Nonionic Surfactant Media for Smartphone-based Colorimetric Determination of Protein

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The potential of using an organized medium based on nonionic surfactants for the smart colorimetric determination of proteins using Coomassie Brilliant blue G has been investigated. The optimal conditions for generating the dyecolorimetric signal in a nonionic surfactant medium have been established. It has been shown that, in a nonionic surfactant medium, the analytical signal of the reagent and analyte is significantly increased due to solubilisation and stabilisation of the colloidal-chemical state of solutions. Micelle-forming surfactants of the Triton series, in particular Triton X-305 at a concentration close to the critical micelle concentration, demonstrate the highest efficiency. The optimal conditions for registration of the protein colorimetric signal in aqueous-alcohol solutions of reagent–nonionic surfactant have been established. An eco-friendly, miniaturised technique for the smart colorimetric determination of protein (LOD = 1.5 mg L^{-1}) has been developed, tested, and successfully applied to analyse albumin content in urine. The proposed approach demonstrates the effectiveness of using nonionic surfactant solutions to enhance the sensitivity of colorimetric analysis. It can serve as a basis for improving the metrological characteristics of other reagent systems in solution.

Keywords: nonionic surfactant, proteins, smartphone, dye, Coomassie Brilliant blue G, colorimetric determination

Середовище на основі неіонних поверхнево-активних речовин для смарт-кольорометричного визначення білка

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У роботі було досліджено ефективність використання організованого середовища на основі неіонних поверхнево-активних речовин для смарт-кольорометричного визначення білків за допомогою Coomassie Brilliant blue G. Було встановлено оптимальні умови формування кольорометричного сигналу барвника в середовищі неіонних поверхнево-активних речовин. Показано, що в середовищі неіонних поверхнево-активних речовин аналітичний сигнал реагенту та аналіту збільшується завдяки розчиненню та стабілізації колоїдно-хімічного стану розчинів. Найвищу ефективність демонструють міцелоутворюючі поверхнево-активні речовини ряду Triton, зокрема Triton X-305 при концентрації, близькій до ККМ. Встановлено оптимальні умови реєстрації кольорометричного сигналу білка у водно-спиртових розчинах реагент–неіонна поверхнево-активна речовина. Розроблено та апробовано екологічно безпечну мініатюризовану методику смарт-кольорометричного визначення білка (LOD = 1.5 мг/л), яку успішно застосовано для аналізу вмісту альбуміну в сечі. Запропонований підхід демонструє ефективність використання розчинів неіонних поверхнево-активних речовин як середовища для підвищення чутливості кольорометричного визначення, та може слугувати основою для покращення метрологічних характеристик інших реагентних систем у розчинах.

Ключові слова: неіонні поверхнево-активні речовини, білок, смартфон, барвник, Coomassie Brilliant blue G, кольорометричне визначення

Introduction

Smartphones are increasingly being used as portable chemical analysers due to their affordability, miniaturisation, broad functionality, and digital image processing capabilities. In modern analytical instrumentation, smartphones function as external sensor modules or signal detectors. Their application is being actively adopted in diagnostics, forensic analysis, product quality control, and environmental monitoring [1]. Digital colorimetry with smartphones (smart colorimetric) is actively developing. It is a modern, competitive analytical method that enables the quantitative assessment of the color of solid surfaces (e.g. reagent paper or sorbents) and colored solutions [2–5]. Smart colorimetry is regarded as a contemporary, environmentally friendly, and affordable alternative to traditional laboratory methods. However, the analysis of solutions requires the standardisation of shooting and lighting conditions, camera parameters, and the properties of the reagent medium to ensure the formation of a reproducible analytical signal.

Determining protein concentration is a routine procedure in life science laboratories worldwide. This task is essential in biochemical, medical, food, and pharmaceutical research. Various methods are used to assess protein content, including spectrophotometry, fluorometry, colorimetry, immunoassays, mass spectrometry, and rapid test systems [6–10]. Among these, methods based on color reactions between reagents and analytes are particularly common [11]. Colorimetry, in particular, enables rapid monitoring of protein content and facilitates product quality control.

The Bradford method is one of the colorimetric techniques for the quantitative determination of proteins in solution. The method is based on the reaction of protein with Coomassie Brilliant blue G-250 (CBBG) [12]. The possibility of using the standard Bradford protocol in colorimetry with a smartphone has been demonstrated [13–14]. Despite its high sensitivity and ease of implementation, this reagent has several limitations [15]. In particular, the high hydrophobicity of CBBG leads to its aggregation in aqueous media, which adversely affects the reproducibility of the determination. To enhance and stabilise the analytical signal, additional electrolytes are often employed. Although CBBG aggregation in aqueous solutions is typically mitigated by the addition of organic solvents, this approach compromises both the environmental friendliness and reliability of the method [16].

