



*Dedicated to the memory of  
Academician Bogdan C. Simionescu (1948–2024)*

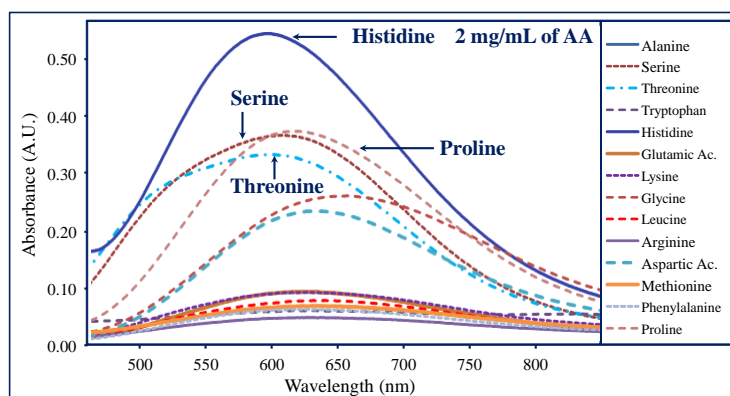
## IMPROVED PROTEIN ASSAY BASED ON THE BIURET REACTION: INTERFERENCE OF PROTEINOGENIC AMINO ACIDS

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Received February 28, 2025

Determining the protein content of a sample is a key step in protein analysis. However, no one single method is suitable for all experimental measurements of proteins in all laboratory settings. Protein quantification by UV-Vis spectrophotometry has numerous applications in chemistry and biochemistry laboratories, medicine and the food industry. Thus, proteins in biological samples can be measured by the classical biuret method using a reagent with copper sulfate, sodium hydroxide and Seignette salt. Micromethods based on the biuret reaction are simple, highly selective and reliable, but there are multiple interferences from free amino acids, peptides, Tris, dextrose and dextran. EDTA also interferes with the tartrate reagent in color development. In this paper, we have introduced an improved version of the biuret method using insoluble copper phosphate, potassium hydroxide and ethyl alcohol that is capable of measuring proteins in both liquid and solid samples. Absorbance of the biuret complex was recorded around 545 nm against a reagent blank, but also at 560 nm with a microplate reader and in the UV range. Amino acid interference was investigated at the same concentration as proteins (2 mg/mL). The interference of amino acids and peptides can be abolished by precipitating the proteins with 10% trichloroacetic acid (TCA), followed by centrifugation and dissolving the pellets in an alkaline-alcoholic solution prior to the biuret reaction. In addition, the degree of interference of many proteinogenic amino acids was calculated at the absorption wavelength of the biuret complex.



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### INTRODUCTION

There is a great variety of protein determination methods based on a wide number of detection mechanisms.<sup>1</sup> All of these techniques, the Dumas and Kjeldahl methods, Berthelot's and Lowry's assays, Bradford, Nessler and Folin-Ciocalteu

procedures, the dye binding test, direct alkaline distillation, near infrared reflectance (NIR) measurement, bicinchoninic acid (BCA) method, the biuret method, etc., were much improved over the last decades.<sup>2,3</sup> The micro-Kjeldahl and Dumas methods are based on nitrogen determination, which is proportional to the protein content of the

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samples.<sup>4,5</sup> Although the Kjeldahl's and Lowry's methods are widely applied for protein determination, the first method has a complex operation and is time-consuming, while the latter, despite its high sensitivity, is susceptible to interference and requires extremely precise time control.<sup>6</sup> However, the protein content can also be determined in the ultraviolet (UV) range at 214, 220, or 280 nm.<sup>7,8</sup> Indeed, the UV-Vis spectrophotometric methods are cheap, easy-working and the most common way to quantitate protein concentrations. Protein concentrations are now also determined by reading the sample absorbance at a wavelength of 540 nm for the biuret method, 750 nm for the Lowry assay and 595 nm for the Bradford assay.<sup>9</sup>

Generally, the peptide content can be determined by the biuret method, while the amino acids are stained with ninhydrin.<sup>10</sup> Although, the biuret method was highly recommended to measure the total protein in serum,<sup>11</sup> we assumed that amino acids could seriously interfere when they are in high amounts in other samples. In fact, proteins extracted from environmental samples may contain polypeptides and amino acids, which could interfere with the biuret absorption. In addition, the extraction of proteins from environmental samples may be associated with protein hydrolysis to amino acids and peptides.<sup>12</sup>

