Quantitation of polyhexamethylene biguanide by photometric titration with Naphthol Blue Black dye

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Abstract: A convenient and reliable method for quantitative determination of polyhexamethylene biguanide in aqueous solutions has been developed, based on complexation of the polymer with Naphthol Blue Black dye. Spectrophotometric titration measuring absorbance at 600 nm leads to characteristic zigzag curves, with the inflexion point at dye to polymer mers molar ratio 1:2. The photometric titration endpoints are characterized with good repeatability, resulting in low variance values. As a result, the limit of polyhexamethylene biguanide determination has been found to be equal to 50 and 150 μmol/dm^3 of mers for direct and reverse titration, respectively.

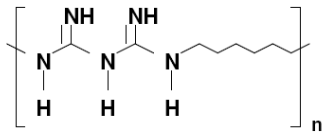
Keywords: polyhexamethylene biguanide, quantitation, titration, Naphthol Blue Black dye.

Oznaczanie biguanidu poliheksametylenowego metodą fotometrycznego miareczkowania roztworem Czerni Amidowej 10B

Streszczenie: Opracowano nieskomplikowaną, dokładną metodę ilościowego oznaczania biguanidu poliheksametylenowego w roztworach wodnych opartą na reakcji kompleksowania polimeru z barwnikiem Czerń Amidowa 10B. Zmiany widma UV-Vis sugerują, że przy pewnym stosunku molowym stężeń polimeru (w przeliczeniu na mer) i barwnika tworzą się słabo rozpuszczalne kompleksy. Wynikiem miareczkowania spektrofotometrycznego przy długości fali 600 nm są zygzakowate zależności absorbancji od ilości analitu z punktem przegięcia przy stosunku molowym barwnik:polimer wynoszącym 1:2. Zastosowana metoda wyznaczania punktu końcowego miareczkowania charakteryzuje się dobrą powtarzalnością. Zakres oznaczania merów biguanidu poliheksametylenowego wynosi 50 i 150 μmol/dm^3, odpowiednio, dla miareczkowania prostego i odwrotnego.

Słowa kluczowe: biguanid poliheksametylenowy, analiza ilościowa, miareczkowanie, Czerń Amidowa 10B.

Cationic polymers containing amino groups are known as biocides of wide application spectrum [1, 2]. One of them, polyhexamethylene biguanide [PHMB, formula (I)] easily deactivates wide variety of bacteria, fungi and viruses as well [3—6].



(I)

PHMB is well soluble in water and has low biocide concentrations, causes minor foaming and does not leave visible residues on surfaces of different materials. Due to good germicide properties and low toxicity PHMB is widely used as preservative in cosmetics, personal care products, antimicrobial hand washes, wet wipes *etc.* [7]. PHMB is also used as sanitizer for equipment, hard surfaces and sanitary porcelain in food handling institutions and hospitals [7, 8]. Medical uses of PHMB include also prevention of microbial contamination in wound irrigation and sterile dressings as well as disinfection of skin and incisions. Polyhexanide is known as a typical component of contact lens care solutions [7]. Farmers use PHMB for disinfection of equipment and drinking water for animals as well. One more but not least application of PHMB is preservative to control microorganisms and algae in swimming pools and hot tubs [7, 9]. As the polymer is resistant to sunlight, temperature, pH and water hardness, a pool may be properly maintained for about ten days before additional antiseptic is added.

Analytical method for routine determination of PHMB in swimming pools should be both non-complicated and sensitive enough to monitor residual concentration, maintained usually at about 10 mg/dm^3 [9]. Such effective methods as capillary electrophoresis [10] and solid phase extraction coupled with HPLC [11] are too sophisticated. More simple spectrophotometric method using nickel(II) and 1,2-cyclohexanedionedioxime (nioxime) is sensitive to the resting time between addition of

