Annals of the University of Craiova
The Chemistry Series
Volume 51, No. 1 (2025) 60-66
homepage: chimie.ucv.ro/anale/
10.52846/AUCCHEM.2025.1.08

Interaction of Bovine Serum Albumin with the Azo Dye Methyl Red: A UV-Vis Spectrophotometric Study☆

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Received: 20.10.2025 / Accepted: 25.10.2025 / Published: 06.11.2025

Abstract:
Protein denaturation is a process that significantly influences the structural integrity and biological activity of proteins. This paper presents an investigation into the denaturation and aggregation mechanisms of bovine serum albumin (BSA) and its interaction with the azo dye Methyl Red under controlled pH and temperature conditions. The study was conducted using UV-Vis spectrophotometry to observe conformational changes and complex formation.

Keywords: protein denaturation, bovine serum albumin, Methyl Red, UV-Vis spectroscopy, azo dyes

1. INTRODUCTION

Most proteins, in their native state, are folded into well-defined, three-dimensional, and generally rigid structures. For most proteins, this structure is compact and globular, as exemplified by the well-known structures of myoglobin, lysozyme, ribonuclease, and chymotrypsin.

☆Paper presented at the XVIIth Edition of the National Chemistry Symposium, Craiova, November 7, 2025

In the case of a few proteins, it can be observed that the native substance is rod-like in shape, or consists of a mixture of rod-shaped and globular structures. Myosin is an example of the latter.

The definition of denaturation, which many earlier authors found problematic due to the lack of knowledge about native protein structures, can now be clearly stated: it is simply a major alteration of the original native structure without modification of the amino acid sequence — that is, without the breaking of primary chemical bonds that link one amino acid to another [1]. Denaturation is, therefore, a process that disrupts the ordered structure of proteins, often resulting in the loss of their biological function. It can be induced by heat, pH variations, or chemical agents and is accompanied by modifications in solubility, viscosity, and optical properties.

Proteins found in bovine milk are soluble proteins that are separated from casein during cheese production. The main whey protein is β-lactoglobulin (β-Lg), representing about 50% of total whey proteins, while the remainder includes α-lactalbumin (α-La, 20%), bovine serum albumin (BSA, 8%), immunoglobulins (Igs, 12%), and minor components such as lactoferrin, lactoperoxidase, lysozyme, and growth factors [2].

β-Lactoglobulin is the major whey protein in milk and exists as a non-covalent dimer in solution at neutral pH (5.5–7.5) and concentrations above 50 mM. There are nine genetic variants of bovine β-Lg. The dimeric conformation is stable at pH 5.5–7.5 and temperatures below 40 °C but dissociates into two identical monomers either at pH < 3.5, pH > 7.5, or at temperatures above 40 °C. Each monomer contains two disulfide bridges (Cys66–Cys160 and Cys106–Cys119) and one free thiol group (–SH) from cysteine 121, which is buried in the protein structure. These disulfide bonds and thiol groups play an essential role in the reversibility of β-Lg denaturation. Heat-induced denaturation and aggregation of globular whey proteins are generally caused by exposure of the free SH group, followed by intermolecular reactions leading to complex aggregate formation.

Albumins are globular proteins that play an essential role in maintaining plasma pressure and nutritional balance. Various compounds are transported by binding to albumin in the blood. Serum albumins are the most abundant (52–62%) proteins in the total water-soluble fraction of blood plasma. BSA is composed of a single polypeptide chain that includes 583 amino acid residues. The 17 disulfide bridges cross-linking the cysteine (Cys) amino acid residues stabilize the structure [3]. BSA was studied within the temperature range of 25-90 °C, and the temperature-induced changes in secondary structure and conformation were analyzed using FTIR spectroscopy [4].

The interaction of Sudan II and Sudan IV dyes with BSA was studied using UV-Vis spectra [5]. Both Sudan II and Sudan IV primarily interact with BSA thru Van der Waals forces. In BSA, the strong absorption peak at 208 nm reflects the protein's framework conformation, and the weak absorption peak appears at 276 nm due to the aromatic amino acids (Trp, Tyr, and Phe) in the protein. The intensity of the peak at 208 nm decreases with the addition of Sudan dye because the protein backbone unfolds. At 276 nm, absorption increases with the addition of Sudan dyes, indicating that the dyes alter the microenvironment of aromatic amino acids within the protein. It is also observed that Sudan IV has a greater effect than that of Sudan II. Additionally, the spectrum indicates that the secondary or tertiary structure is influenced by the addition of dyes.

In this work, it was used to study the interaction of BSA with Methyl Red (MR) dye at pH 3.5.