Bovine serum albumin (BSA) is normally used to standardize the biuret method. However, the biuret method is sensitive in the range 0.5 to 2.5 mg protein per assay, while the Lowry method is 1 to 2 orders of magnitude more sensitive (5 to 150 µg).<sup>13</sup> Nevertheless, the main disadvantage of the Lowry method is the number of interfering substances.<sup>13</sup> Interference in the classical biuret assay is seen with free peptides, amino acids, Tris, dextrose, and dextran.<sup>14</sup> EDTA can also influence color development with the tartrate reagent. The classical biuret assay measures the reaction of cupric ions with peptide bonds forming a complex with tartrate, which absorbs at 540–550 nm.<sup>14</sup> Yet, protein determination based on the biuret reaction is commonly used as a reference method.<sup>6</sup> The biuret reaction-based measurement of the total protein has been recommended for many biological materials,<sup>14</sup> including cerebrospinal fluid,<sup>15</sup> synovial fluid,<sup>16</sup> saliva,<sup>17</sup> and serum.<sup>6</sup> Brooks and co-workers found the biuret reaction superior to other methods for the determination of protein content in whole body homogenates.<sup>18</sup> Nevertheless, the biuret method is less sensitive

than other methods. Okutucu and co-workers reported a linear range of 500 µg/mL to 4.0 mg/mL.<sup>3</sup> In addition, the applicability of the colorimetric biuret procedure for protein determination at 540 nm to a variety of biological preparation may be affected by the presence of lipids.<sup>19</sup> Use of suitable controls eliminates the interfering opalescence in biuret color measurements, which considerably improves the method.<sup>20</sup>

In this work, we showed that proteins solubilized by alkaline solutions can extract copper ions from insoluble precipitates to form the biuret complex, and that the color absorbance of the resulted solution is proportional to protein concentration. In addition, because the content of copper ions mobilized by proteins from insoluble copper phosphate powder is relatively low and proportional to the amounts of protein, the biuret complex absorbance can be monitored in the UV range. Moreover, copper ions do not interfere so that the biuret complex absorbance can be measured in the UV range. In addition, we compared our results obtained with the proposed biuret micro-method with the classical biuret method described in literature, and investigate the amino acid interference. Rapid spectrophotometric measurement with a microplate reader was also investigated.

## RESULTS AND DISCUSSION

### The biuret reaction

The sensitivity of the biuret reaction is applicable to protein concentrations from 0.01 to 5.00 mg/mL.<sup>21</sup> Therefore, also in this experiment, UV-*vis* spectra were plotted to construct the calibration curve using a stock albumin solution of 5.00 mg/mL BSA (Fig. 1). The protein mobilized copper ions from the insoluble copper phosphate powder and formed the biuret complex. The alcohol solution solubilized the resulting biuret complex. After decantation, the reaction mixture remained opalescent and had to be centrifuged at 5000–18000 rpm, depending on the particle size involved in this procedure. The absorption spectra in the wavelength range 300–700 nm of BSA solutions of different concentrations are shown in Fig. 1. Two absorption maxima are visible in this figure, at 326 nm and 545 nm, respectively.

Since copper ions were extracted from insoluble copper phosphate in proportion to the protein concentration, the maximum at 326 nm could be important to determine smaller amounts of protein. Thus, the absorbance values at this wavelength were four times higher than those at 545 nm. The corresponding calibration curves at the two wavelengths are shown in Fig. 2. Here, we demonstrated that, by using modern spectrophotometers, it is possible to accurately measure absorbance measurements greater than 1.0 absorbance unit (A.U.). Thus, the calibration curve was linear up to 2.5 A.U., although the spectra may be slightly distorted at higher protein concentrations, probably due to the reduced intensity of light passing through the sample. Thus, the calibration curve plotted at 326 nm was linear

up to about 2.5 A.U. The linearity of the measurement was determined using a 5-point concentration series. The calibration curve was linear between 1 and 5 mg/mL, with a correlation coefficient (Pearson  $R^2$ ) of 0.990 (Fig. 2). A regression equation,  $A_{326} = 0.4328 \cdot C + 0.1886$ , was calculated, where  $A_{326}$  was the absorbance at 326 nm and C, the concentration of BSA expressed in mg/mL.

Compared with existing spectrophotometric methods, the measurement of the biuret complex absorbance at 326 nm seems to be advantageous in terms of simplicity, speed, cost-effectiveness and improved sensitivity and accuracy. However, further investigations are needed to advance a robust method for protein determination by biuret at this wavelength.

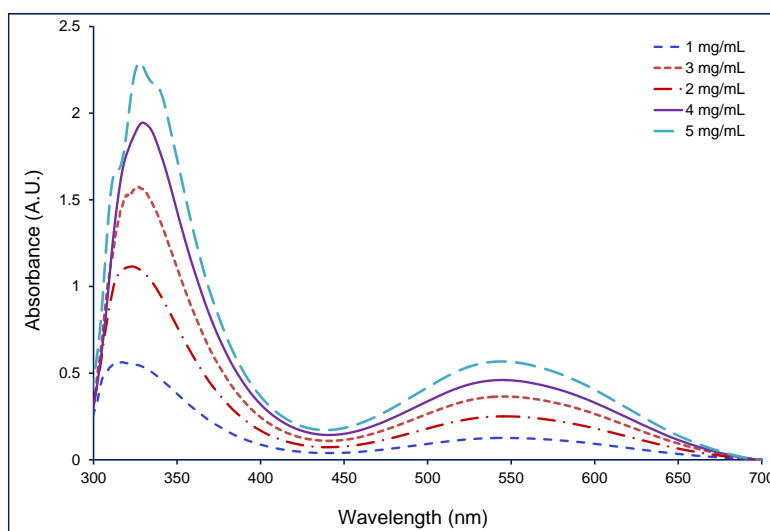


Fig. 1 – Biuret UV-vis spectra of differently concentrated BSA solutions.

