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# Optical spectroscopy studies of the interaction between thiophanate methyl and human serum albumin for biosensor applications

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ABSTRACT

Optical properties of the interaction between thiophanate methyl and human serum albumin have been investigated for biosensor applications. The interaction between human serum albumin (HSA) and thiophanate methyl (MT) was investigated by UV–Vis absorption spectra and atomic force microscopy. The optical constants (refractive index, absorption index, band gap and dielectric properties) of HSA, MT and MT + HSA films were determined using absorbance, transmittance and reflectance spectra. The refractive index dispersion curve (>530 nm) exhibits the normal dispersion. The refractive index of the MT + HSA is higher than both HSA and MT alone due to the highest reflectance of the mixture of MT and HSA. This behavior is indicative of the complex formation between the MT and HSA.



## 1. Introduction

Human serum albumin (HSA), represents the major soluble protein constituent of the circulatory system. It is the most abundant drug carrier protein which plays an important role in the transportation and deposition of many endogenous and exogenous drugs/ligands in blood [1,2]. Many drugs, chemicals and bioactive small molecules can bind reversibly to albumin and other serum components, which implicate their role as carriers [3–5]. HSA can bind and carry many drugs and chemical materials which are soluble or poorly soluble in water. The distribution, free concentration and the metabolism of various drugs can be obviously altered upon binding with HSA [1]. Modern agricultural production in many countries largely depends on intensive use of pesticides. Indiscriminate applications of pesticides resulted in accumulation of pesticide residues in fruits and vegetables [6–9]. Thiophanate methyl (MT) is a thioallophanate compound, which is widely used to control the fungal diseases of crops due to its broad spectrum activity [10,11]. Apart from the beneficial effects associated with the use of pesticides, the pesticides may also pose potential hazards to humans and the environment [12,13]. MT is a known category-III acute inhalation toxicant, likely to be carcinogenic to humans and is widely used for the control of some important fungal diseases of crop [14–16]. It is a potential spindle poison and impairs the polymerization of tubulin [17] besides causing delayed cellular proliferation and increase in the frequency of apoptosis. Recently, Saquib et al. [18,19] demonstrated its genotoxic potential and capacity to induce DNA strand breaks, micronuclei and 8-oxodG formation in human lymphocytes due to ROS generation in vitro. The interactions between bio-macromolecules and chemicals have attracted increasing research interest in recent years especially for the health and environmental protection [20,21]. Among various bio-macromolecules, serum albumins are the most abundant soluble proteins in the body circulatory system of a wide variety of organisms and have many indispensable physiological functions. Serum albumins are serving as a depot protein and a transport protein for many exogenous compounds [14,21]. Therefore, the absorption, distribution, metabolism and excretion properties, as well as the stability and toxicity of chemical substances can be significantly affected because of their binding to serum albumins [14–21]. Moreover, there is evidence of conformational changes of serum albumin induced by its interaction with low molecular weight molecules, which appear to affect the secondary and tertiary structure of albumins [14–21]. Therefore, the studies on interaction of chemicals with serum albumin are of great importance. Binding of chemicals with protein helps in understanding the metabolism and transport process besides assessing the structure–function relationship of the protein [20]. Thus, it is necessary to investigate the interaction of MT with HSA, to

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demonstrate the MT–protein complexation and provide important insight into the nature of interaction. The interaction has been studied by means of spectrophotometric studies and AFM. UV–Vis–NIR spectra of transmittance/reflectance/absorbance were measured in the wavelength range 250–800 nm. Optical constants of HSA, MT and complex interaction between them are calculated and analyzed in the light of the optical spectroscopy for the first time to characterize the interaction between MT and HSA.

AFM micrographs as a powerful tool provide more useful information about the complex interaction between MT and HSA.

## 2. Experimental procedures

### 2.1. Chemicals and materials

HSA was purchased from Sigma–Aldrich and MT was obtained from D-86199 Augsburg (Germany).

### 2.2. Reagents

HSA solution was prepared in pH 7.4 buffer solution as ($3.0 \times 10^{-5}$ mol/L) and HSA stock solutions were kept in the dark at room temperature (293 K). The stock solution ($1.0 \times 10^{-3}$ mol/L) of MT was prepared in ethanol.

### 2.3. Instrumentation

Thin film of HSA and MT and MT + HSA were prepared through Xe-100 Desk-top Spin Coater at 1000 rpm and dried at 60 °C for 5 min. The transmittance/reflectance/absorbance spectra of the HSA, MT and MT + HSA thin films were obtained using a Shimadzu UV-VIS-NIR 3600 spectrophotometer. The morphology of MT and MT + HSA thin films was investigated using Park System XE-100E atomic force microscope.

