PROTEIN DETERMINATION BY BICINCHONINIC ACID METHOD (BCA): DOES THE TIME MEASUREMENT HAVE AN EFFECT IN THE RESULTS?

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BCA is a simple colorimetric assay for protein determination; however, it is not a true end-point method, because the color continues to develop through time. This fact posed a question: What is the effect in the absorbances, when samples are measured one by one using a spectrophotometer? The aim of this work was to determine if the time between measurements generates a significant error during milk protein quantification. BSA at 0.24 to 1.92 g/L was used. The first measurement after incubation and cooling was performed in triplicate, and successive measurements were carried out at 5, 10, 15 and 20 minutes. For a high concentration (1.92 g/L) at 5 minutes the difference in calculated concentration was 1.7% and at 20 minutes was 6.9%. For an intermediate concentration (0.72 g/L) at 5 min the difference was 2.4% and 9.5% at 20 minutes. The higher difference was found for the lowest concentration sample (0.24 g/L): 3.4% at 5 minutes and 13.4% at 20 minutes. In all cases, the protein content was over-estimated (being these differences significant for times over 5 minutes). Therefore, when more time was used for sample set measure, errors were higher. This is typical for a large set of samples, when 20 or more minutes are necessary for the all measures. This time error difference between measurements can be avoided if all the samples are measured at once. In conclusion, a microplate reader is recommended to avoid this kind of error.

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