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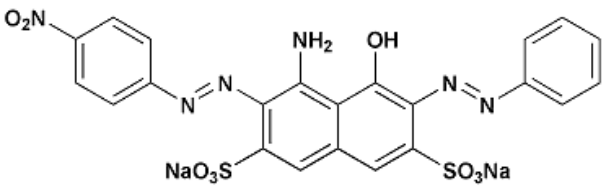
the reagents and absorbance measurement [12]. Very simple colorimetric assays with Eosin Y [13, 14] and Bromophenol Blue [12] dyes enable to measure PHMB concentrations in the range between 6 and 10 mg/dm^3, however they both are very sensitive to the sample pH. The most appropriate method appears to be polyelectrolyte titration by potassium polyvinyl sulfate [15, 16]. This method has been reported to be as highly sensitive as 1 μmol/dm^3 when using potentiometry with a custom-made cationic surfactant-selective electrode for the endpoint recognition [15] and 2 μmol/dm^3 when using Crystal Violet dye as the indicator [16].



(II)

The present paper describes application of Naphthol Blue Black (NBB) dye [formula (II)] for colloid titration of PHMB in aqueous solutions. The NBB diazodye (known also as Amido Black 10B) is known as an analytical reagent for determination of proteins in biochemistry [17, 18] and detection of blood traces in forensics [19]. The proposed method is based on complexation of the anionic dye with the cationic polymer resulting in spectral changes.

EXPERIMENTAL

Materials

Polyhexamethylene biguanide hydrochloride [$(C_8H_{17}N_5HCl)_m$], has been obtained from Acrylmed (Poland) as 20 wt. % solution of density 1.04 g/cm^3. Stock solution containing 0.3 mmol/dm^3 of PHMB mers has been prepared and stored at 8 °C.

The Naphthol Blue Black (NBB) dye has been purchased from Sigma-Aldrich and was used without additional purification.

Analytical method

Absorption spectra were determined by spectrophotometer Spectroquant Pharo 300 (Merck) using quartz cuvette with 1 cm optical path. The photometric titrations have been performed measuring absorbance at 600 nm by means of spectrophotometer Spekol 11 (Carl Zeiss Jena) equipped with magnetically stirred 30 cm^3 titration cuvette with 2 cm optical path. The direct titration procedure has been performed as follows: 15 cm^3 of PHMB solution of known concentration of mers in the range from 0.0533 to 0.1067 mmol/dm^3 (obtained by mixing the stock PHMB solution with distilled water) has been titrated with 0.1 mM NBB solution. The inverse titration has been performed as follows: 15 cm^3 of 0.033 mM NBB solution has been titrated with PHMB solutions of known concentration of mers ranging from 0.1 to 0.3 mmol/dm^3. Variance values were determined using tenfold repeating of the titration procedures at the same initial conditions.

RESULTS AND DISCUSSION

Absorption spectra

Absorption spectra of the NBB dye, shown in Figure 1, contain two peaks, one at the higher wave length of 622 nm and the second at the lower one of 325 nm (not shown). In turn, spectra of the NBB-PHMB mixtures still contain two absorption peaks, but the shape of the main one at 622 nm is changed with the increase of PHMB concentration.

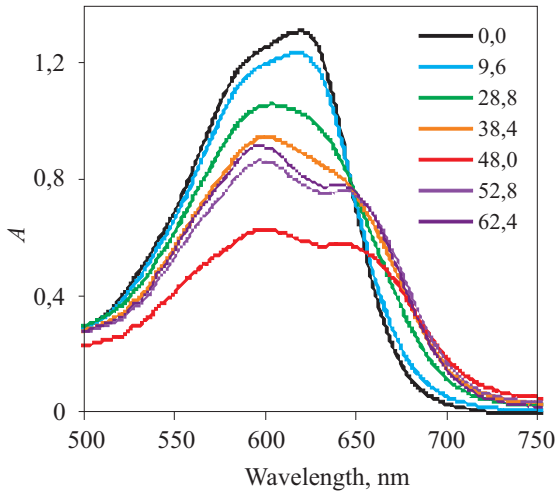


Fig. 1. UV-Vis spectra of NBB-PHMB mixtures obtained for 25 μmol/dm^3 solution of NBB and the indicated PHMB concentrations (given in μmol/dm^3 of mers)