Of course, the protein determination at 545 nm was more sensitive than the classical biuret measurement using copper sulfate and tartrate, due to the lower volume of reagent used. In addition, the correlation coefficient

between absorbance and concentration was slightly higher ( $R^2 = 0.996$ ) and the calibration curve was linear and passed through the concentration value 0 (Regression equation:  $A_{545} = 0.1164 \cdot C$ ).

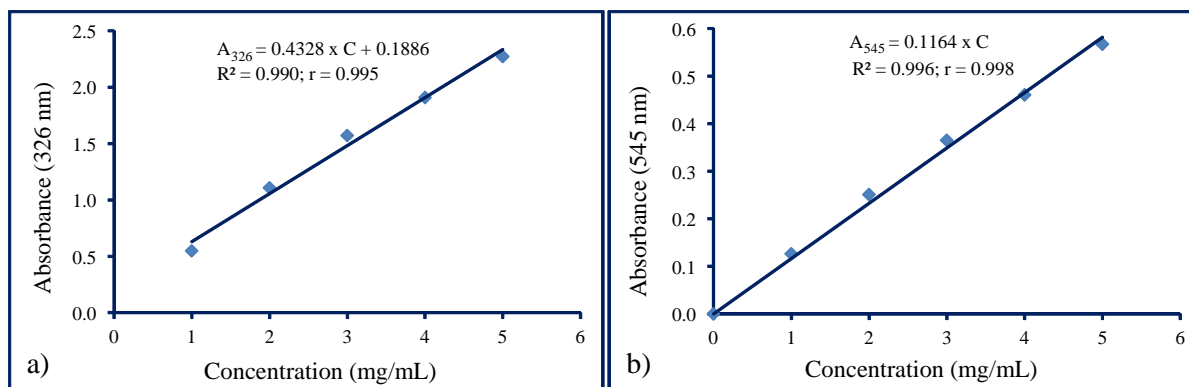


Fig. 2 – Calibration curves for protein determination at 326 nm (a) and at 545 nm (b).

### Microplate reader-based quantitation of proteins

Between the absorbance obtained with the microplate reader and those of the UV-Vis spectrophotometer (Fig. 3), a highly significant correlation was found ( $y = 0.8761x$ ,  $r = 0.998$ , where  $y$  = the biuret complex absorbance values at 560 nm from the microplate reader,  $x$  = the values from UV-Vis instrument, and  $r$ , the correlation coefficient). Microplate reading increased the sensitivity of protein determination by 3.33 times, compared to 545 nm-reading. The microplate reader provides the analyst with the advantage of using smaller volumes of protein solutions and lower amounts

of reagents, as well as an increased speed in absorbance reading.

Since microplate measurements at 560 nm can only be used for color readings of clear solutions, insoluble copper phosphate powder may interfere with the biuret color measurement by giving opalescence. The Eppendorf vials containing the reaction mixtures must therefore be centrifuged at a minimum of 15,000 rpm. The standard curves were also drawn using BSA solutions with concentrations ranging from 0 to 5 mg/mL. Since the absorbance was read at 560 nm in the microplate reader, the calibration curves should be made for each kind of experiment (545 nm or other maxima of UV-Vis spectra versus 560 nm microplate reading).

