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Digital image colorimetry method for determination of glucose using silver nanoparticles immobilized into polymethacrylate matrix

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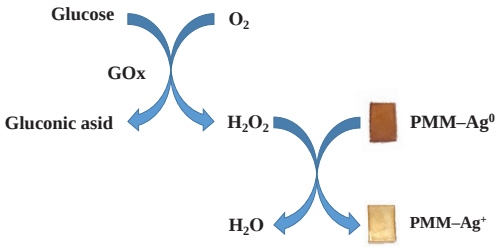
We suggest Ag^0 nanoparticles immobilized into transparent polymethacrylate matrix (PMM–Ag^0) as a simple colorimetric sensor for determination of glucose. We demonstrate the capabilities of a smartphone to process the images and further analyze the information obtained by determination of glucose using PMM–Ag^0 sensor. The method can be employed for glucose concentrations of 0.1–4.3 mmol dm^{-3}, while its detection limit is 0.05 mmol dm^{-3}.



Keywords: glucose, glucose oxidase, hydrogen peroxide, polymethacrylate matrix, silver nanoparticles.

Silver nanoparticles (Ag^0 NPs) combined with glucose oxidase test are widely used for determination of glucose.[1–5] The glucose oxidase test is based on oxidation of glucose by the ambient oxygen assisted by glucose oxidase:

$$\text{glucose} + O_2 + H_2O \rightarrow \text{gluconic acid} + H_2O_2.$$

The amount of hydrogen peroxide educed by oxidation of glucose under the influence of glucose oxidase is in direct proportion to the initial concentration of glucose. Ag^0 NPs are used as a chromogenic agent for quantitative determination of the hydrogen peroxide.[6–8] Oxidation of Ag^0 nanoparticles to Ag^+ ions by hydrogen peroxide results in attenuation of the surface plasmon resonance absorption band in the visible spectrum,[9,10] depending on the shape, size and aggregation intensity of nanoparticles.[11]

The technological development and the spread of various electronic devices designed to register images of colored objects make it possible to use colorimetry for evaluation of colorimetric characteristics[12,13] of colored compounds in the framework of quantitative chemical analysis. This method uses smartphones with in-built cameras and specialized software for image processing as an analytical signal detector.[14,15]

We developed a colorimetric sensor for simple determination of glucose using the glucose oxidase method involving the use of Ag^0 NPs immobilized into polymethacrylate matrix (PMM–Ag^0).† It was demonstrated that the response of this silver-based sensor increased with the increase of the hydrogen peroxide concentration. The goal of this study was to explore the possibility of colorimetric glucose determination by interpretation of a smartphone scanned digital image of PMM–Ag^0 material after its contact with a biological sample to be analyzed.

The color coordinates (*R*, *G*, *B*), the color difference value (ΔE), the effective absorption values (*A*) for the red, green and blue color channels and the color ratio CR [equations (1)–(5)] were used as analytical parameters in the interpretation of the colorimetric data:

$$\Delta E = \sqrt{\Delta R^2 + \Delta G^2 + \Delta B^2}, \quad (1)$$

$$A_R = -\lg(R_s/R_b), \quad (2)$$

$$A_G = -\lg(G_s/G_b), \quad (3)$$

$$A_B = -\lg(B_s/B_b), \quad (4)$$

where R_s, G_s, B_s are the averaged trichromatic coordinates of the image of PMM–Ag^0 plate after its contact with the analyzed

† *Procedure of glucose determination*. The PMM–Ag^0 material was synthesized by thermal reduction of Ag^+ ions immediately in PMM. Silver ions were immobilized into PMM plates from an aqueous solution of silver nitrate for 2–4 min, then the plates were taken out from the solution and placed into a drying cabinet at 140 °C for 3–5 min. The PMM–Ag^0 samples are yellowish-brown in color and have the surface plasmon resonance absorption band with a maximum at 425 nm. Absorption spectra and absorbance of PMM matrix and solutions were recorded using an Evolution 600 spectrophotometer (Thermo Fisher Scientific Inc., USA) against a blank polymer plate prepared under the same conditions. In order to build a calibration graph for glucose concentration from 0.1 to 0.8 mmol dm^{-3}, glucose oxidase (GOx) was added to the analyte sample, then the sample was heated at 37 °C for 10 min. At the second stage of the experiment, acetate buffer with pH 4 was added to the solution, then the volume of the sample was brought to 500 ml with distilled water, the PMM–Ag^0 plates were placed into the solution and the sample was stirred at 50 °C for 30 min. Next, the PMM–Ag^0 plates were taken out from the solution, placed on a sheet of white paper and their image was scanned by a smartphone camera in scanner mode at the distance of 25 cm. The obtained digital images were processed using 'ColourGrab' mobile application developed for determination of the *R*, *G*, *B* color coordinates.

sample; R_b, G_b, B_b are the color coordinates of the PMM–Ag^0 image in the reference sample;

$$CR = (R_s/R_r + G_s/G_r + B_s/B_r)/3, \quad (5)$$

where *r, s* and *b* indexes pertain to the reference, real and blank samples, respectively.

The intensity level of the gray scale was used as the analytical parameter,[16] for this the colored image was translated into the gray scale steps using the brightness algorithm $0.3R + 0.59G + 0.11B$. Optical darkening coefficient (ODR) was calculated using the following equation:

$$ODR = (I_b - I_s)/I_b, \quad (6)$$

where I_b and I_s are the intensity levels of the gray scale for the reference and the analyzed samples, respectively.

