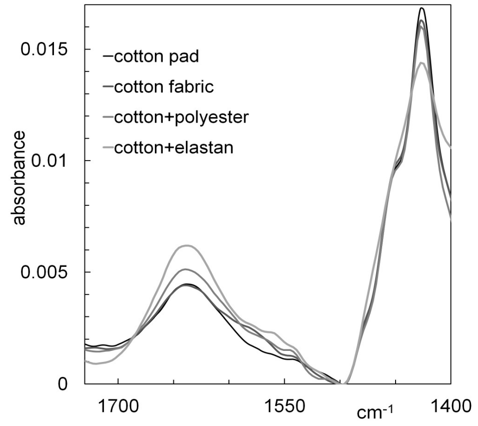

Figure 4: Comparison of ATR-FTIR spectra of the studied materials in the range of analytical measurements

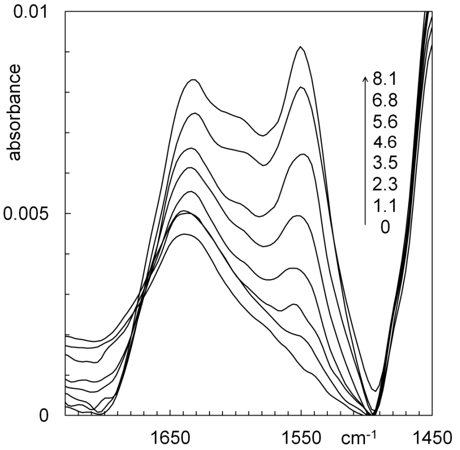

Figure 5: Increase of the analytical peak with the increase of PHMB content

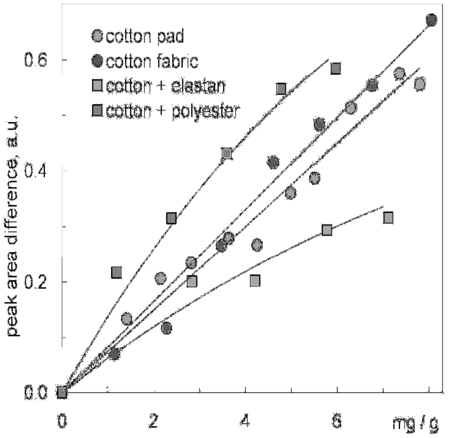

Figure 6: Analytical peak area vs. PHMB content

In full agreement with the preliminary observations, the largest differences in the spectra of cotton fabric after impregnation with PHMB were registered in the wavenumber range from 1580 to 1490 cm^{-1} (fig. 5). For that reason, just this spectral range was used for quantitative determination of the polymer amount. The graphs shown in fig. 5 indicate clearly that the peak height increases with the polymer content increase. The peak area plotted against the PHMB content resulted in almost straight-line dependences for pure cotton samples (fig. 6), wherein the obtained graphs had somewhat different slopes for cotton pads and cotton fabric. On the other hand, the calibration graphs for the mixed cotton fabrics proved to be slightly curvilinear (fig. 6). Polynomials of degree 1.8 were found to have a good fit for both obtained graphs. Ten repeated spectral measurements performed for pure cotton fabric bearing PHMB (2.28 mg/g) resulted in mean recovery equal to 81.6% and standard deviation equal to 1.07 mg/g (RSD = 47%). The decreased accuracy and precision of the FTIR method, as compared to the dye-binding procedure, may result from difficulties to perform ATR measurements on fibrous textile surface. Apparently, infrared laser beam undergoes multiple scattering on the cellulose fibers resulting in increased variance values.

CONCLUSION

Polyhexamethylene biguanide content on cotton fabric surface was determined via bonding of the Naphthol Blue Black dye, followed by spectrophotometric measurement of the residual dye concentration. This indirect method assures high accuracy and precision. However, rather long time (24 h) is required to attain equilibrium of the dye adsorption. Another method consists in direct determination of imine groups on the cotton surface by means of ATR-FTIR spectrophotometry. The polyhexamethylene biguanide content is related to the peak area obtained by integration of the IR absorbance curve in the range of wavenumbers from 1490 to 1580 cm^{-1}. The ATR-FTIR method is fast and convenient in use, also it does not cause damage to the cotton sample. Unfortunately, both the accuracy and the precision of the ATR-FTIR method are lower than those of the dye-binding method.

REFERENCES

[1] F. Pinto, J.-Y. Maillard, S. P. Denyer, *Am. J. Infect. Control*, **38**, 393 (2010).

[2] S. R. Passic, M. L. Ferguson, B. J. Catalone, T. Kish-Catalone, V. Kholodovych *et al.*, *Biomed. Pharmacother.*, **64**, 723 (2010).

[3] L. Timofeeva, N. Kleshcheva, *Appl. Microbiol. Biotechnol.*, **89**, 475 (2011).

[4] K. Kaehn, *Skin Pharmacol. Physiol.*, **23**, 7 (2010).

[5] R. S. Blackburn, A. Harvey, L. L. Kettle, J. D. Payne and S. J. Russell, *Langmuir*, **22**, 5636 (2006).

[6] C. Canal, F. Gaboriau, S. Villeger, U. Cvelbar, A. Ricard, *Int. J. Pharm.*, **367**, 155 (2008).

[7] K. Moore, D. Gray, *Wounds UK*, **3**, 96 (2007).

[8] M. L. Wallace, *AATCC Rev.*, **1**, 18 (2001).

[9] Y. Gao, R. Cranston, *Text. Res. J.*, **78**, 60 (2008).

[10] J. H. Chen-Yu, D. M. Eberhardt, D. H. Kincade, *Cloth. Text. Res. J.*, **25**, 258 (2007).

[11] E. Chadeau, C. Brunon, P. Degraeve, D. Leonard, C. Grossiord *et al.*, *J. Food Safety*, **32**, 141 (2012).

[12] AATCC Test Method 100-2012. Antibacterial Finishes on Textile Materials: Assessment of.

[13] C. Brunon, F. Bessueille, C. Grossiord, E. Chadeau, N. Oulahal *et al.*, *Surf. Interface Anal.*, **43**, 604 (2011).

[14] R. Cheng, S. Ou, B. Xiang, Y. Li, F. Chang *et al.*, *Desalination and Water Treatment*, **16**, 176 (2010).

[15] M.-E. Ignat, V. Dulman, T. Onofrei, *Cellulose Chem. Technol.*, **46**, 357 (2012).

[16] Y. Gao, X. Yu, A. P. Pierlot, R. J. Denning, R. Cranston, *J. Mater. Sci.*, **46**, 3020 (2011).

[17] S. Ashraf, N. Akhtar, M. A. Ghauri, M. I. Rajoka, Z. M. Khalid *et al.*, *Nanoscale Res. Lett.*, **7**, 267 (2012).

[18] T. Topalovic, V. A. Nierstrasz, L. Bautista, D. Jocic, A. Navarro *et al.*, *Cellulose*, **14**, 385 (2007).

[19] C. Chung, M. Lee, E. K. Choe, *Carbohyd. Polym.*, **58**, 417 (2004).

[20] Q. Wang, X. Fan, W. Gao and J. Chen, *Carbohyd. Res.*, **341**, 2170 (2006).