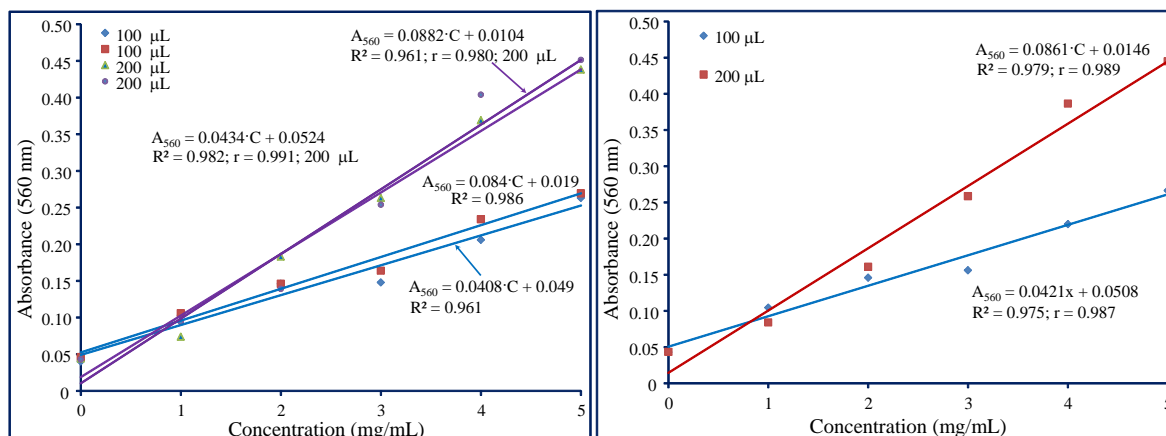


Fig. 3 – Calibration curves using BSA (0–5 mg/mL) and a microplate reader: a) two different measurements out of three, the supernatant biuret volume being 100 µL and 200 µL respectively; b) average of three measurements under the same conditions as in (a).

### Amino acid interference

Because proteins extracted from biological samples may have a high content of polypeptides and amino acids, these can interfere with protein determination methods. Here we investigated the interference of proteinogenic amino acids only in visible light at 545 and 560 nm using both a normal spectrophotometer and a microplate reader. Therefore, we first investigated the biuret absorption of the proteinogenic amino acids at their maximum wavelength as well as at 545 nm or other wavelengths of interest (Fig. 4). There was a serious interference of some amino acids in the determination of pure protein in the solution analyzed at a concentration of 2 mg/mL of both BSA and each amino acid. However, the shape of the spectra also shows that this interference may be slightly stronger at 560 nm than at 545 nm.

The values presented here suggest that it is necessary to draw calibration curves at the wavelength specific to each complex biuret of the proteins analyzed. BSA can be used as a standard in the determination of other proteins. The same figure also shows that free amino acids extract copper ions from insoluble copper phosphate as well to form complexes with different absorption maxima (tryptophan – 628 nm, serine – 602 nm, the amino acid concentration being also 2 mg/mL). The maximum absorption values of these complexes were also completely different (tryptophan – 0.032 A.U.; serine – 0.273 A.U.). The possible interference of the two amino acids was evaluated, as if they were present in the protein solutions at the same concentrations as the proteins (*i.e.* 2 mg/mL). Thus, the biuret absorbance in BSA determination would increase from 0.463 A.U. to 0.490 A.U. (+5.83%) in the presence of tryptophan, but to 0.711 A.U. (+53.56%) due to the presence of serine.

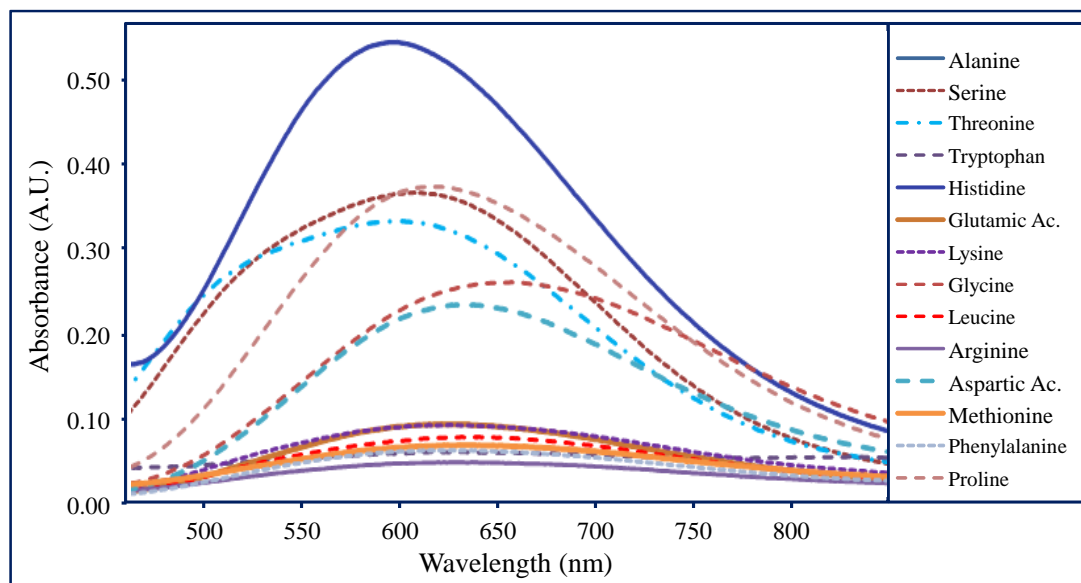


Fig. 4 – Absorption spectra of proteinogenic amino acids subjected to the biuret reaction (2 mg/mL amino acids).