In this context, the use of surfactants, in particular nonionic surfactant (n-Surf) solutions, as a supramolecular medium for modifying analytical systems is relevant. Reagent systems in organized surfactant medium and the manifestation of supramolecular correspondence effects provide new opportunities for improving the sensitivity and selectivity of analytical techniques. When reactions are carried out in surfactant-modified medium, the intensity of the analytical signal typically increases. The stability of the analytical form of the substances also improves. In such systems, the colloidal-chemical state of solutions can be stabilized, and the proteolytic properties of reagents and analytes can be controlled [17–18]. The corresponding changes occur due to the binding of surfactants to the analyte, sample components, and the reagent system through association and solubilisation processes. Nonionic surfactants are used to modify organic reagents. Moreover, n-Surf solutions are also used as a medium for analytical reactions through solubilisation processes [19–20]. When analytical reactions are performed in n-Surf medium, an increase in the analytical signal and an overall improvement in metrological characteristics is generally observed. The linearity of the calibration curve also expands, and the limit of detection of the analyte decreases.

Previous research has shown an improvement in the metrological parameters of colorimetric and luminescence determination of analytes with CBBG in the presence of nonionic surfactants [21–22]. It has also been established that the effectiveness of nonionic surfactant association with a reagent or analytical form depends on the hydrophobic and spatial compatibility between the components [23]. In the rational search or development of reagents for the determination of large hydrophobic particles, proteins, or biologically active compounds, it is advisable to choose more hydrophobic reagents bearing an opposite charge. This enhances the efficiency of hydrophobic and electrostatic interactions. However, the potential of such media in smart colorimetry of solutions remains understudied, which became the subject of our study.

Therefore, the aim of this study was to explore the feasibility and establish the optimal conditions for smart colorimetric protein determination using Coomassie Brilliant blue G in liquid organized media based on a nonionic surfactant. Special attention was given to studying the influence of micelle-forming and high-molecular-weight surfactants on the protein colorimetric signal.

Experimental part

The triphenylmethane dye Coomassie Brilliant blue G (“for electrophoresis”, Sigma-Aldrich, Germany) was used in the work. The aqueous solutions of the reagent were prepared by dissolving an exact weight of the compound in distilled water.

Polyoxyethylated alkylphenols of varying degrees of oxyethylation: Triton X-100 (of laboratory grade with a purity of >98.0%, Merck USA; TX-100) and Triton X-305 (70% in H_2O, Merck USA; TX-305) were used as nonionic surfactants. Polyoxyethylene sorbitan monolaurate Tween 20 (tested according to Ph. Eur., Merck, USA) was also used. High molecular weight surfactants Polyethylene glycol 115 (Kyiv Plant of Reagents, Indicators and Analytical Products “RIAP”) and Polyethylene glycol 6000 (Ph. Eur. Sigma-Aldrich, Germany) were used.

Bovine serum albumin (basic substance content >96.0% Sigma-Aldrich, Germany; BSA) and egg albumin were used as proteins. Aqueous solutions of proteins were prepared by dissolving the exact weight in distilled water.

The RGB characteristic was used to assess the color intensity of the studied solutions. In the RGB colorimetric model, the spectral ranges covered by the R, G, and B channels are 550-750 nm, 450-650 nm, and 400-550 nm, respectively [24]. In the vector representation, (0,0,0) and (255, 255, 255) represent complete absorption and no absorption (complete reflection) of the incident radiation, respectively. The sum of the colorimetric coordinates of the system was calculated using formula (1). The colorimetric signal of the analyte was determined as the sum of the changes in color coordinates (2):

$$\sum RGB = R+G+B \quad (1)$$

$$\sum|\Delta|RGB = \sum(|\Delta|R+|\Delta|G+|\Delta|B) = \sum((|R-R_{blank}|)+(|G-G_{blank}|)+(|B-B_{blank}|)) \quad (2)$$

A comparison of the behaviour of the analyte with the reagent in solutions containing different surfactants was carried out using the normalised analytical signal $\sum|\Delta|RGB/\sum|\Delta|RGB^0$, where $\sum|\Delta|RGB^0$ colorimetric signal of a solution without surfactants.

The colorimetric signal was detected with a smartphone camera using the "Color Picker" program, v.7.4.2. Smart colorimetric measurements were performed in a white photobox to obtain a stable and reproducible signal. The survey was conducted by photographing solutions in a Petri dish (d=3 cm) with a smartphone camera from above. The photobox was equipped with circular diode lighting (SMD 2835 LEDs, 3000 K). In the photobox, the solutions under study were placed on the central geometric axis in the photobox-lighting-smartphone camera system. Utilising a rotary table within the photobox, the solutions were positioned at a designated fixed point.

The absorption spectra of the solutions were recorded using a spectrophotometer UV2401 PC Shimadzu. The acidity of the solutions was measured using an OrigaMeter OpH218 pH meter (OrigaLys, France). An aqueous solution of sulfuric acid was used to establish the pH.

