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A new method of spectrophotometric determination of poly(diallyldimethylammonium chloride) concentration

RAPID COMMUNICATION

Summary – Spectrophotometric method of protein determination, widely known as the Bradford method, has been applied for quantitative determination of poly(diallyldimethylammonium chloride) (PDDA) monomer units in aqueous solution. In phosphoric acid environment the PDDA polymer stabilizes blue form of the Commasie Briliant Blue G-250 dye having a broad absorption band. Increase in absorbance at wavelengths in the range from 570 to 630 nm follows the increase in monomer units of polymer concentration up to 0.042 mmole/dm^3. Calibration curves fulfilling second-degree polynomial equations can be determined for PDDA monomer units concentrations not exceeding 0.03 mmole/dm^3.
Keywords: poly(diallyldimethylammonium chloride), Coomassie Brilliant Blue G-250.

NOWY SPOSÓB SPEKTROFOTOMETRYCZNEGO OZNACZANIA STĘŻENIA POLI(CHLORKU DIALLILODIMETYLOAMONOWEGO)
Streszczenie — Metoda Bradford, używana do spektrofotometrycznego oznaczania białek za pomocą barwnika błękitu brylantowego Comassie G-250, została zastosowana do ilościowej analizy merów poli(chlorku dimetylodialliloamonowego) w roztworach wodnych. Wykazano, że szerokie maksimum absorpcji kompleksu barwnik-PDDA stwarza możliwość oznaczenia stężenia merów polimeru w dużym zakresie długości fal, tj. od 570 do 630 nm (rys. 1). Wzrost absorbancji występuje wraz ze wzrostem stężenia merów polimeru do 0.042 mmola/dm^3 (rys. 2). Krzywe kalibracyjne spełniające równania wielomianowe drugiego stopnia dają możliwość precyzyjnych oznaczeń wtedy, gdy stężenia merów PDDA nie przekraczają 0.03 mmola/dm^3. Linearyzację krzywej wzorcowej można przeprowadzić w zakresie stężeń merów polimeru od 0 do 0.019 mmola/dm^3, jednak zabieg ten powoduje spadek dokładności dopasowania danych doświadczalnych i linii trendu.
Słowa kluczowe: poli(chlorek dimetylodialliloamonowy), błękit brylantowy Coomassie G-250.

Poly(diallyldimethylammonium chloride), usually abbreviated as PDDA or PDADMAC, is a well-known cationic polyelectrolyte. PDDA applied in wastewater treatment causes neutralization of negatively charged colloidal particles [1, 2]. Both PDDA and diallyldimethylammonium chloride copolymers may be also used in water clarification processes to remove mineral suspension particles, algae and humic substances [3—5]. However, while applying PDDA care should be taken, because it is not neutral for the human health. Moreover, disinfection of wastewater containing residues of PDDA may lead to formation of highly toxic *N*-nitrosodimethylamine [6]. For that reason measurements of PDDA concentration in clarified water require special precision and reliability. Concentration of PDDA in water solution can be determined with different analytical procedures. The non-selective method is measurement of total organic carbon [7]. The selective analytical procedures are potentiometric titration with ion-selective electrode [8], polyelectrolyte titration with streaming current detector [9] and spectrophotometry with triphenylmethane dyes [10]. Direct turbidimetry of interpolymer complexes may be applied as well [11]. Unfortunately, all these methods often do not provide enough precise results.

The aim of this work was to find rather simple analytical procedure for accurate determination of monomer units of PDDA concentration in water solutions. For this reason an attempt to adapt the spectrophotometric

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method originally used in biochemistry to determine protein concentration (widely known as the Bradford method [12]) has been undertaken. The Bradford method is based on change of spectral absorbance of the Coomassie Brilliant Blue G-250 dye (CBB) when complexed with proteins in acid environment.