Table 1

Interference of several amino acids in protein determination using the proposed biuret variant<sup>1</sup>

Amino acid	$\lambda_{\max}$	$A_{\max}$	$A_{545}$	$A_{560}$	$A_{545}/A_{BSA}$	$A_{560}/A_{BSA}$
Alanine	633	0.010	0.008	0.009	0.017	0.020
Arginine	633	0.023	0.012	0.015	0.026	0.033
Asparagine	592	0.202	0.163	0.185	0.355	0.410
Aspartic acid	639	0.186	0.089	0.115	0.194	0.255
Glycine	658	0.210	0.095	0.121	0.208	0.268
Glutamic acid	625	0.062	0.034	0.043	0.074	0.095
Glutamine	655	0.042	0.025	0.030	0.054	0.067
Histidine	598	0.457	0.367	0.410	0.800	0.910
Leucine	633	0.048	0.027	0.033	0.059	0.073
Lysine	628	0.061	0.039	0.047	0.086	0.103
Methionine	632	0.040	0.023	0.028	0.050	0.062
Norvaline	540	0.251	0.250	0.243	0.545	0.539
Phenylalanine	634	0.034	0.019	0.024	0.041	0.053
Proline	617	0.309	0.196	0.235	0.427	0.522
Serine	602	0.273	0.257	0.273	0.561	0.606
Threonine	600	0.273	0.248	0.258	0.541	0.572
Tryptophan	628	0.032	0.024	0.026	0.053	0.059
Tyrosine	621	0.043	0.029	0.035	0.063	0.078
BSA Protein	547	0.459	0.459	0.451	1.000	1.000

<sup>1</sup>) Here, 1 mL of 2 mg/mL of each amino acid or BSA was treated with 0.5 mL of alkaline-alcohol solution and approximately 50 mg of copper phosphate powder. Based on the biuret spectra in Fig. 4, the absorbance at various wavelengths is tabulated (where,  $\lambda_{\max}$  is the wavelength at which absorption is maximum ( $A_{\max}$ ), and  $A_{545}$  and  $A_{560}$  are its values at 545 nm and 560 nm respectively;  $A_{BSA}$  means the absorption of BSA at either 545 nm or 560 nm).

From Table 1 it can be seen that amino acid complexes with copper ions mobilized from copper phosphate absorb at longer wavelengths than BSA ( $A_{\max}$  547 nm). For this reason, their interference at 545 nm and 560 nm is stronger than that at 545 nm. Histidine exhibited the strongest influence on the reaction of biuret through its complex with copper ions at both 545 nm and 560 nm ( $A_{\max}$  = 0.457 A.U.;  $A_{545}$  = 0.367 A.U.;  $A_{560}$  = 0.410 A.U.). The maximum wavelength is also important, because each copper-

amino acid complex absorbs at specific  $\lambda_{\max}$ . Thus, histidine formed a copper complex due to nitrogen atoms, which absorbs at  $\lambda_{\max}$  = 598 nm ( $A_{598}$  = 0.457 A.U.), whereas its absorbance at 545 nm or 560 nm was lower ( $A_{545}$  = 0.367 A.U. and  $A_{560}$  = 0.410 A.U., respectively). Similarly, the proline complex with copper ions showed relatively high absorbance at 617 nm (0.309 A.U.). It is noteworthy that the presence of an HO group in an amino acid molecule significantly increases the

absorbance of the amino acid complex with copper ions. For example, alanine, which does not form a copper complex but rather a copper salt, has very low absorption at 633 nm, while serine and threonine show significant absorption around 600 nm (0.273 A.U.). These two amino acids form copper complexes involving oxygen atoms.

### Real samples

Previously, a variant of the biuret test for determining proteins in corn was reported, in which an alkaline reagent with sodium dodecyl sulfate was used, but which required heat treatment.<sup>22</sup> Nevertheless, such method uses copper sulfate and sodium tatarate, and starch, fiber, oil and other non-protein substances in the turbid solution to be analyzed have been found to hinder the colorimetric methods.

Consequently, we advanced and tested our biuret variant on 20 maize seed samples (Fig. 5). As we expected, a better correlation coefficient was found between the absorbance of the biuret complex and the pure protein content of the analyzed samples ( $r = 0.982$ ) than between the 545 readings and the crude protein content ( $r = 0.975$ ). Crude protein means pure protein plus non-protein compounds such as nitrates, nitrites, ammonia and other nitrogen-containing compounds.

The crude protein percentage (CP%) was proportional to the biuret absorbance of the corn samples (BA). Nevertheless, the correlation coefficient  $r = 0.975$  was rather low because the biuret reaction refers only to pure proteins and peptides, except dipeptides. The value of this coefficient increased when pure protein content (PP%) was measured ( $r = 0.982$ ).