Aggregation in CBBG solutions was assessed by their turbidity and using the turbidity spectrum method. Turbidity (τ) was determined by the absorbance of solutions at λ = 800 nm - at a wavelength ≥100 nm from the position of maximum absorption of the reagent. The turbidity value was calculated by the formula: where A_λ – is the optical density of the solution, l – is the thickness of the absorbing layer [25].

The sizes of colloidal aggregates in aqueous solutions were estimated using the scattering factor (F_r). The F_r value was calculated as the tangent of the slope of $\ln A = f(\ln\lambda)$ auxiliary function for the long-wave region of the spectrum (λ=800-850 nm).

Results and discussion

Coomassie Brilliant Blue G – BSA solutions. The absorption spectra of aqueous solutions of CBBG-BSA were studied in this work. The absorption in the long-wavelength region (λ = 640 nm) increases, accompanied by a simultaneous hypsochromic shift of the maximum, when BSA is added to the CBBG solution (Fig. 1). The maximum analytical signal of the protein is observed in acidic solutions. Under the experimental conditions at pH=1, CBBG exists in three forms in solution: HL^-, H_2L and H_3L^+, based on the pK values of the reagent [26]. Proteins associate with the dye, stabilising its blue anionic form (λ_{max} = 595 nm). Stable complexes between CBBG and the amino acid residues of BSA are formed through hydrogen bonding, Van der Waals forces, and hydrophobic interactions. At the same time, the obtained calibration curves for the spectrophotometric determination of BSA with CBBG at λ=595 nm exhibit some degree of non-linearity, a phenomenon also observed in other studies. This effect may be caused by the coexistence of different forms of CBBG, a decrease in the availability of free dye with increasing protein concentration, and protein aggregation at higher concentrations [13, 27].

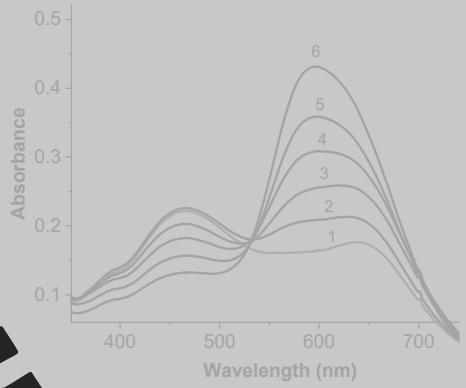

Figure 1. Absorption spectra of CBBG–BSA aqueous solutions. C_{CBBG} = $2.0 \cdot 10^{-5}$ M, C_{BSA}: 0(1), 2 (2), 4 (3), 8 (4), 12 (5), 20 (6) mg L^{-1}. λ=350-850 nm, l=1.0 cm, pH= 1.0.

The dependence of the CBBG-BSA colorimetric signal on albumin concentration was observed. The reflected radiation is inversely proportional to the color intensity and, therefore, to the concentration of the absorbing substance. The signal intensities of the Red, Green, and Blue components change with increasing protein concentration (Fig. 2). The most pronounced change is observed for the R component. The intensity of the red color is inversely proportional to the rise in protein concentration. Plateauing of the B and G components, along with a reduced change in the R component, was observed at a BSA concentration of more than 12 mg L^{-1}.

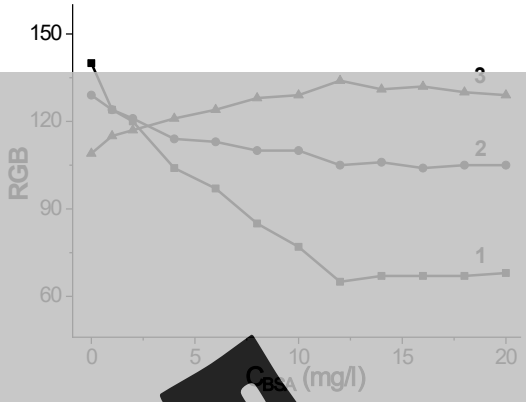

Figure 2. Colorimetric signal R(1), G(2), B(3) of CBBG–BSA aqueous solutions. C_{CBBG}=2.0·10^{-5} M, pH=1.0.