The absorption spectra of PMM–Ag^0 sample after its contact with glucose solutions of various concentrations are shown in Figure 1. The PMM transparent sensors can be used for direct spectrophotometry, unlike modern opaque sensors. Such results have higher metrological characteristics compared to scattering or diffuse reflection methods.[17] However, the achieved characteristics correspond to digital image processing in colorimetric methods. The change in absorbance occurs through oxidation of Ag^0 nanoparticles to Ag^+ free ions, which is accompanied by visible change of the color of PMM plate from yellowish-brown to pale yellow. The process might be reversible under certain conditions, which yet needs to be further investigated. The calibration curves were built for determination of glucose (Table 1) with the use of various colorimetric parameters (R, G, B, ΔE, A_R, A_G, A_B, CR, ODR).[18] The effective colorimetric parameters for determination of glucose are the absorption value for the blue color channel A_B and the color ratio CR.

Table 1 Analytical characteristics of the colorimetric glucose determination.

Signal	Regression equation	Correlation coefficient	Linearity range/ mmol dm^{-3}	Detection limit/ mmol dm^{-3}
R	$R = 140 + 37c_{gl}$	0.967	2.4–3.5	0.7
G	$G = 80 + 42c_{gl}$	0.908	1.1–3.0	0.4
B	$B = 0.3 + 28c_{gl}$	0.899	0.1–2.8	0.04
ΔE	$\Delta E = 6 + 62c_{gl}$	0.956	0.9–1.0	0.3
A_R	$A_R = -0.12c_{gl}$	0.910	0.3–0.5	0.2
A_G	$A_G = -0.25c_{gl}$	0.737	0.1–0.3	0.06
A_B	$A_B = -0.99c_{gl}$	0.998	0.1–4.3	0.05
CR	$CR = 0.99 + 1.8c_{gl}$	0.998	0.3–2.6	0.1
ODR	$ODR = -0.53c_{gl}$	0.976	0.1–3.0	0.09

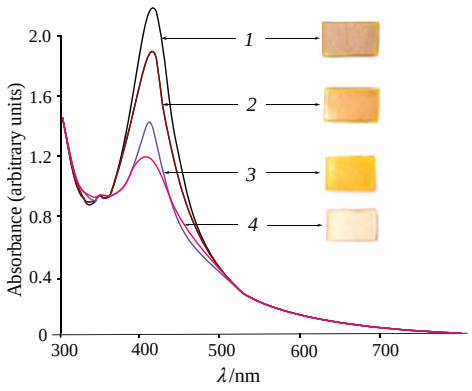


Figure 1 Absorption spectra of PMM–Ag^0 plate after its contact with glucose solution with various concentrations (mmol dm^{-3}): (*1*) 0, (*2*) 0.25, (*3*) 0.50, and (*4*) 0.75.

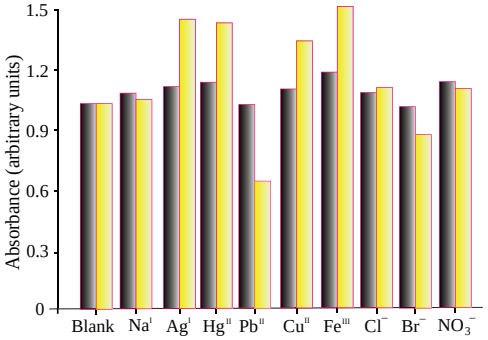


Figure 2 Effect of the extraneous ions on the determination of hydrogen peroxide, $c(H_2O_2)$ = 0.40 mmol dm^{-3}, n = 3, ratio H_2O_2 : ion = 1:10.

The effect of cations and anions present in solution on the hydrogen peroxide determination was evaluated as relative deviation of the analytical signal of hydrogen peroxide at fixed concentration with different concentrations of the extraneous components (at the ratio 1:10) in the sample solution (Figure 2). It was revealed that Hg^{II}, Fe^{III}, Pb^{II} and Cu^{II} significantly affect the determination of hydrogen peroxide, as the relative deviation of the analytical signal exceeds 10% in the presence of these ions. The results of hydrogen peroxide determination using solid-phase spectrophotometry and Glucose-UTS Kit as well as the comparison of the significance of discrepancy in the assay results obtained by the both methods are given in Table 2. Levels of glucose detected by the PMM–Ag^0 sensor were a bit lower than those obtained using the commercial assay kit when samples were not diluted. Comparable values were obtained when samples were double diluted, which seems to be directly related to reducing the effect of matrix at high dilutions.

Table 2 The results of glucose determination in real samples (n = 4–7, P = 0.95).[a]

Sample	PMM–Ag^0		Assay Kit	
	Found/ mmol dm^{-3}	s_r (%)	Found/ mmol dm^{-3}	s_r (%)
Non-diluted 1	0.42 ± 0.04	3.8	0.46 ± 0.07	5.8
Double dilution 1	0.22 ± 0.05	9.2	0.16 ± 0.07	17.6
Quadruple dilution 1	0.1 ± 0.05	20.1	0.08 ± 0.05	25.2
Non-diluted 2	0.61 ± 0.04	2.6	0.55 ± 0.05	3.7
Double dilution 2	0.26 ± 0.04	6.2	0.31 ± 0.06	7.8
Quadruple dilution 2	0.11 ± 0.05	18.3	0.17 ± 0.08	18.9

[a] Samples 1 and 2 are saliva samples of two healthy persons; n denotes number of parallel determinations; s_r is the relative standard deviation rate.

In summary, we proposed a simple method using PMM–Ag^0 sensor for the solid-state spectrophotometric determination of 0.1–4.3 mmol dm^{-3} of glucose with the limit of detection of 0.05 mmol dm^{-3} calculated by the 3s criterion. The suggested method is easy to implement and it requires only the standard spectrophotometric equipment. The PMM–Ag^0 NPs were stable and did not show any aggregation even after six months.

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