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### **Fluorescence spectroscopy: Theoretical aspects and application in the study of interactions between caseins and molecules of interest**

Spectroscopy focus in the study of the interaction between electromagnetic radiation with matter. The application of a specific spectroscopy method varies based on species to be analyzed, kind of radiation-matter interactions and the region of the electromagnetic spectrum. The most common spectroscopy methods applied in food systems deals with the emission or absorption of radiation in ultraviolet, visible, infrared and nuclear magnetic resonance frequency ranges. Molecular luminescence is the emission of light from any molecule. When the excitation source is light, the phenomenon is classified as phosphorescence or fluorescence. The difference between the two phenomenon is the change in the orientation of the electron spin. In fluorescence, a molecule absorbs energy from an electromagnetic radiation source that promotes the excitation of an electron, which moves from the ground state to an excited state. After a small amount of time, in order of  $10^{-9}$  seconds, the electron returns to the ground state and the energy gained in the excitation process is released also as electromagnetic radiation. These transitions process can be illustrated by the Jablonsky diagram. It is relevant to notice that vibrational relaxation also is responsible for dissipation of the absorbed energy, which causes loss of energy as heat. For this reason, the emission spectrum shifts toward higher wavelengths, this phenomenon is known as Stokes shift. The chemical species able to emit fluorescence are called fluorophores. In organic compounds, fluorescence occurs mainly in aromatic structures. The fluorophores may be extrinsic, *i.e.* added to the sample, such as rhodamine and intrinsic *i.e.* naturally occurs in the sample. In proteins, tryptophan is the main fluorophore and it absorbs radiation at 280 nm and emits at 340 nm. The intensity of fluorescence can be decreased by some mechanisms, this decrease is called quenching. Among other factors, quenching can occur as result of complexation of molecules and the determination of bind constants can be achieved by measuring the quenching resulting from the interaction between the molecules. The data is adjusted to mathematic models and thermodynamic parameters also can be estimated. Caseins are natural milk occurring proteins and consisting of proximately 80% of cow milk proteins. In milk, these casein fractions self-assemble in structures called casein micelles by

hydrophobic interactions and calcium phosphate binding. Many works have suggested the use of caseins as delivery system for several bioactive molecules, including vitamins, salicylic acid, ibuprofen, insulin and anthocyanins. Therefore, it is fundamental the understanding of the interactions between the caseins with the molecules of interest. By this way, fluorescence spectroscopy raises as a tool able to estimate the physicochemical parameters of the interactions.

## References


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