Effect of Ethanol on the RGB signals of CBBG solutions. Coomassie Brilliant blue is a highly hydrophobic reagent, which causes it to aggregate in aqueous solutions. We investigated the effect of ethanol on CBBG aggregation in an aqueous medium [21]. By monitoring solution absorbance at λ = 800 nm, it was found that, as the ethanol content increased, the turbidity of CBBG-EtOH solutions decreased, while the size of the aggregated particles in the solution increased. However, it was observed that for ethanol concentrations ranging from 2% to 30% (v/v), no turbidity was observed in CBBG solutions

Ethanol additions also influence the signal intensity of the dye itself. The effect of ethanol on the colorimetric signal of CBBG in solution was investigated. A breakpoint in the dependence occurred at an alcohol content of 7.5% (v/v) (Fig.3). It is universal and manifests itself in different alcohol concentration dependencies. Changes in the colorimetric signal of the CBBG-EtOH system are insignificant when adding 2.5-7.5% (v/v) alcohol. Further addition of ethanol reduces the system signal by 10-30%. The minimum value of the RGB signal of CBBG in solution (decrease ≈ 30%) is observed at an ethanol content of 27.5% (v/v).

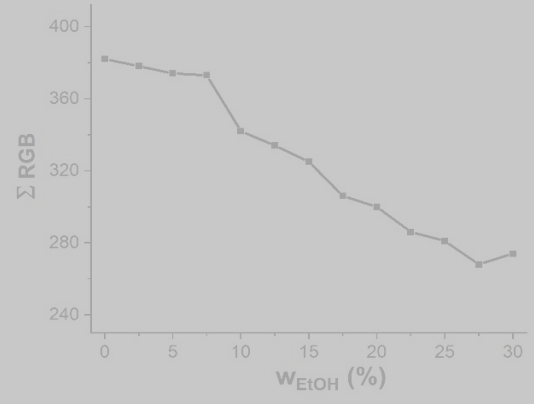

Figure 3. Colorimetric CBBG signal on the EtOH concentration. C_{CBBG}=2.0·10^{-5} M, pH=1.0.

Effect of Ethanol on the spectrophotometric and RGB signals of protein in reaction with CBBG. The effect of alcohol on the colloidal-chemical state of CBBG-BSA solutions and on the intensity of the spectrophotometric and colorimetric signal of BSA was investigated. The colloidal-chemical state of the solutions directly affects the reproducibility of the analytical signal. It was found that even low alcohol concentrations of ≤10% (v/v) reduced the turbidity of CBBG-BSA solutions, and the effect becomes significantly more pronounced at ethanol content of >20% (v/v). However, the addition of alcohol up to 30% (v/v) does not result in the complete removal of turbidity. The analytical form studied in this work is an association of CBBG with a protein, whose stability is due to electrostatic and hydrophobic interactions. The latter show the maximum effect in polar media, in particular in aqueous solutions. Logically, the introduction of organic solvents generally reduces the absorption of associates. The addition of alcohol enhances the spectrophotometric signal of BSA in dye solutions. The BSA signal is enhanced by 30–40% at an alcohol content of 2–7% (v/v). However, when the alcohol content is >10% (v/v), the BSA signal is decreased, and at w(EtOH) = 22–27% (v/v), the protein signal is not detected.

The colorimetric curves of the R, G, and B signals of CBBG-BSA aqueous-alcohol solutions on the EtOH concentration show the separation of the components. An increase in the analytical signal of BSA is observed up to w(EtOH)=7.5% (v/v). With a further increase in the alcohol content, the intensity of the albumin colorimetric signal decreases (Fig. 4). Ethanol increased the colorimetric signal of BSA by 20% at w(EtOH) = 7.5% (v/v). Thus, the addition of alcohol at concentrations between 2.5–7.5% (v/v) is recommended when constructing colorimetric systems for the determination of BSA in solutions with CBBG.

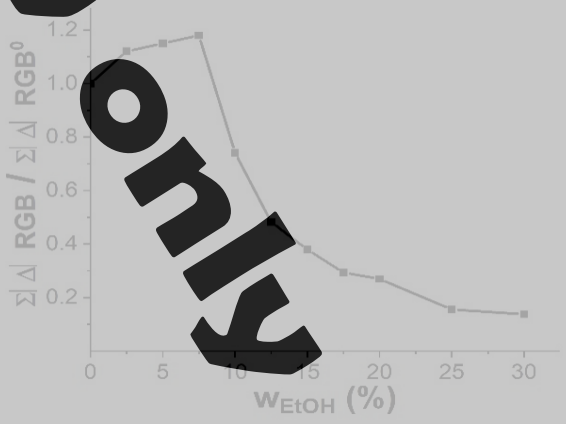

Figure 4. Normalised RGB-signal of BSA in the reaction with CBBG in solution on the ethanol concentration. C_{CBBG}=2.0·10^{-5} M, C_{BSA}=5 mg/l, pH=1.0.

Effect of reagent concentration on the RGB signal of protein in reaction with CBBG. The effect of CBBG

concentration on the RGB signal of its aqueous and water-alcohol solutions was studied to optimise the reagent concentration. The colorimetric signal of BSA increases with increasing reagent concentration in aqueous and water-alcohol solutions of CBBG. The maximum BSA signal was observed at a dye concentration of $4.0 \cdot 10^{-5}$ M in aqueous solutions. In alcohol solutions, the maximum BSA colorimetric signal was recorded in the range of dye concentrations $(2.0–6.0) \cdot 10^{-5}$ M (Fig. 5). However, further increase in CBBG concentration was found to result in a decrease in the analytical signal. Subsequent colorimetric studies were conducted at a CBBG concentration of $2.0 \cdot 10^{-5}$ M, as excessive dye concentrations promote colloidal aggregation in solution.