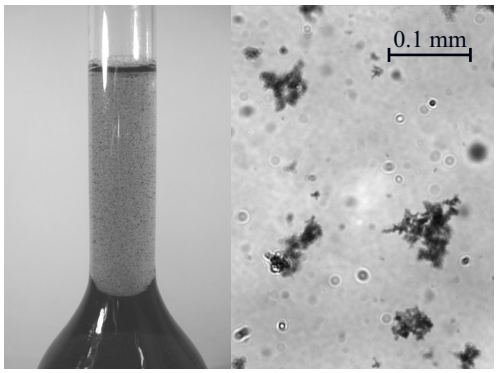


Fig. 2. Photography of NBB-PHMB suspension, which is formed at dye-to-polymer mers molar ratio equal to 1:2 (left) and microphotograph of sediment (right)

The isosbestic point visible on the plots may indicate that only two species contribute to the spectral changes. Taking in account that polyhexamethylene biguanide does not absorb visible light, one can assume a complex between the cationic polymer and the dianionic dye is formed. Apparently, the polymer-dye complex has lowered solubility and remains in solution for a long time as a fine dispersed suspension (Figure 2).

Dye molecules in suspension particles have spectral characteristics somewhat different from those in solution, which results in observed changes of the main absorption peak (Figure 1). Thorough analysis of the spectral data presented in Figure 1 reveals an interesting feature that the mean height of the peak depends non-monotonically on the PHMB concentration. This dependence is better visible when absorbance values are plotted against the polymer concentration as it is shown in Figure 3.

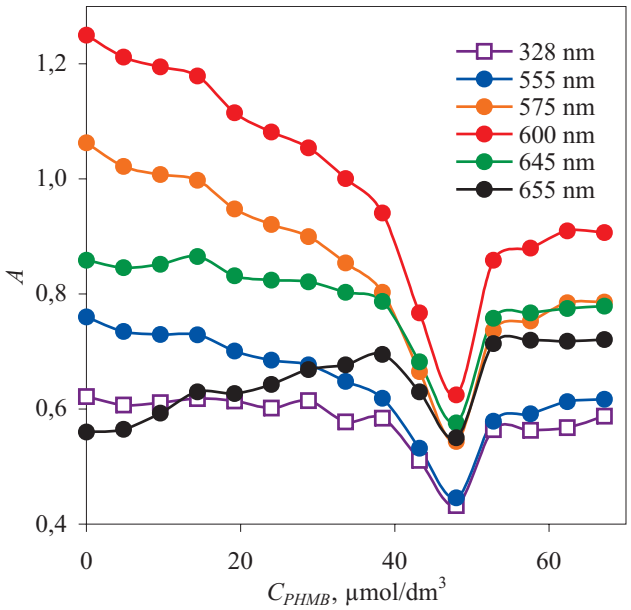


Fig. 3. Dependences of absorbance (A) determined at various wavelengths on PHMB mers concentration (C_{PHMB}) in solutions containing 25 μmol/dm^3 of NBB dye

The most pronounced decrease in the absorbance is observed at mers of polymer concentration equal approximately to 0.05 mmol/dm^3, which corresponds to molar ratio of NBB to PHMB mers equal to 1:2. Apparently, at this ratio all the dye anions in solution become complexed by the cationic macromolecules. As a result, the fine particles of PHMB-NBB complexes become larger and for that reason the solution is to some extent clarified. Further increase in PHMB concentration results in stabilization of the colloidal particles and the absorbance is increased again. The observed non-monotonic absorbance changes suggest that quantitative analysis of PHMB may be performed using the spectrophotometric titration technique.