EXPERIMENTAL

Materials

The dye, Coomassie Brilliant Blue G-250, of chemical formula $C_{47}H_{48}N_3O_7S_2Na_2$ has been supplied by Carl Roth GmbH. The Bradford reagent has been prepared according to the standard recipe [12] by dissolving 87.7 mg of the dye in 50 cm^3 of 95 % CH_3OH, adding 100 cm^3 of 85–87 % H_3PO_4 solution and filling up with distilled water to 200 cm^3. The Bradford reagent has been stored at 4 °C in the dark. In such conditions the solution is stable for about two months.

Poly(diallyldimethylammonium chloride) (PDDA) of average molecular mass of 100—200 kg/mole has been obtained from Aldrich as a 20 % water solution with density of 1.04 g/cm^3. The stock solution of PDDA with concentration of 0.322 mmole(mer)/dm^3 has been prepared by diluting the basic solution with distilled water and stored in the dark. The sample solutions have been prepared in volumetric flasks, taking 10 cm^3 of the Bradford reagent, adequate quantity of the stock solution and distilled water up to the volume of 50 cm^3. Preliminary measurements proved that samples prepared in the described way are stable for 20 min.

Methods

Light absorption measurements have been performed with spectrophotometer Pharo 300 (Merck) in the wavelength range from 450 to 650 nm using quartz cuvettes with 10 mm optical path. Distilled water was used as a reference. All measurements have been performed in two series with six month intervals, twice each time. Results obtained this way are presented as the mean values taken from four measurements.

RESULTS AND DISCUSSION

The vis spectra of mixtures of PDDA with the Bradford reagent, presented in Figure 1, proved that the CBB dye and PDDA form a complex (CBB-PDDA) having extinction coefficient value from 48000 to 21000 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$. Absorbance of the CBB-PDDA complex depends on the concentration of polymer monomer units. Intensity of the short-wavelength maximum of CBB absorption (visibly at about 475 nm) decreases with increase in concentration of PDDA monomer units in the solution. On the other hand, a new broad absorption band occurs at 560—620 nm and increases with concentration of PDDA monomer units. One can conclude that PDDA acts like water-soluble proteins and stabilizes blue anionic form of the CBB dye [12]. The isosbestic point occurs around 520—530 nm (Fig.1) suggesting the formed complex has rather stoichiometrical character.

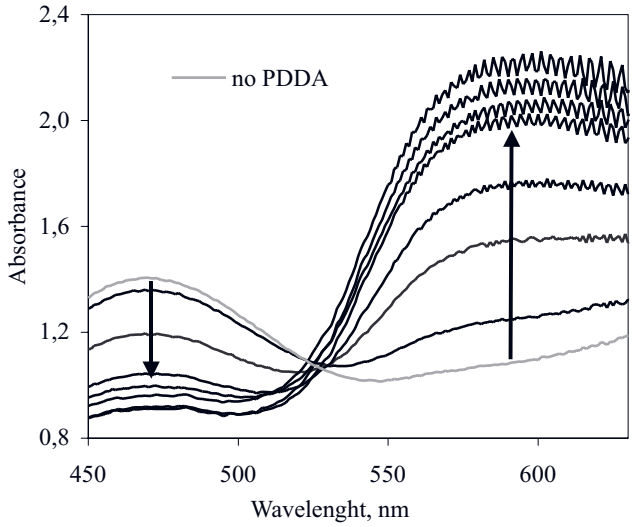

Fig. 1. Absorption spectra of CBB-PDDA complexes for increasing concentrations (in the direction of the arrows) of PDDA monomer units

The dependences between concentration of PDDA monomer units and solution absorbance have been determined upon the absorption spectra. Exemplary plots have been presented in Figure 2. As it can be seen, the relationship between light absorption and PDDA monomer units concentration exists from 0 to about 0.02 mmole/dm^3 in the range of short-wavelenght absorption maximum as well as to about 0.04 mmole/dm^3 in the range of long-wavelenght one. Increase of PDDA monomer units concentration results in stabilization of