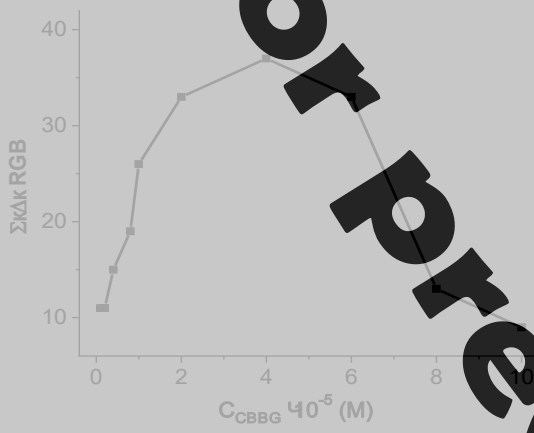

Figure 5. Colorimetric signal of BSA in the reaction with CBBG on the reagent concentration. C_{BSA} = 5 mg·L^{-1}, w(EtOH) = 5% (v/v), pH=1.0.

Effect of Triton X-100 on the RGB signal of CBBG solution. The effect of different types of surfactant media on the spectrophotometric and colorimetric characteristics of dye solutions was investigated in this study. The n-Surf medium was considered as a substitute for ethanol to prevent aggregation and improve the metrological characteristics of the smart colorimetric method. We showed some features of the influence of TX-100 on CBBG in the spectrophotometry method in a previous publication [21]. Premicellar concentrations of TX-100 had a negligible effect on the absorbance of CBBG solutions (curve 1, Fig. 6). At the same time, a sharp increase in the absorbance of solutions is recorded at an n-Surf concentration above the critical micelle concentration (CMC) and is explained by the solubilisation of the reagent. In general, the introduction of TX-100 reduces the turbidity of CBBG solutions. This effect is most pronounced in micellar solutions. The turbidity of the solutions is greatly reduced, and the size of the aggregated particles increases.

The dependence of the colorimetric signal of CBBG-TX-100 solutions on n-Surf concentration was investigated (curve 2, Fig. 6). A slight increase in the colorimetric signal of the system was observed at premicellar concentrations of TX-100. The maximum increase signal (≈4%) was recorded at a stoichiometric ratio of reagent: n-Surf=1:1, at a surfactant concentration of $2.0 \cdot 10^{-5}$ M. A sharp change in the colorimetric signal of CBBG-TX100 solutions was observed at a surfactant concentration greater than the CMC. The maximum decrease in system signal in the micellar region was 35%. This is due to the effects associated with micellar formation in the system. The dependence of the colorimetric signal of TX-100 on surfactant concentration made it possible to record the formation of associates with the ratio of surfactant: CBBG = 1:2 and 4:1.

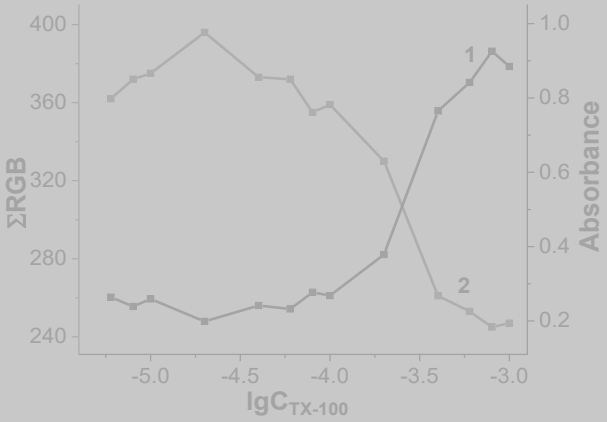

Figure 6. Spectrophotometric (1) and colorimetric (2) signals of CBBG in solution on the TX100 concentration. $C_{CBBG}=2.0 \cdot 10^{-5}$ M, $C_{TX-100}=6.0 \cdot 10^{-6}–1.0 \cdot 10^{-3}$ M, pH=1.0, λ=640 nm.

The effect of alcohol and TX-100 on the intensity of the colorimetric signal of the system is nearly identical. However, while the addition of alcohol completely prevents the turbidity of CBBG solutions, TX-100 only eliminates turbidity at micellar concentrations ($\approx 4.0 \cdot 10^{-4}$ M). Therefore, it was reasonable to consider the effect of alcohol and n-Surf on the system in their combined presence.