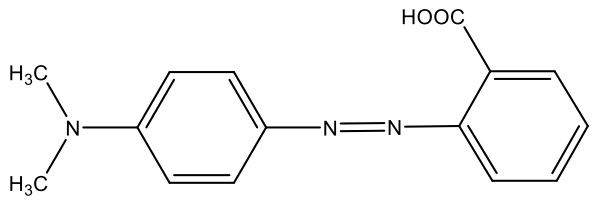
2. MATERIALS AND METHODS

The MR dye was used as an aqueous solution (0.1 g/L), while the BSA solution had a concentration of 0.1 g/L. The pH value of 3.5 was adjusted using a 0.1 N HCl solution. The interaction between the dye and albumin was facilitated by stirring with a magnetic stirrer at 800 rpm, which also ensured the maintenance of a constant

temperature. The UV-Vis spectra were recorded using a Varian Cary 50 UV-Vis spectrophotometer with the Cary Win software.

3. RESULTS AND DISCUSSION

Methyl Red (2-(N,N-dimethyl-4-aminophenylazo)benzoic acid, CI Acid Red 2, Scheme I) is a well-known azo dye and has been widely used in textile dyeing and paper printing, or as a pH indicator [6].



Scheme I. Chemical structure of MR.

In the electronic spectrum of BSA in solution at pH 3.5, a maximum at 280 nm is observed, corresponding to the $\pi \rightarrow \pi^*$ transitions of the aromatic nuclei of amino acid residues (tryptophan, tyrosine) (Figure 1).

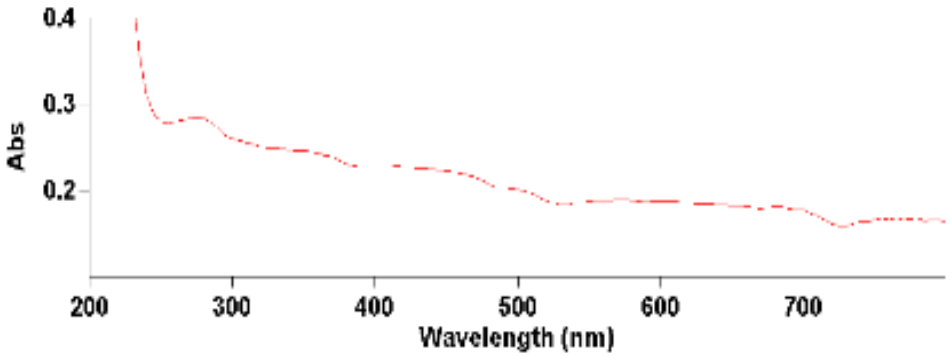


Figure 1. UV-Vis absorption spectrum of BSA solution.

In the presence of increasing concentrations of MR, the absorbance attributed to the protein increases, and a hyperchromic shift occurs. This effect is due to protein unfolding and the change in

the polarity of the environment around the aromatic amino acid residues in the protein structure [11]. These findings demonstrate that the protein can bind the dye to form a BSA–dye complex, thereby altering its structure (Figure 2). The bands in the visible region at 525 nm are due to the presence of the azo chromophore (-N=N-) in the dye molecule. The increase in absorbance in the visible range is expected and results from the higher dye concentrations.

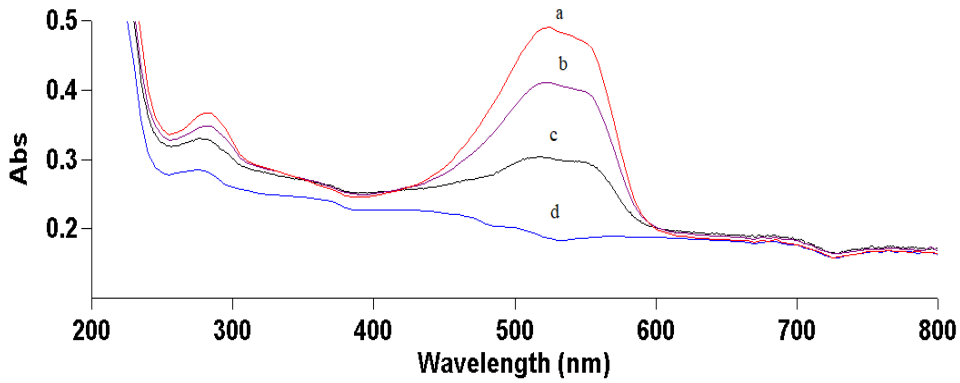


Figure 2. UV-Vis absorption spectra of BSA–MR systems at pH 3.5 and varying dye concentrations.