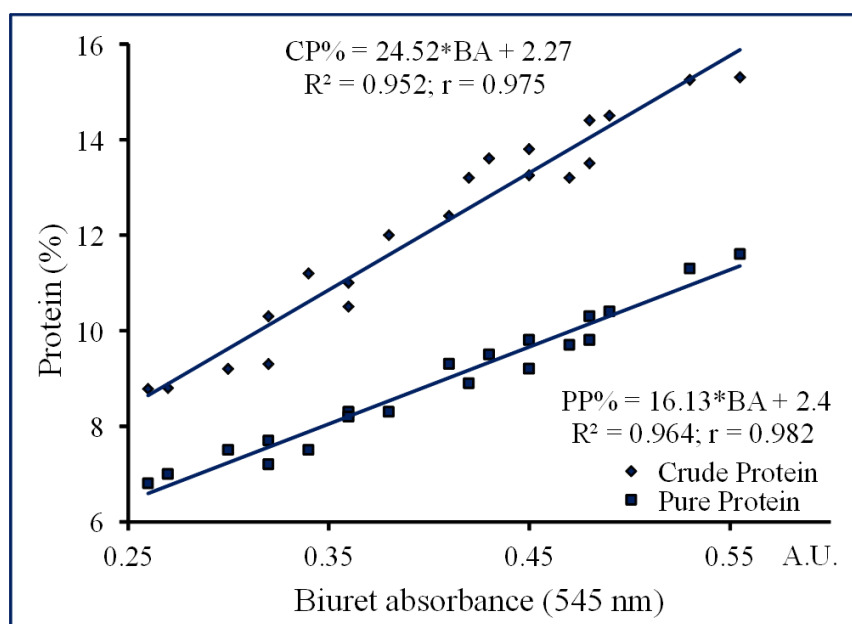


Fig. 5 – The relationship between the absorbance of the biuret complex and the crude and pure protein content in some maize seeds.

Figure 6 shows the relationship between the absorbance of the biuret complex absorbance at 545nm of the finely ground seed samples (particle size 100  $\mu$ m) and the duration of sonication of the Eppendorf vials containing the reaction mixtures. All samples consisted of 200 mg of maize meal of the same maize variety. One of these samples was not sonicated, but manually stirred for one minute and showed an absorbance of only  $0.639 \pm 0.151$  A.U. The maximum absorbance was read after 30 min of sonication ( $A_{545} = 1.537 \pm 0.232$  A.U.), while 1 mL of 5 mg/mL BSA dissolved in alkaline-alcohol solution

had 1.57 A.U. When BSA was used as the standard protein, the regression equation was  $A_{545} = 0.2964 \cdot C$  ( $R^2 = 0.998$ ), where C is BSA concentration expressed as mg/mL. Therefore, the protein concentration of this sample was 9.80%, which is normal for maize seeds. However, since we weighed 200 mg of maize flour and not 50 mg as shown in figure 5, the crude protein content should be  $CP\% = (24.52 \times 1.537 + 2.27) \times 50/200 = 9.98\%$ , while the pure protein content was  $PP\% = (16.13 \times 1.537 + 2.4) \times 50/200 = 6.79\%$ , and these values are normal for maize.

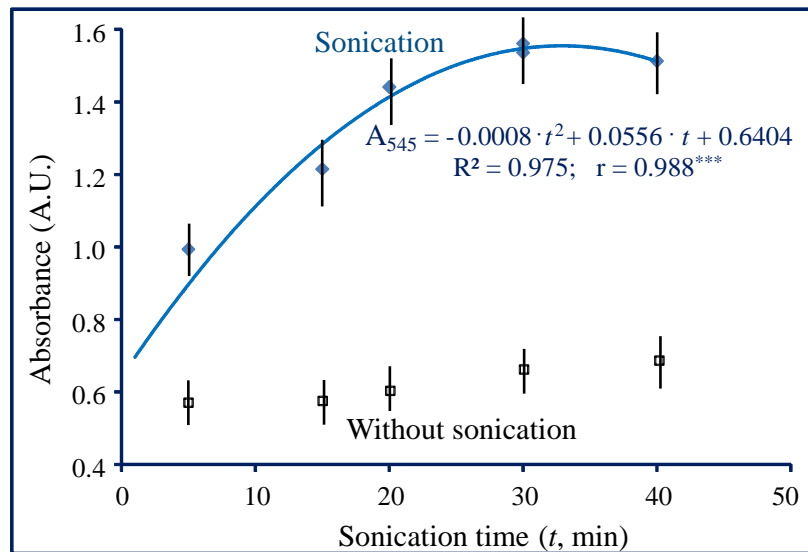


Fig. 6 – Determination of extraction time of proteins from maize samples.

The biuret variant developed and presented in this paper may thus provide an alternative method for the rapid determination of proteins in cereal grains, including maize.

It was found that amino acids interfere, but they and other soluble compounds can be removed by precipitating proteins with 10%

TCA (Fig. 7). Thus, the absorbance of BSA and serine, as well as their mixture, was measured following their reaction with copper ions. Such results show that when constructing calibration curves with BSA, BSA solutions must be treated under the same conditions as sample solutions.