In general, the colorimetric signal of CBBG-TX100 aqueous-alcohol solutions decreases with surfactant concentration. This dependence is characterised by a local maximum in the CMC region. An increase in the optimum surfactant concentration was observed in the experiment. A sharp decrease in the colorimetric signal of the solutions was observed at micellar concentrations of n-Surf. The combined effect of TX-100 and alcohol on the turbidity of CBBG solutions also studied. The nonionic surfactant TX-100 in premicellar solutions increased the turbidity of an alcohol solution of CBBG at a surfactant concentration of $(2–4) \cdot 10^{-5}$ M and at the CMC. At the same time, in micellar solutions of TX-100, the turbidity of the dye alcohol solution is not recorded.

Effect of TX-100 on the protein signal in reaction with CBBG. The effect of organized media at premicellar and micellar concentrations of TX-100

on the absorption spectra of CBBG–BSA solutions was investigated (Fig. 7). The organized medium of a nonionic surfactant increases the intensity of the absorption maximum in the long-wavelength region (λ = 620 nm). A minor bathochromic shift ($\Delta\lambda_{max}$ = 11 nm) of the maximum position is observed in the premicellar region. A threefold increase in the absorbance of CBBG-BSA solutions is recorded at TX-100 concentrations exceeding the CMC. The dependence of the analytical signal of BSA on the concentration of the nonionic surfactant is characterised by the presence of local maxima at stoichiometric ratios of CBBG : TX-100 = 1:1, 3:1 and in the region of the CMC of TX-100. As the surfactant concentration further increased, the photometric signal of BSA is no longer recorded. In general, the TX-100 medium reduces the turbidity of CBBG-BSA solutions. However, the corresponding concentration dependence passes through a maximum at the surfactant concentration near the CMC. In the micellar medium of the nonionic surfactant, turbidity is not recorded; the scattering factor decreases, and the size of the aggregated particles increases.

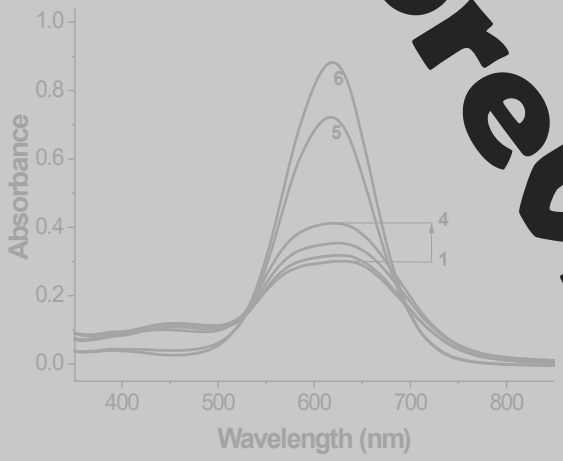

Figure 7. Absorption spectra of aqueous solutions of C_{CBBG}=2.0·10^{-5} M, C_{BSA}=5 mg L^{-1}, C_{TX-100} = 6.0·10^{-6} (1), 1.0·10^{-5} (2), 1.0·10^{-4} (3), 2.0·10^{-4} (4), 4.0·10^{-4} (5), 1.0·10^{-3} (6) M, pH=1.0, l=1cm.

The dependence of the BSA colorimetric signal on the concentration of the nonionic surfactant was investigated. It was found that the BSA colorimetric signal increased (≈20%) at the premicellar concentration of TX-100, whereas at micellar concentrations of the nonionic surfactant, it decreased. The maximum colorimetric signal of the protein in reaction with CBBG was recorded in the TX-100 concentration range of (2.0–6.0)·10^{-5} M.

At the optimum concentration of the nonionic surfactant, TX-100 does not yet eliminate turbidity, whereas at micellar concentrations it disappears. Therefore, an alcohol additive should be used in further studies to establish optimum conditions for the colorimetric determination of protein. In this case, the colorimetric signal of the system with alcohol is more stable, which is due to the stabilisation of the colloidal-chemical state. Importantly, the maximum colorimetric signal of the protein in reaction with CBBG was observed at the CMC of nSurf in the alcohol solution.

Influence of the type of nonionic surfactant on the RGB signal of the protein in reaction with CBBG. The effect of nonionic surfactant media of varying nature on the colorimetric signal of BSA in its reaction with Coomassie Brilliant blue was investigated. Polyoxyethylated alkylphenols with different degrees of oxyethylation, polyoxyethylene sorbitan monolaurate, and high-molecular-weight surfactants were used in the study.

The increase in the colorimetric signal of the protein in reaction with CBBG in the medium of the nonionic surfactant TX-305 (degree of oxyethylation m ≈ 30) is greater compared to the effect of TX-100 (m≈9.7). The colorimetric signal of BSA increases in the region of the premicellar surfactant concentration in the TX-305 media (curve 1, Fig. 8). At the same time, the RGB signal of the protein is maximal in the region of the CMC, within the concentration range of (0.6–2.0)·10^{-4} M Triton X-305. Similar to n-Surf TX-100 solutions, micellar solutions of TX-305 eliminate system turbidity.