Direct titration

Titration of the polymer solution with the NBB dye solution (so called direct titration) gave zigzag curves presented in Figure 4. Location of the characteristic inflexion point on the obtained curves, as well as the height of these bends, depends on the content of polymer in the analyzed solution. Two methods of titration endpoint determination have been tested as illustrated in Figure 5. The first one assumes that the endpoint is the half-distance between the lines indicating the extremes of the titration curve. The second method lets to determine the titration endpoint based on the second derivative plot, which has

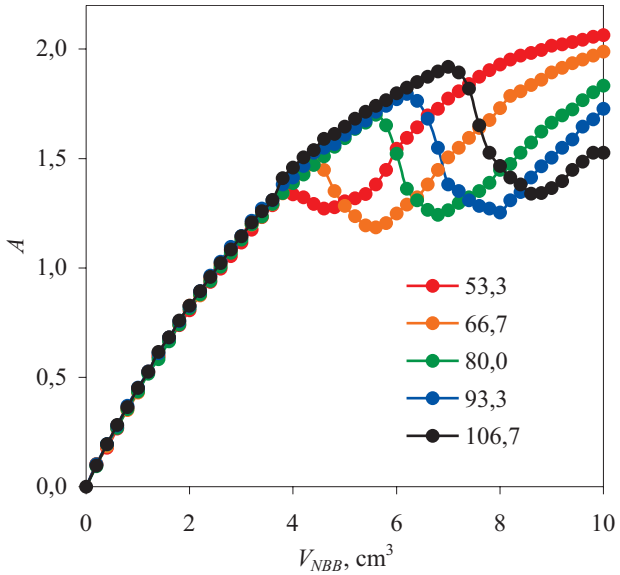


Fig. 4. Spectrophotometric direct titration curves obtained for different PHMB mers concentrations (given in μmol/dm^3) in the analyzed solution using the dye solution with constant concentration

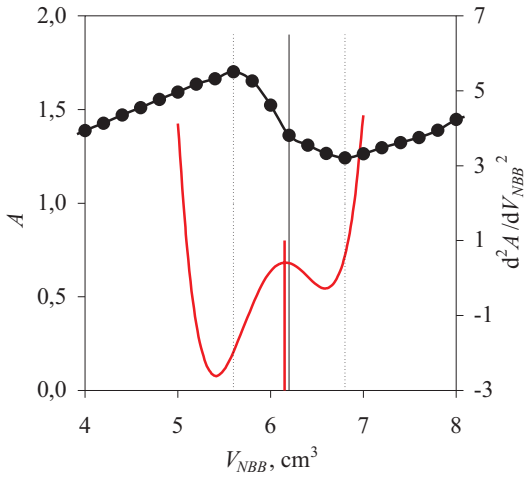


Fig. 5. The illustration of graphic methods of endpoint determination for direct titration (black lines — half distance method, red lines — second derivative method)

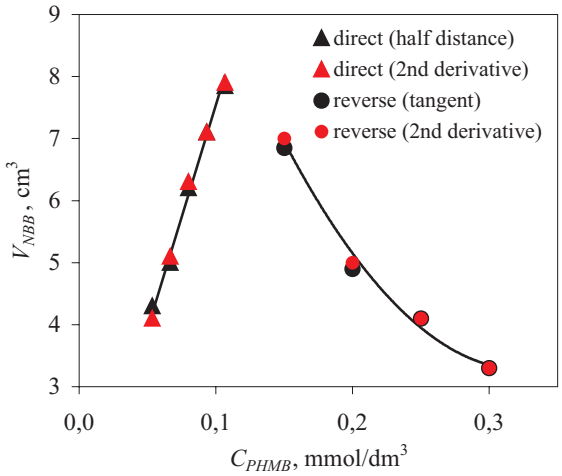


Fig. 6. Dependence between PHMB mers concentration and titrant volume at the endpoint for direct and inverse titration

been obtained from the polynomial function describing the titration curve.

It turned out that the molar ratio of the NBB dye to the PHMB polymer at the titration endpoint was 1:2 leading to quite simple equation for PHMB concentration calculation:

$$C_{PHMB} = \frac{2 \cdot C_{NBB} \cdot V_{NBB}}{V_{PHMB}} \quad (1)$$

where: C_{PHMB}, C_{NBB} — concentrations of the polymer mers and the dye, respectively, V_{PHMB}, V_{NBB} — volumes of the polymer and the dye solutions, respectively.

As it can be seen in Figure 6 the dependence between PHMB concentrations and titrant volume at the endpoint has rectilinear character.