## 3. Results and discussion

### 3.1. AFM micrographs of MT and MT + HSA thin films

The atomic force microscopy (AFM) has been exploited as a surface characterization technique for examination of biomolecules in biomaterial research. This technique offers the significant advantage of probing in high detail the surface topography qualitatively (surface images) and quantitatively (surface roughness) due to its nanometer-scale spatial resolution. It has proved to be very helpful for the determination and verification of various morphological features and parameters. The interaction between HSA and MT in thin film form was examined. Two and three dimensional AFM micrographs of $40 \times 40\ \mu m^2$ and $10 \times 10\ \mu m^2$ of MT and MT + HSA are shown in Fig. 2. The MT thin film micrographs dried at 60 °C have the shape of tree leafs with roughness 85.977 nm. The mixed MT and HSA showed the roughness of 75.369 nm, suggestive of strong binding energy between MT and HSA. Li et al. [6] and also reported the interaction between MT and HSA via the UV absorption spectra, the fluorescence emission and Fourier transform infrared (FT-IR) spectroscopy and the molecular modeling studies. The effect of MT on HSA caused considerable changes in the protein secondary structure, and the AFM data support the interaction between MT with human serum protein. These results corroborate with our earlier studies on circular dichroism (CD) analysis [22], which have explicitly demonstrated the MT-induced conformational changes in the secondary structure of protein. The significant reductions in the mean residue ellipticity (MRE) in deg $cm^2$ $dmol^{-1}$, suggested alterations in protein helicity upon MT–HSA complexation. The MT concentration dependent decrease in band intensities at 209 and 222 nm in the far-UV region reflected a change in the protein secondary structure, primarily due to reduction in α-helical content of treated protein. At a molar ratio of 1:0.04, the perturbation has been determined to be 8% with loss in protein helicity from 54.8% in native untreated HSA to 50.6% upon MT treatment [22].

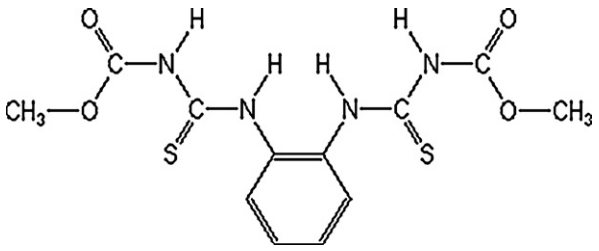

**Fig. 1.** The chemical structure of MT.

### 3.2. Optical Transmittance/reflectance/absorbance of HSA, MT and MT + HSA

Optical absorbance/transmittance/reflectance spectra were used to determine the optical constants (refractive index, absorption index, band gap and dielectric properties) of HSA, MT and MT + HSA for optical biosensor applications. Both the absorbance and the reflectance were recorded on UV–Vis–NIR double beam spectrophotometer at room temperature in the range 250–800 nm. The transmittance/reflectance spectra of HSA, MT and MT + HSA are shown in Fig. 3a. The data revealed that the HSA, MT and MT–HSA complex exhibit a transparent region through a wide range of wavelengths as shown in Fig. 1a. It is clear that the transmittance of a mixture (MT + HSA) is lower than the MT and HSA alone, indicating that the interaction occurred between them. The reflectance of MT + HSA was significantly higher than MT and HSA alone, which has affected the calculated optical constants of MT, HSA and the mixture (MT + HSA). UV–Vis absorption measurement is a very simple but effective method to explore the structural change and the complex formation. Fig. 4b represents the UV–Vis–NIR absorption spectra of HSA, MT and MT–HSA complex binding in which the most absorption area of this materials are in UV region as shown in the inset of this figure. Also, the HSA exhibited higher absorbance than both MT and MT + HSA. It is also found that the complex interaction between HSA and MT led to the enhancement the absorbance to be increased than MT alone and lower than HSA spectra. Accordingly, the MT–HSA complex formation upon mixing MT with HSA results in the change in protein conformation due to change in the micro-environment around HSA. The results correspond well with our earlier studies, which have suggested that the MT–HSA complex formation occurs mainly due to binding with aromatic amino acids, preferably the tryptophan moiety on sub-domain IIA due to the alkyl group transfer from MT to amino acid functional group [22]. The complex formation has also been validated by cyclic voltammetric analysis, where a significant reduction in the cathodic peak current (ipc) was noticed upon addition of HSA to MT solution. The reduction in peak current is attributed to the HSA interaction with MT to form a non-electrochemical complex, which blocks the electron transfer between the quasi-reversible peaks of MT and electrode [22].

Refractive index measurement plays a vital role in many areas of biophysics, biochemistry and biomedicine. The complex refractive index and dielectric function characterize the optical properties of thin materials. The optical properties of the thin films have been investigated by spectrophotometric measurement of transmittance/reflectance/absorbance at the wavelengths range of 250–800 nm. The refractive index was calculated through the following equations. The normal reflectance