The modifying effect of surfactants from the Tween group and high-molecular-weight PEGs on the BSA signal was less effective. They did not contribute to enhancing the colorimetric signal of BSA in the CBBG reaction. The structural features of the relevant surfactants can explain this. The elongated hydrocarbon radical, which acts as an “anchor” in hydrophobic association with CBBG, is absent in the molecular structure of Tween 20. The absence of such an ‘anchor’ reduces the ability of the surfactant to form associations with the reagent. The BSA signal decreases in the entire range of nonionic surfactant concentrations studied (curve 2, Fig. 8). Similar results were obtained for PEGs of different molecular weights. Polyethylene glycols (PEG-115, PEG-6000) do not contain a hydrophobic fragment. They do not form classical micelles and are incapable of solubilising substrates. As a result, PEGs had a weak effect on the turbidity of CBBG–BSA solutions, and the colorimetric signal of BSA did not increase. Only PEG-6000 was capable of reducing the turbidity of CBBG–BSA solutions at higher concentrations (w ≤ 7%). However, no increase in the analytical signal of the protein was observed.

For the first time, it has been demonstrated that a nonionic surfactant medium can serve as an effective matrix for generating a stable and reproducible CBBG signal in the smart colorimetry method. An optimal nonionic surfactant-based medium for hydrophobic Coomassie dye and its protein analytical forms must meet specific criteria. The nonionic surfactant should possess a single extended hydrocarbon radical combined with a sufficiently long polyoxyethylene

chain. The unbranched aliphatic radical acts as a hydrophobic "anchor", enabling the surfactant molecule to bind to the analytical form. The long polyoxyethylene fragment of the surfactant, in turn, ensures the stability of the analytical form in solution, particularly from a colloidal-chemical perspective. Among the tested surfactants, TX-305 proved to be the most effective due to its higher number of polyoxyethylene fragments. The optimum concentration of TX-305 for the colorimetric determination of protein is close to the CMC. Therefore, the combined use of the nonionic surfactant TX-305 and ethanol is appropriate.

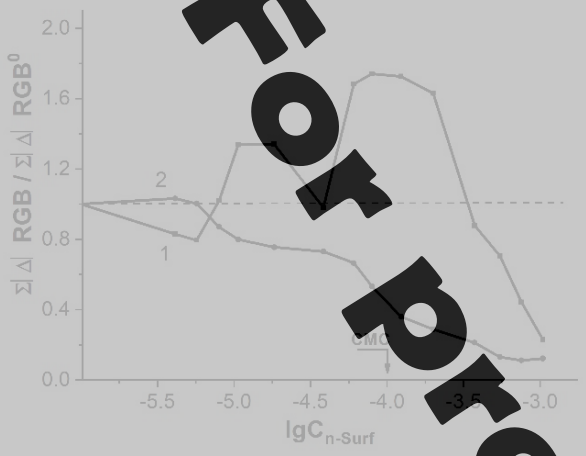

Figure 8. Normalised dependences of the BSA analytical signal on the concentrations of TX-305 (1) and Tween 20 (2). C_{CBBG}=2.0·10^{-5} M, C_{BSA}=5 mg L^{-1} $C_{n\text{-}Surf}$=4.0·10^{-6}–1.0·10^{-3} M, w(EtOH)=5% (v/v), pH=1.0.

Colorimetric determination of proteins with CBBG in the presence of EtOH and TX-305. The optimal conditions for the smart colorimetric determination of protein with CBBG in anaqueous-alcohol medium of n-Surf were determined by studying the corresponding concentration dependencies: C_{CBBG}=2.0·10^{-5}M, $C_{TX\text{-}305}$=1.5·10^{-4} M, and w(EtOH)=5% (v/v).

Calibration curve. An appropriate volume of albumin solution was placed in 10.0 mL tubes containing CBBG, Triton X-305 and ethanol solutions. The contents of the tubes were brought to a final volume of 10.0 mL, mixed thoroughly, the pH was set to 1.0, and the colorimetric signal of the solutions was measured. The calibration curve of the albumin signal was plotted against albumin concentration. The linearity range of the calibration curve for the colorimetric determination of albumin in the CBBG-EtOH-TX-305 system is 1.0–14.0 mg L^{-1}, LOD(3σ)= 1.5 mg L^{-1}, ΔR=(-2.28±0.14)·$C_{albumin}$, mg L^{-1} for egg albumin; 1.0–8.0 mg L^{-1}, ΔR=(-5.67±0.11)·CBSA, mg L^{-1}, LOD(3σ)=0.3 mg L^{-1}, r=0.999 for BSA.