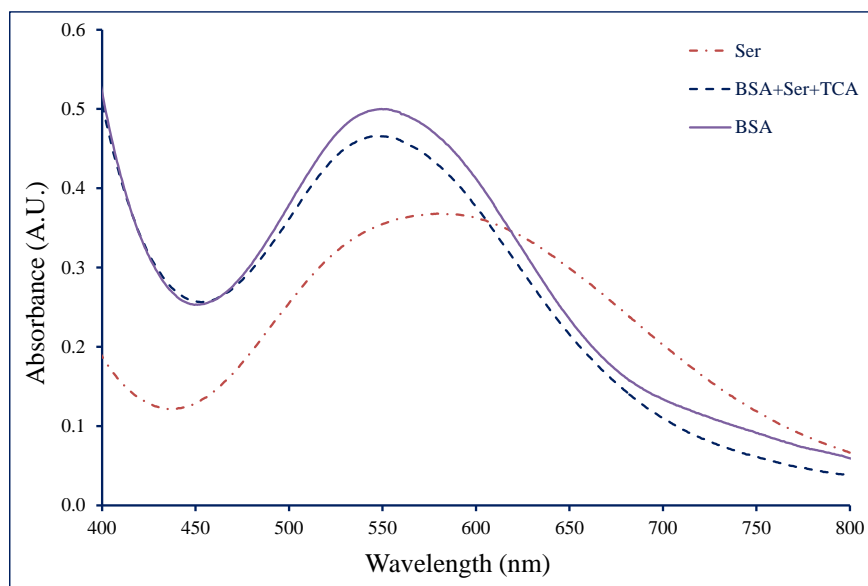


Fig. 7 – Absorption spectra of the biuret complex obtained in the reaction of BSA and serine, as well as their mixture with copper ions after precipitation with 10% TCA.

## DISCUSSION

Previously, we considered the option of determining proteins by the biuret method only at 545 nm, not at 326 nm.<sup>23</sup> However, our results suggested that the protein determination in at 326 nm could be feasible and a new investigation

is needed to characterize it. Nevertheless, the mobilization of copper ions by proteins in alkaline solution is not new, but numerous research data have been acquired in this field that allow the biuret method to be improved.<sup>24–26</sup> In fact, the research started by binding copper ions to peptides, when we showed that peptides extract copper ions

from the insoluble precipitate depending on the pH values of their solutions.<sup>24,27,28</sup>

The classic biuret reagent is composed, for example, of copper sulfate pentahydrate  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  (3.25 g/L), potassium sodium tartrate  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4 \text{H}_2\text{O}$  (9.02 g/L), and NaOH (24 g/L). Another biuret reagent contains 9.00 g/L potassium sodium tartrate, 0.024 g/L sodium hydroxide, 1.90 g/L anhydrous copper sulfate, and 5.00 g/L potassium iodide.<sup>29</sup> However,  $\text{CuSO}_4$  interferes with biuret reading in the ultraviolet range, whereas starch produces opalescence in some biological samples, such as cereals.<sup>24,30</sup>

The Bradford assay is based on a metachromatic shift of the dye on binding to a protein.<sup>31</sup> The high sensitivity of this method is dependent on the quality of the protein, the solvent and the reaction conditions. A linear range of 20–500  $\mu\text{g}/\text{mL}$  was reported.<sup>3</sup>

The interference of ammonium ion with protein estimation by the biuret reaction will be investigated in the next work. When the classical biuret reagent containing a large amount of copper sulfate is used, the ammonium ion forms complexes with copper ions and interferes strongly with the biuret color formation.<sup>32,33</sup> In the method proposed by us in this work, the protein extracts proportional amounts of copper ions. Therefore, a detailed study of the ammonium ion interference in the biuret reaction is required, with measurements being made at several wavelengths, both in the visible and in the UV.

We have thus improved the biuret method replacing tartrate and copper sulfate with insoluble copper phosphate and ethyl alcohol. The interference of amino acids can be completely eliminated by protein precipitation with 10% TCA, centrifugation and pellet solubilization in an alkaline-alcoholic solution before the biuret reaction.

## EXPERIMENTAL

### Materials

*Chemicals.* The reagents used in our experiments were of analytic purity, purchased from commercial sources and used as received unless otherwise specified. The solutions were prepared with MilliQ grade water (18.2  $\text{M}\Omega \cdot \text{cm}$ ) from a Millipore water purification system (Millipore, Bedford, MA, USA). Bovine serum albumin (BSA) from Fluka was employed as the

standard protein for all measurements. Amino acids were bought from Merck. Copper sulfate, disodium phosphate, sodium hydroxide, and potassium hydroxide were purchased from Merck (Darmstadt, Germany). Ethyl alcohol was from Chemical Company S.A. of Iasi, Romania.

Copper phosphate was prepared by treating of a 1 M solution of disodium phosphate with a 1 M of copper sulfate solution (1:1, v/v), and the resulted precipitate was washed three times with distilled water on filter paper, dried, and grinding up to a fine powder.