Both the methods of endpoint determination provide very similar results (Table 1). The calculated recovery values [C_{PHMB}(determined) · 100/C_{PHMB}(prepared)] in most the cases fit the recommended range 95—105 %. Both the half distance and the second derivative methods provide low values of variance, which are equal to $3.5 \cdot 10^{-6}$ and $5.9 \cdot 10^{-6}$, respectively (at $C_{PHMB} = 0.0800$ mmol/dm^3). The limit of determination for the direct titration procedure has been found equal to about 0.05 mmol/dm^3 of mers.

Reverse titration

Titration of the NBB dye solution with the polymer solution (so called reverse titration) has resulted in Z-shaped curves, shown in Figure 7, having marked inflexion sufficient for end point determination. Similarly as in the case of direct titration, both the inflection point and the bend height depend on the titrant concentration. Two ways of the endpoint determination, illustrated in Figure 8, have been compared. The first one assumes that the tit-

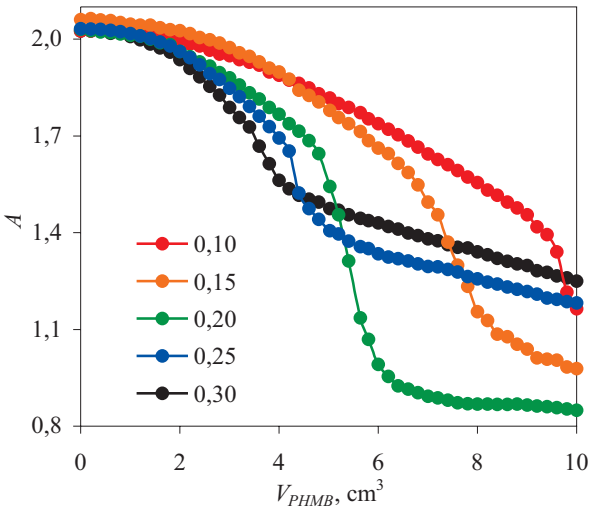


Fig. 7. Spectrophotometric reverse titration curves obtained for different PHMB mers concentrations (given in mmol/dm^3) in the analyzed solution using the dye solution with constant concentration

T a b l e 1. Titrant volumes at the endpoint and recovery values for direct and reverse titration procedures

C_{PHMB} (prepared) mmol/dm^3	V of titrant cm^3	C_{PHMB} (determined) mmol/dm^3	Recovery %	V of titrant cm^3	C_{PHMB} (determined) mmol/dm^3	Recovery %
direct titration						
	half-distance method			second derivative method		
0.0533	4.3	0.0573	107.5	4.1	0.0547	102.5
0.0667	5.0	0.0667	100.0	5.1	0.0680	102.0
0.0800	6.2	0.0827	103.3	6.3	0.0840	105.0
0.0933	7.1	0.0947	101.4	7.1	0.0947	101.4
0.1067	7.85	0.1047	98.1	7.9	0.1053	98.8
reverse titration						
	tangents intersection method			second derivative method		
0.15	6.85	0.1445	96.4	7.0	0.1414	94.3
0.20	4.9	0.2020	101.0	5.0	0.1980	99.0
0.25	4.1	0.2415	96.6	4.1	0.2415	96.6
0.30	3.3	0.3000	100.0	3.3	0.3000	100.0

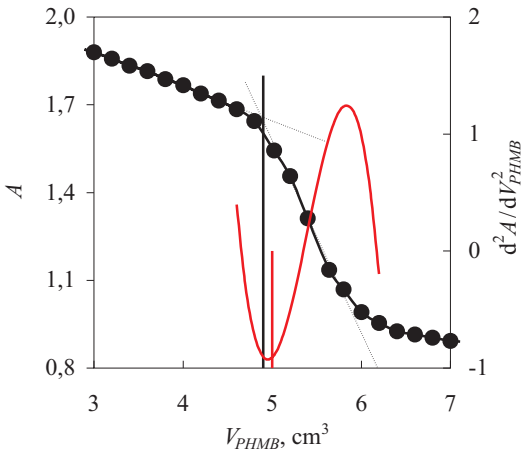


Fig. 8. The illustration of graphic methods of the endpoint determination for reverse titration (black lines — tangent method, red lines — second derivative method)

ration endpoint is determined as the point of intersection of tangent lines in the curve inflection vicinity. The second method uses the second derivative plot obtained from the polynomial function describing the titration curve. Results collected in Table 1 show that both methods provide very similar values of concentration.