The proposed conditions have been used for the colorimetric determination of albumin in urine. The modified systems proposed in this work, namely CBBG-nonionic surfactant-EtOH, can also be used to determine the protein content in milk serum (RSD = 0.053, n = 5).

Determination of albumin in urine. Human urine was collected in a special medical container and filtered through Whatman filter paper grade 4 (Maidstone, England) with a pore size of 20–25μm to remove coarse particles. Appropriate sample volumes were added to 10.0 mL tubes containing the required amounts of Coomassie Brilliant blue (1.0 mL, C_{CBBG}=2.0·10^{-4} M), nonionic surfactant TX-305 (1.0 mL $C_{TX\text{-}305}$=1.5·10^{-3} M), and EtOH (0.5 mL). The pH was adjusted to 1.0 with H_2SO_4, and distilled water was added to reach the final volume. The contents of the tubes were mixed thoroughly. The colorimetric signal of the solutions was measured using a smartphone in a photo box.

The results of the smart colorimetric determination of albumin in urine are presented in Table 1. The accuracy of the technique was verified by the arbitration method based on the results of the Synevo medical laboratory.

The obtained results indicate that the developed technique for the colorimetric determination of albumin may be a promising alternative for the determination of protein in urine. This technique is competitive in terms of sensitivity compared to molecular spectroscopy and colorimetric methods (Table 2). The method is easy to perform, rapid and demonstrates satisfactory accuracy of the colorimetric determination of albumin in urine. In addition, the developed method allows for screening analysis.

Table 1. Results of smart colorimetric determination of albumin content in urine, C_{CBBG}=2.0·10^{-5} M, w(EtOH)=5% (v/v), $C_{TX\text{-}305}$=1.5·10^{-4} M, pH=1.0, n=5, P=0.95.

Sample urine*	According to the developed method		Arbitration method**
	Found albumin, mg L^{-1}	RSD	Found albumin, mg L^{-1}
1	49.3 ± 3.4	0.075	47.0 ± 1.7
2	35.7 ± 2.6	0.056	36.5 ± 1.1
3	41.0 ± 3.0	0.064	39.0 ± 1.2

*- urine samples were voluntarily provided by adult donors with informed consent.

**- according to the results of the «Synevo» European medical laboratory in Ukraine.

Table 2. Comparison of the analytical performance for determination of the albumin in urine with different methods.

Reagent system	Technique used	Linear/working range, mg L^{-1}	LOD, mg L^{-1}	Ref.
Hemicyanine dye	Fluorescent probe NIR-HSA	27-2750	1.74	[28]
Diketopyrrolopyrrole	Fluorescence sensor	10-100	3.1	[29]
Poly(thymine) (poly T)-templated copper nanoparticles	Spectrofluorimetry	10-166	5.45	[30]
CBBG/ trichloroacetic acid	Spectrofluorimetry	2 -60	2.0	[31]
Bromocresol green/Jaffé reaction/PADs	Colorimetric	10 - 350	7.1	[32]
Albumin Tester	Colorimetric	10-200	5	[33]
Antigen–antibody reaction based on immunoassay	Smartphone-based photoelectrochemical biosensing system	9.9-500	1.5	[34]
Commercial colorimetric strips	Smart colorimetry	0-500	7.5	[35]
Immune latex microspheres	Smart colorimetry	7-450	5.9	[36]
CBBG – EtOH –TX-305	Smart colorimetry	1-14	1.5	This work
CBBG – EtOH –TX-100	Spectrophotometry	1-8	0.5	This work

Conclusions

The effectiveness of using a nonionic surfactant-based medium for the colorimetric determination of protein in reaction with Coomassie Brilliant blue G in solutions is demonstrated. It has been established that the medium formed by n-Surf plays an important role in the formation and stabilisation of the colloid-chemical reagent–protein system. The specificity of the effect of solvents and surfactants on the colloidal-chemical properties of CBBG-protein systems necessitates their combined use. The simultaneous presence of a nonionic surfactant and a solvent, as well as the nature of the surfactant, significantly affects the measurement results. A nonionic surfactant enhances the protein signal while reducing the turbidity of the system, which is important for increasing the stability of the analysed systems. The maximum colorimetric signal of the reagent and protein in the reaction with CBBG is observed in the CMC region of the surfactant. It is also important to note that the selection of n-Surf should be based on its structural properties. The most effective are n-Surf-based media containing an elongated hydrocarbon radical that acts as an “anchor” and a long polyoxyethylene fragment to stabilise the system. The results obtained were used to develop a colorimetric technique for the determination of protein with CBBG in a nonionic surfactant medium TX-305 (LOD = 1.5 mg L^{-1}, S_r = 0.075). The developed technique was tested for the determination of albumin in urine and is characterised by acceptable metrological performance. Thus, non-ionic surfactant-based media offer new prospects for the development of sensitive and stable smart colorimetric methods.

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