Protein precipitation was carried out with 10% (w/v) trichloroacetic acid (TCA) prepared by dissolving 500 g TCA (as shipped) into 350 ml MilliQ grade water, store room temperature, and diluting to the required concentration.

*Biological material.* Commercial corn seeds with a residual moisture of 12% were ground to a fine flour powder that passed through the 100  $\mu\text{m}$  mesh of a sieve.

### Instruments

The UV-visible spectra were performed on a Biochrom® Libra S35 PC UV\Visible spectrophotometer with 1 cm matched cells of quartz. Spectrophotometric analyses for proteins were also carried out at 560 nm on a 96-well microplate spectrophotometer (Modulus™ microplate reader, Turner Biosystems, Sunnyvale, USA). Sonication and the biuret reaction were performed on an ultrasound bath cleaner (J.P. Selecta Ultrasons system, 40 kHz; Barcelona, Spain). Centrifugation at 15,000 rpm was performed using a Hettich Mikro 22R centrifuge (Tuttingen, Germany).

### Procedure

Typically, to 1 mL of protein-containing solution, 0.5 mL of alkaline-alcoholic solution was pipetted into an Eppendorf vial of plastic under shaking. Then, 20–30 mg of copper phosphate powder was added and the resulted mixture obeyed to vigorous shaking, followed by ultrasonic stirring for at least 15 min. Finally, the Eppendorf vials were centrifuged at 15,000 rpm, and the absorbance of each supernatant was measured in the range from 340 to 840 nm with the spectrophotometer. Each time, a control using only reagents was used. Only spectra and absorbance in the wavelength range of interest, such as 300–

350 nm, 400–700 nm, or 545 nm and 560 nm, were discussed. In addition, a comparison between biuret measurement using microplates and normal UV-Vis spectrophotometry was done. Thus, the volume of sample introduced in the Eppendorf vial was reduced to only 300  $\mu$ L and only 150  $\mu$ L of alcohol-alkaline solution was pipetted; then, 5–10 mg of copper phosphate was added and the procedure for the biuret reaction and centrifugation was performed as mentioned above. Then, 200  $\mu$ L of supernatant was transferred into a 96 well-plate, and the absorption signal proportional to the protein content was detected at 560 nm with a Modulus™ microplate reader (Turner Biosystems, Sunnyvale, USA). The results were expressed as mean relative light units (RLU) and compared with those obtained by the normal UV-vis spectrophotometry at 545 nm.

The calibration curves were plotted at the maximum absorption wavelength of biuret, around 545 nm, as well as at 560 nm or even at 326 nm.

In the case of proteins from cereals and other vegetable seeds, samples of 50 mg of fine vegetable powder were taken and placed in Eppendorf vials. Then, while stirring, 1 mL of alkaline-alcoholic solution and approximately 50 mg of copper phosphate powder were added. The Eppendorf vials were sonicated for at least 30 minutes. After centrifugation at 15,000 rpm for 5 minutes, the clear supernatant was measured in a spectrophotometer in 1 cm cuvettes (plastic cuvettes were used in the visible spectrum) compared to the control made only with reagents and treated in a similar manner. The calibration curve was performed either with an alkaline-alcoholic BSA solution (1 mL) or with protein concentrations determined by the micro-Kjeldahl method. A standard nitrogen-protein conversion factor of 6.25 was applied to calculate the protein content of maize.

### Statistics

Results of triplicate measurements were reported as mean  $\pm$  SD. The standard deviation (s), standard deviation of the mean ( $s_x$ ), t parameters and coefficient of variation, CV%, were determined to compare the methods and obtained results.

### CONCLUSIONS

Here, the traditional biuret method based on the use of copper sulfate and sodium potassium tartrate

was modified by replacing these reagents with an alkaline-alcoholic solution and insoluble copper phosphate. Our results show that the proteinogenic amino acids and peptides interfere in both the classical biuret method and the modified biuret variant, and, therefore, we investigated this interference in both biological materials and artificial solutions. To completely eliminate interference from amino acids and peptides, proteins can be precipitated with 10% TCA, centrifuged, and the sediment dissolved using an alkaline-alcoholic solution before the biuret reaction. The sensitivity of the new biuret variant is increased by 4 times by reading absorption values in the UV range at about 326 nm. Better results were obtained in the determination of pure protein in maize than for crude protein. Calibration curves can be plotted both with BSA and protein contents determined by the micro-Kjeldahl method.

*Acknowledgement:* We are indebted to the anonymous reviewers who greatly improved the quality of this work through their pertinent suggestions. The authors wish to thank the financial support from the Roumanian Government through UEFISCDI Bucharest (PN-III-P2-2.1-PED2019-2484, Contract PED494, BioPASCAL). G.D. thanks for the funding from Al. I. Cuza University of Iasi through the FPR grant through the University's Research Department.

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