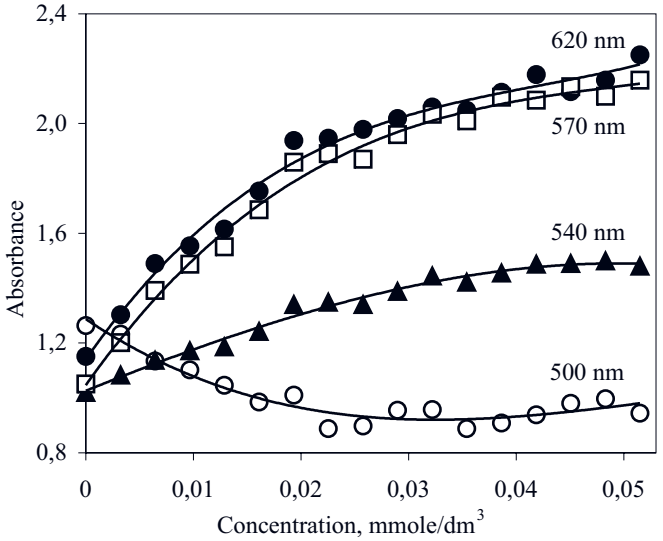

Fig. 2. Dependence of absorbance at different wavelengths on concentration of PDDA monomer units

absorbance values at all wavelengths. The nonlinear character of dependences presented in Fig. 2 is the reason of a broad range of extinction coefficient values calculated for the CBB-PDDA complex.

To increase accuracy of analysis the absorbance values should not exceed 2 (see Fig. 1). For that reason the suggested maximum of PDDA monomer units concentration in solution should be 0.03 mmole/dm^3. The calibration equations in such concentration range for chosen wavelengths together with corresponding coefficients of determination (r^2) have been presented in Table 1.

T a b l e 1. Calibration equations describing the dependence of absorbance (*A*) on concentration of PDDA monomer units in aqueous solutions (*c*); optical path equals to 10 mm, the applicable concentration range of monomer units ranges between 0.003 and 0.03 mmole/dm^3.

Vawelength, nm	Calibration equations	r^2
570	$A = 1.06 + 50.8 \cdot c - 655.8 \cdot c^2$	0.987
580	$A = 1.07 + 56.3 \cdot c - 778.3 \cdot c^2$	0.981
590	$A = 1.09 + 52.4 \cdot c - 627.3 \cdot c^2$	0.986
600	$A = 1.10 + 52.7 \cdot c - 638.6 \cdot c^2$	0.982
610	$A = 1.12 + 52.8 \cdot c - 704.3 \cdot c^2$	0.982
620	$A = 1.15 + 49.5 \cdot c - 662.7 \cdot c^2$	0.986
630	$A = 1.19 + 42.7 \cdot c - 513.3 \cdot c^2$	0.989

The data collected in Table 1 show that all the experimental points can be well described with a polynomial equation of second degree with quite high accuracy ($r^2 >$ 0,98). Inconvenience of the polynomial data description is that somewhat complicated calculations are needed. However, employment of a linear equation for interpolation of experimental data results in considerable decrease of the r^2 value. As a consequence, the applicable concentration range is reduced by half.

Comparison of the results obtained in four measuring series can lead to the conclusion that repeatability and reproducibility of the method is satisfactory. The variance and standard deviation are equal to 0.001 and 0.036, respectively, while mean recovery of the proposed method equals to about 103 %.

CONCLUSIONS

The Bradford assay, previously known as the method of protein determination, has been proved to be useful for quantitative analysis of poly(diallyldimethylammonium chloride) monomer units in water solutions. In phosphoric acid environment the Coomassie Brilliant Blue G-250 dye reacts with poly(diallyldimethylammonium) chloride resulting in blue complex which is stable within 20 minutes The absorption band of the PDDA-CBB complex is rather wide and extends from 570 to 630 nm. The complex absorbance increases following the increase of the polymer amount. Using the Bradford reagent and cuvettes of 10 mm thickness, one can determine the concentration of PDDA monomer units in the range from approximately 0.003 to 0.03 mmole/dm^3.

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