The fluorescence spectroscopic studies of fluoxymestrone and acetaminophen upon interaction with human serum albumin

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1.Introduction

Human serum albumin (HSA) is the most abundant protein in the circulatory system (0.6 mmol dm^{-3}) and one of the most extensively studied proteins. It is used for the transportation and distribution of drug in the body and is very important to explain interaction mechanism, pharmaco-kinetics and toxicity of drug. Hence, the studies on binding of drug and serum albumin become an important research field in chemistry, life science and clinical medicine [1-3]. Here, the interaction of acetaminophen and fluoxymestrone with HSA was investigated under physiological conditions by utilizing the fluorescence spectroscopy. The number of binding sites and affinity were determined according to the relevant fluorescence intensity.

2.Materials and methods

HSA, fluoxymestrone and acetaminophen purchased from Sigma Chemical Company, was used without further purification.

All HSA solutions were prepared in the pH 7.4 buffer solution. Buffer solution consists of phosphate buffer (0.05 M)). All reagents were used through out all the experiments. Fluorescence spectra were performed with a Hitachi F-2500. The emission spectra were recorded between 300 and 600 nm (excitation wavelength 280 and 295 nm).

3.Result and discussion

The fluorescence intensity of HSA was decreased regularly and the fluorescence maxima showed blue shift by increasing the fluxymestrone concentration, which validates the interaction of fluoxymestrone with HSA leading to the quenching of the fluorescence of HSA and suggests that the chromophore of protein was placed in a more hydrophobic environment after addition of fluxymestrone [4]. It is well known that quenching occurs through the static or dynamic quenching process, both of which can result in a linear Stern-Volmer plot. To analyze the data from the quenching experiments, we used the Stern-Volmer equation (Eq. (1)) [4,5].

$$F_0 / F = 1 + K_q \tau_0 [Q] = 1 + K_{SV} [Q] \qquad (1)$$

Where F_0 and F are the fluorescence intensities of HSA in the absence and presence of the quencher, respectively. K_q is the quenching rate constant of the biomolecule. K_{SV} is the Stern-Volmer dynamic quenching constant, τ_0 is the average lifetime of the biomolecule without quencher, and [Q] is the concentration of quencher.