Title: **Determination of the binding constants and thermodynamic parameters of different drugs with serum proteins**

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Serum Albumin (SA) and Serum Fetuin (SF) are two of the main plasma protein synthetized in the liver and secreted into the bloodstream. Either SA or SF have been implicated as carriers of biologically active molecules or endogenous substances such as fatty acids, thyroid hormone, metal ions, metabolites, bile acids and amino acids. Moreover, they carry a large number of exogenous substances such as nutrients and drugs. Due to their physico-chemical properties, the majority of drugs are low soluble in the bloodstream and need a specific protein bearer to reach the target organ. To ensure the bioavailability, the knowledge of the drug-protein interaction parameters is essential. This interaction demonstrates the affinity of the drug for proteins so that the drug binding is strong enough to be efficiently distributed and weak enough to be released in the target organ. It does also depend on several other factors such as the affinity of the drug for the protein binding site and the presence of other drugs competing for the same site.

In the present work, Fluorescence Spectroscopy (FS) and Isothermal Titration Calorimetry (ITC) were used to study drug-protein interactions. SA and SF show an intrinsic fluorescence as they contain fluorescent amino acids (phenylalanine, tryptophan or tyrosine) in their structures. When the drug-protein complex is formed, the fluorescence tends to decrease (Quenching) and this phenomenon can be related to the binding process by means of the Stern-Volmer equations. ITC evaluates the binding event by measuring the heat exchange when the reaction takes place.

Experiments between the anti-inflammatory drug, diflunisal, and SA (Bovine, BSA, and Human, HSA) were performed by means of ITC using PBS as buffer. The stoichiometry of the reaction was 1:1 while the binding constant was 10^5 M^{-1}. Then, an antidepressant, imipramine, was evaluated with BSA and BSF using FS and HEPES as buffer. This antidepressant-protein interaction showed a stoichiometry relation 1:1 and a binding constant round 10^3 M^{-1}. Another

antidepressant, trazodone, was tested. However, it was not soluble in HEPES in the working conditions.

Keywords: Drug discovery, ITC, FS, BSA, HSA, BSF, diflunisal, imipramine.