The molar ratio of the dye and the polymer mers at the titration endpoint is 1:2. So, as it is shown in Figure 6, the PHMB mers concentration is inversely proportional to its volume at the titration endpoint.

The recovery values in most the cases fit the recommended range 95—105 %. Low values of variance, equal to $1.0 \cdot 10^{-5}$ and $3.5 \cdot 10^{-6}$ for the tangent intersection and second derivative methods, respectively (calculated at C_{PHMB} = 0.2 mmol/dm^3) confirm good repeatability. The limit of determination for the reverse titration procedure is about 0.1 mmol/dm^3 of mers.

CONCLUSIONS

The anionic dye Naphthol Blue Black interacts with polyhexamethylene biguanide resulting in reduction of solution absorbance at the molar ratio of NBB:PHMB mers equal to 1:2. Based on this interaction, the new method of quantitative analysis of PHMB in water solutions has been proposed, which consists in spectrophotometric titration at 600 nm wave length. Both direct and reverse titration procedures are applicable with the first one characterized as more sensitive. The limit of determination (50 μmol/dm^3 of mers) is sufficient for polyhexamethylene biguanide assay in swimming pools.

REFERENCES

[1] Timofeeva L., Kleshcheva N.: *Appl. Microbiol. Biotechnol.* **2011**, *89*, 475.
[2] Chylińska M., Kaczmarek H.: *Polimery* **2012**, *57*, 3.
[3] Kim B. R., Anderson J. E., Mueller S. A., Gaines W. A., Kendall A. M.: *Wat. Res.* **2002**, *36*, 4433.
[4] Linyan Feng, Fuwang Wu, Jing Li, Yueming Jiang, and Xuewu Duan: *Postharvest Biol. Tec.* **2011**, *61*, 160.
[5] Pinto F., Maillard J.-Y., Denyer S. P.: *Am. J. Infect. Control* **2010**, *38*, 393.
[6] Passic S. R., Ferguson M. L., Catalone B. J., Kish-Catalone T. *et al.*: *Biomed. Pharmacoter.* **2010**, *64*, 723.
[7] Kaehn K.: *Skin Pharmacol. Physiol.* **2010**, *23*, 1, 7.
[8] http://crystalbiocides.com/links_pdf/Vantocil IB in Food Application.pdf
[9] Goeres D. M., Palys T., Sandel B. B., Geiger J.: *Water Res.* **2004**, *38*, 3103.
[10] Abad-Villar E. M., Etter S. F., Thiel M. A., Hauser P. C.: *Anal. Chim. Acta* **2006**, *561*, 133.
[11] Lucas A. D., Gordon E. A., Stratmeyer M. E.: *Talanta* **2009**, *80*, 1016.
[12] Rowhani T., Lagalante A. F.: *Talanta* **2007**, *71*, 964.
[13] Dawson M. W., Brown T. J., Till D. G.: *New Zel. J. Mar. Fresh* **1983**, *17*, 3, 305.
[14] Turner N. A., Russell A. D., Furr J. R., Lloyd D.: *J. Appl. Microbiol.* **2004**, *96*, 1287.
[15] Masadome T., Yamagishi Y., Takano M., Hattori T.: *Anal. Sci.* **2008**, *24*, 415.
[16] Masadome T., Miyanishi T., Watanabe K., Ueda H., Hattori T.: *Anal. Sci.* **2011**, *27*, 817.
[17] Yonan C. R., Duong P. T., Chang F. N.: *Anal. Biochem.* **2005**, *338*, 159.
[18] Okutucu B., Dınçer A., Habib Ö., Zıhnıoglu F.: *J. Biochem. Biophys. Methods* **2007**, *70*, 709.
[19] Lawley R.: *J. Forensic Ident.* **2003**, *53*, 4, 404.

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