In the second part of the study, we investigated the effect of temperature on the BSA–MR interaction. First, we recorded the UV-Vis spectrum of MR for a solution kept at room temperature (20 °C) and for a solution heated to 50 °C and then cooled (Figure 3). Analyzing the two spectra, no significant changes were observed, indicating that temperature does not affect the visible absorption of the dye.

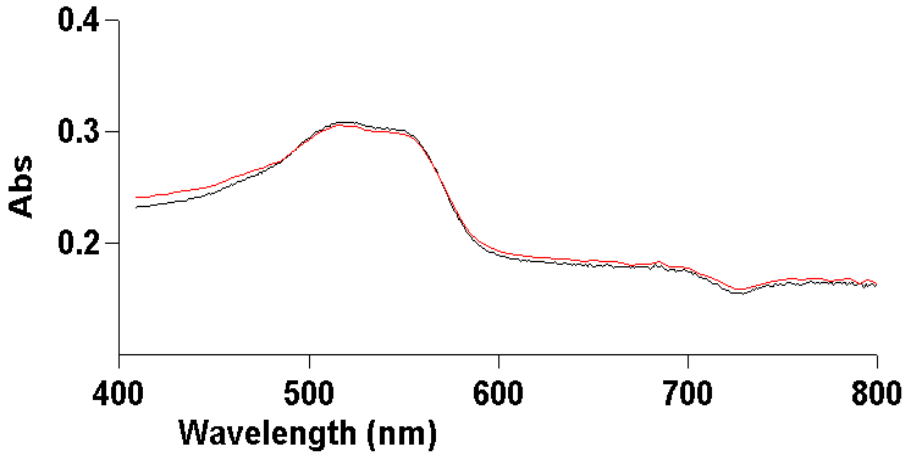


Figure 3. UV-Vis absorption spectra of BSA–MR complexes recorded at 20 ° (---) and/ 50 °C (---).

Next, BSA and MR solutions were heated to 50 °C and 40 °C, respectively, at pH 3.5, and after cooling, their UV-Vis spectra were recorded (Figure 4). Curve a corresponds to the dye–protein solution obtained at room temperature. The visible maximum at 525 nm corresponds to the dye, while the UV maximum at 280 nm corresponds to the protein. Curve b corresponds to a MR solution of low concentration, showing the same absorption maximum due to the azo group at the same wavelength but with a lower absorbance. Curves c and d were obtained from the UV-Vis spectra of BSA–MR solutions at pH 3.5, heated to 40 °C and 50 °C, respectively. The disappearance of the 525 nm maximum (due to the dye's azo group) and the appearance of a new maximum at 432 nm indicate the formation of a complex between the dye and the protein, which also implies a structural modification of the protein.

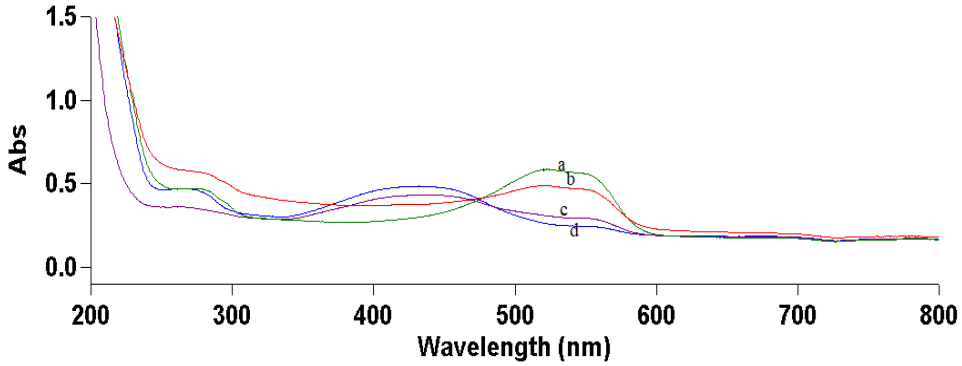


Figure 4. UV-Vis absorption spectra of BSA–MR complexes recorded at pH=3.5 and different temperatures

4. CONCLUSION

- The interaction between azo dyes and proteins can be studied using UV-Vis spectrophotometry.
- BSA interacts with the azo dye MR, leading to structural modifications of the protein.
- Increasing concentrations of MR alter the ultraviolet absorption of BSA.
- Rising temperature causes the disappearance of the characteristic absorption maxima of the dye and the protein, accompanied by the appearance of a new absorption maximum corresponding to the BSA–MR complex formed.
- The structure of BSA is affected by both the presence of MR and temperature.

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